# HIV-1 DRUG RESISTANCE MUTATIONS IN CHILDREN WHO FAILED NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR-BASED ANTIRETROVIRAL THERAPY

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**Abstract.** Non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens have recently been used in HIV-1 infected children in resource-limited settings. Treatment failure with this regimen has become more common. A second regimen needs to be prepared for the Thai national program. Genotypic resistance testing was conducted among HIV-1 infected children who experienced virological failure with antiretroviral therapy (ART) using NNRTI-based regimens. Patterns of resistance mutations were studied and options for a second regimen were determined. There were 21 patients with a median (IQR) age of 4.1 (1.9-7.7) years. Sixteen patients were males. The median CD4 cell count and HIV-1 RNA at the time of virological failure were 647 cells/mm$^3$ and 5.3 log copies/ml, respectively. The prevalences of patients with ≥1 major mutation conferring resistance to NRTIs and NNRTIs were 52% and 43%, respectively. Thymidine analoque mutations, M184V/I, and Q151M were observed in 38%, 33%, and 5%. The patterns of resistance mutations suggest that 48% of patients need a protease inhibitor-based regimen for the second regimen and didanosine+lamivudine is the most required nucleoside reverse transcriptase inhibitor backbone.

## INTRODUCTION

Combined antiretroviral therapy (ART) has changed the course of human immunodeficiency virus type 1 (HIV-1) disease, with a substantial reduction in morbidity and mortality (Palella *et al*, 1998; Manosuthi *et al*, 2006). This benefit is also well established in HIV-1 infected children (Granados-Sánchez *et al*, 2003; Viani *et al*, 2004; Puthanakit *et al*, 2007; Patel *et al*, 2008). Access to HIV care and ART for HIV-infected children has greatly improved in recent years in resource-limited settings. The recommendation for ART in children is a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) and one of the non-nucleoside reverse transcriptase inhibitors (NNRTIs) or a protease inhibitor (PI) (WHO, 2007; DHHS, 2008). A NNRTI-based regimen is preferred particularly in resource-limited settings secondary to its simple regimen, tolerability and accessibility. HIV-1 antiretroviral resistance is a major cause of

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treatment failure (Kuritzkes, 2004; Gallant, 2006). The extensive mutation rate, prolonged use of antiretroviral agents and incomplete viral suppression leads to the development of drug resistant viruses and the management of treatment failure represents a serious challenge to the management of HIV-1 infected children.

HIV-1 genotypic resistance testing detects resistance mutations in reverse transcriptase (RT) and protease (PR) genes by comparing the gene sequences of a resistant virus to those of a wild-type strain and is the most useful method for patients failing an initial regimen to identify the presence of resistance to drugs in the current regimen, as well as cross resistance to other drugs in the same class (Haupts *et al*, 2003; Hirsch *et al*, 2003; Johnson *et al*, 2008). However, the cost of the test limits the accessibility of resistance testing in resource-limited settings.

The present study aimed to demonstrate the patterns of genotypic resistance mutations in pediatric patients who failed an initial regimen of NNRTI-based ART and determine the options for a second regimen in this population. The results from the present study should be useful for planning with the national ART program for HIV-1 infected children and for considering ART options in patients who fail an initial regimen of NNRTI-based ART and cannot afford genotypic resistance testing.

## MATERIALS AND METHODS

### Patients

An observational study was conducted among HIV-1 infected children who were diagnosed with virological failure from an initial regimen of an NNRTI-based ART between January 2000 and December 2007 at a teaching hospital. Inclusion criteria were: (1) HIV-1-infected patients <15 years of age, (2) having been treated with NNRTI-based ART as an initial regimen, (3) having virological failure documented by HIV-1 RNA assay of greater than 1,000 copies/ml. Patients who had received duotherapy or a protease inhibitor (PI) were excluded. HIV-1 genotypic resistance testing was conducted and mutations critical to the HIV-1 RT and protease sequences were reviewed from the results of genotypic resistance tests. Patterns of resistance mutations were studied and the options for a second antiretroviral regimen were determined. The study was reviewed and approved by the institute review board.

### Genotypic resistance assay

HIV RNA was isolated from plasma samples using the QIAamp viral extraction kit (Qiagen, Chatsworth, CA). The TRUGENE HIV-1 Genotyping Assay (Grant *et al*, 2003; Kuritzkes *et al*, 2003) was used in conjunction with the Open Gene automated DNA sequencing system (Visible Genetics, Toronto, Canada) to sequence the RT and PR regions of the HIV-1 cDNA. Testing involved simultaneous clip sequencing of protease and codons 35-244 of the RT from the amplified cDNA in both the 3´ and 5´ directions. Sequences were aligned and compared with a lymphoadenopathy-associated virus type 1 (HIV-B-LAV1) consensus sequence using Visible Genetics Gene Librarian software. We focused on mutations at positions in the polymerase gene previously reported to be associated with ARV resistance by the International AIDS Society-USA Drug Resistance Mutations Group (Johnson *et al*, 2008). In this study, M41L, K65R, D67N, insertion 69, K70R, L74V, L100I, K103N, V106A/M, V108I, Y115F, Q151M, Y181C/I, M184V/I, Y188C/L/H, G190A/S, L210W, T215Y/F, K219Q/E, and P225H in the RT gene and D30N, V32I, L33F, M46I/L, I47V/A, G48V, I50V, V82A/T/F/S, I84V, and L90M in the PR gene were considered as major drug resistance mutations.

**Statistics**

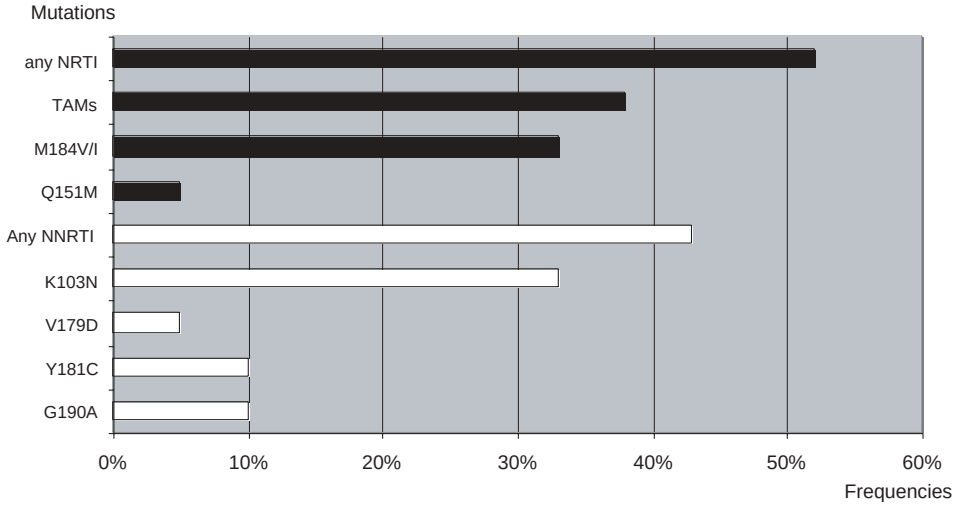
Mean (± standard deviation, SD), median (interquartile range, IQR) and frequencies (%) were used to describe patient characteristics in each group. The Student's *t*-test was used to compare means of continuous variables with normal distribution between the two groups and the Mann-Whitney *U* test was used to compare the medians of continuous variables with non-normal distribution. The chi-square test and Fisher exact test were used to compare categorial variables where appropriate. All analyses were performed using SPSS version 14.0. A *p*-value less than 0.05 was considered statistically significant.

## RESULTS

There were 21 patients with a median (IQR) age of 4.1 (1.9-7.7) years. Sixteen (76%) patients were males. Twelve (57%) patients received a nevirapine-based regimen and the others were on efavirenz-based ART. Distributions of the two NRTIs in the regimen were zidovudine + lamivudine (13 patients, 62%), stavudine + lamivudine (5 patients, 24%), zidovudine + didanosine (2 patients, 10%), and stavudine + didanosine (1 patient, 5%). The median (IQR) duration of ART prior to failure was 6.3 (4.6-11.9) months. The median (IQR) CD4 cell count and HIV-1 RNA at the time of virological failure were 647 (198-1,290) cells/mm$^3$ and 5.3 (4.5-5.9) log copies/ml, respectively.

Of 21 patients, 12 (57%) had ≥1 major mutation confering drug resistance to any antiretroviral drug. The prevalences of patients with NRTI and NNRTI reistance mutations were 52% and 43%, respectively. Thymidine analogue mutations (TAMs), M184V/I and Q151M were observed in 38%, 33%, and 5% of patients, respectively (Fig 1). For NNRTI mutations, K103N, V179D, Y181C, and G190A were observed in 33, 5, 10, and 10%, respectively.

Patients who acquired M184V/I had a higher prevalence of NNRTI resistance mutations when compared to patients without




TAMs, thymidine analogue mutations; NRTI, nuceloside reverse transcriptase inhibitor; NNRTI, non-nuceloside reverse transcriptase inhibitor.

Fig 1–NRTI and NNRTI resistance mutations in 21 patients.

M184V/I (86% *vs* 21%, $p = 0.016$). The second regimens were determined based on the resistance mutation patterns. There were 10 (48%) patients who needed PI-based regimens as the second regimen because of NNRTI resistance (43%) or multi-NRTI resistance (5%). The required NRTI backbones for the second regimen were didanosine + lamivudine (38%), zidovudine + didanosine (10%), zidovudine + lamivudine (5%), and tenofovir + lamivudine (5%). Of note, there were 5% of patients (with Q151M) who needed tenofovir in the second regimen.

## DISCUSSION

The results from the present study demonstrate relatively high frequencies of NRTI and NNRTI resistance mutations in patients having virological failure. More than half of patients had genotypic mutations contributing to the resistance of NRTI and/or NNRTIs. Further, these patients acquired drug resistance mutations in a short duration of ART. A previous study in a resource-limited setting showed that children are almost twice as likely to have virological failure compared with adults (Kamya *et al*, 2007). However, nearly half the patients had no drug resistance mutations at the time of virological failure. This finding suggests adherence may be a major issue for HIV infected children.

Among patients with drug resistance mutations, TAMs, M184V/I, and NNRTI resistance mutations were commonly observed in the present study. This finding is concordant with a previous study in adults failing an initial NNRTI-based regimen (Sungkanuparph *et al*, 2007a). The relationship between M184V/I and NNRTI mutations suggests that these two mutations may occur simultaneously. Both NNRTIs and lamivudine have a low genetic barrier that may facilitate the development of high-level resistance in one step (Schuurman *et al*, 1995; Podzamczer and Fumero, 2001). Lamivudine resistance is rapidly acquired if the other components in the regimen are not effective.

Since NNRTI resistance mutations were commonly found in patients failing NNRTI-based regimens, a PI-based regimen is preferred for the second ART regimen. A ritonavir boosted-PI containing regimen has shown virological success in salvage regimens among HIV-1 infected children (Larru *et al*, 2007). Choosing a backbone NRTIs for a second regimen is limited by NRTI resistance mutations in patients. Due to the high rate of M184V/I, lamivudine should not be used as an NRTI in the second regimen unless no other active NRTI is available (Sungkanuparph *et al*, 2007b). Although tenofovir has not been approved yet for children, some small studies have demonstrated good efficacy and tolerability in children through 96 weeks (Viganò *et al*, 2007a,b; Rosso *et al*, 2008). However, monitoring of children who require treatment with TDF is warranted due to the risk of decreased bone mineral density (Papaleo *et al*, 2007; Purdy *et al*, 2008).

In resouce-limited settings where the availability and affordability of antiretroviral agents are limited, early detection of virological failure and drug resistance mutations is crucial. The patients in the present study had better options for a second ART regimen when compared to adult patients in a previous study in which some patients had late detection of failure (Sungkanuparph *et al*, 2007a). In developing countries where the HIV-1 RNA assay is not widely available or not affordable, monitoring of ART and early diagnosis of virological failure is difficult. In order to preserve future options for the second regimen in patients, the accessibility of this tool needs to be scaled-up along with the accesibility of ART.

A limitation of the present study is that the sample size is relatively small. This is due to the small number of HIV-1 infected children compared to adults. However, we conducted the present study at a tertiary care center which is also a center for HIV-1 genotype testing. All children were monitored with the HIV-1 RNA assay and the genotype test when there was virological failure. Thus, we were able to demostrate various patterns of resistance mutations. Another limitation of this study was that the results of the present study may not be applicable to other settings. In medical centers where the HIV-1 RNA assay is not regularly performed and virological failure is detected late, there may be a higher rate of NRTI resistance including multinucleoside resistance. However, the data from the present study may provide useful information for planning a second regimen in the national AIDS program. This information should also be beneficial for physicians who care for HIV-1 infected children failing the initial NNRTI-based regimen but lacking resistance testing. Since HIV-1 resistance is affected by multiple factors, the genotypic resistance patterns are not accurately predictable. Individual resistance testing is still highly recommended whenever available.

In conclusions, NRTI and NNRTI resistance were observed in more than half the patients experiencing virological failure with an initial NNRTI-based regimen. TAMs, M184V/I, and Q151M were observed in 38, 33, and 5% of patients, respectively. The patterns of resistance mutations suggested that 48% of patients needed a protease inhibitor-based regimen for the second regimen; didanosine + lamivudine was the most required NRTI backbone (38%). In resource-limited settings where antiretroviral agents are limited, early detection of virological failure may provide more options and better treatment outcomes.

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