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In Pediatric AIDS Clinical Trials Group 377, antiretroviral therapy–experienced children were randomized to 4 treatment arms that included different combinations of stavudine, lamivudine (3TC), nevirapine (Nvp), nelfinavir (Nfv), and ritonavir (Rtv). Previous treatment with zidovudine (Zdv), didanosine (ddI), or zalcitabine (ddC) was acceptable. Drug resistance (^{R}) mutations were assessed before study treatment (baseline) and at virologic failure. Zdv^R, ddI^R, and ddC^R mutations were detected frequently at baseline but were not associated with virologic failure. Children with drug resistance mutations at baseline had greater reductions in virus load over time than did children who did not. Nvp^R and 3TC^R mutations were detected frequently at virologic failure, and Nvp^R mutations were more common among children receiving 3-drug versus 4-drug Nvp-containing regimens. Children who were maintained on their study regimen after virologic failure accumulated additional Nvp^R and 3TC^R mutations plus Rtv^R and Nfv^R mutations. However, Rtv^R and Nfv^R mutations were detected at unexpectedly low rates.

Treatment with highly active antiretroviral therapy (HAART) can prolong the lives and improve the health of patients with human immunodeficiency virus type 1 (HIV-1) infection. However, the emergence of drug-resistant HIV-1 can limit the efficacy of antiretroviral treatment regimens and a patient's treatment options [1, 2]. Most studies of antiretroviral drug resistance have been in adults [3–5]; however, those results may not be directly applicable to pediatric cohorts, since factors that influence selection of drug-resistant variants may be different in children. Such factors include the heterogeneity of HIV-1 variants in the virus pool before drug therapy, the potential for the patient to have been infected with drug-resistant HIV-1, the pharmacokinetics of antiretroviral drugs across different age groups, and unique barriers to adherence to treatment regimens.

Limited studies in children have confirmed that drug-resistant HIV-1 can emerge during antiretroviral therapy [6, 7] and that emergence of drug-resistant HIV-1 can affect clinical outcome [8]. The number of children who have drug-resistant HIV-1 before initiating antiretroviral therapy probably has increased in recent years, since the use of antiretroviral drugs to prevent vertical transmission has become the standard of care [9–11]. An increasing number of children in developed nations probably have acquired drug-resistant HIV-1, since antiretroviral use has increased, typically as sequential courses of 1- or 2-drug regimens. Infection of a child with drug-resistant HIV-1 by horizontal transmission also has been reported [12].

Here we examined HIV-1 drug resistance in stable, antiretroviral therapy–experienced HIV-infected children enrolled in a randomized, open-label, multiarm controlled trial, Pediatric

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AIDS Clinical Trials Group Protocol (PACTG) 377 [13]. Children in PACTG 377 were randomized to treatment arms that included different combinations of stavudine (d4T), lamivudine (3TC), nevirapine (Nvp), nelfinavir (Nfv), or ritonavir (Rtv) (table 1). Children were followed up for ≤96 weeks for analysis of safety, tolerance, and virologic response. Of the children, 57% had satisfactory initial virologic suppression (see below). The proportion of children who were maintained on their initial study therapy and had sustained HIV-1 RNA suppression (≤400 copies/mL) at week 48 was significantly higher for study arm D, which suggests that the 4-drug combination prolonged the benefits of antiretroviral therapy (P.K., unpublished data).

We evaluated the prevalence of drug resistance mutations in the children before study treatment and the impact of these mutations on virologic response to treatment. We also evaluated whether there was additional selection of drug resistance mutations in children who experienced virologic failure and whether selection was less common in children who received the 4-drug regimen. This is the largest study to date of HIV-1 drug resistance in children and the first to examine drug resistance in a large pediatric cohort in the context of a randomized, controlled trial of HAART.

Materials and Methods

Quantification of HIV-1 RNA. HIV-1 RNA copy numbers (virus loads) were measured by using the Amplicor HIV-1 Monitor Test kit (Roche Diagnostic Systems), with an assay quantification limit of 400 copies/mL.

HIV-1 genotyping. HIV-1 genotyping was done by using the

Table 1. Resistance mutations detected in Pediatric AIDS Clinical Trials Group 377, by trial arm and outcome.

	Arm A			Arms B + Q			Arm C			Arm D		
	d4T + Nvp + Rtv			d4T + 3TC + Nfv			d4T + Nvp + Nfv			d4T + 3TC + Nvp + Nfv		
Resistance mutation	Baseline (n = 32)	Failure (n = 17)	Late failure (n = 9)	Baseline (n = 49)	Failure (n = 19)	Late failure (n = 11)	Baseline (n = 30)	Failure (n = 16)	Late failure (n = 12)	Baseline (n = 24)	Failure (n = 7)	Late failure (n = 3)
Reverse transcriptase												
M41L	13	4	3	19	6	5	10	4	5	10	2	1
E44D	2			4			3			1		
D67N	5	2	2	10	2	1	9	5	4	5	1	
T69D					2	1		1	1		1	1
K70R	5	3	3	11	5	2	6	4	2	5	1	1
L74V	3	1		8	1		6	2	1	4		
V75T				1			1	1	1			
K103N	1	12	7					7	6		1	2
V106A		1	1					1			1	1
V108I										2		
V118I	3			11	3	2	7	2	2	2	1	1
Q151M							1	1	1			
Y181C		5	5					8	6			
M184I								1				
M184V	2	1		1	14	10	1				3	2
Y188L		1							1			
G190A		3	1				1	5	3			1
L210W	7	2	1	12	3	3	7	4	3	7	2	1
T215F	1			5	2		3	1		3		
T215Y	13	7	4	16	6	5	9	5	5	10	2	1
K219Q	3	1	1	8	2	1	4	1		4	1	
Protease inhibitor												
L10I	2	1		3	2	1	2	2	1	1		1
K20R				1		1	1		1			
D30N						1		2	3			
L33F			1									
M36I	6	3	1	3	1	1	7	3	3	3	1	
M46I			1	1		3						
M46L						1			1			
I54V			1									
A71V	1	1				1	2					
A71T			2	2			1		2			1
V77I	8	5	3	12	5	6	11	8	6	4	2	1
V82A		1	3									
I84V			1									
N88D						1			2			
L90M			2	2		4			1			

NOTE. Data are no. of times each mutation was detected in samples of the study arms. The number of children analyzed at each time point in each study arm is shown *(n)*. Data from study arms B and Q are combined; arms B and Q differed in the frequency of Nfv dosing. 3TC, lamivudine; d4T, stavudine; Nfv, nelfinavir; Nvp, nevirapine; Rtv, ritonavir.

ViroSeq HIV-1 Genotyping System (Applied Biosystems). In some cases, the amount of plasma available for analysis was limited, and genotyping was done with less than the recommended 0.5 mL of plasma. In this system, HIV-1 RNA is extracted from plasma samples and reverse transcribed with murine Moloney leukemia virus reverse transcriptase (RT). A 1.8-kb DNA fragment is then amplified in the same tube in a single 40-cycle polymerase chain reaction (PCR) with AmpliTaq gold polymerase and uracil N-deglycosylase decontamination control. PCR products are purified by using spin columns, analyzed by agarose gel electrophoresis, and sequenced with premixed BigDye sequencing reagents in 7 separate reactions. Sequencing products were analyzed by using an ABI 310 or ABI 377 automated sequencer. The resulting sequences were assembled and were analyzed with HIV-1 Genotyping System software (see below).

Phylogenetic analysis. Protease and RT nucleotide sequences (297 and 972 nt, respectively) were aligned. Phylogenetic reconstructions were performed with PHYLIP 3.572c (J. Felsenstein, University of Washington, Seattle), using the neighbor-joining method. Reference sequences were obtained from the 1999 HIV-1 Subtype Reference Alignments of the Los Alamos National Laboratory (http://hiv-web.lanl.gov/), including laboratory strains pNL4-3 and HXB2. There was no evidence of contamination with strains pNL4-3 or HXB2, which were present in some of the genotyping laboratories. Furthermore, sequences from samples derived from individual children collected at different times clustered more closely with one another than with sequences from other children in the study, and no 2 sequences were identical. Therefore, it is unlikely that samples were cross-contaminated (e.g., during PCR) or were misidentified.

Analysis of drug resistance mutations. Mutations associated with resistance to antiretroviral drugs were identified by using the HIV-1 Genotyping Software package v2.1 or v2.2 (Applied Biosystems). Analysis was focused on primary and secondary HIV-1 drug resistance mutations described in a recent consensus report [2]. These mutations and the drugs with which they are associated are as follows (primary mutations are denoted with an asterisk): d4T, V75T; 3TC, E44D*, V118I*, and M184V*; Nvp, L100I, K103N*, V106A*, V108I*, Y181C/I*, Y188C/H/L*, and G190A*; Rtv, K20M/R, V32I, L33F, M36I, M46I/L, I54L/V, A71T/V, V77I, V82A/F/S/T,* I84V, and L90M; Nfv, L10F/I, D30N*, M36I, M46I/L, A71T/V, V77I, V82A/F/S/T*, I84V, N88D, and L90M*; zidovudine (Zdv), M41L, D67N, K70R*, L210W, T215F/Y*, and K219Q; didanosine (ddI), K65R, L74V*, and M184I/V; zalcitabine (ddC), K65R*, T69D*, L74V*, and M184I/V*; and multinucleoside, Q151M, 69 insertion.

Sequences were obtained for protease (aa 1–99) and RT (aa 1–324). Drug resistance mutations present as amino acid mixtures were identified only if the corresponding nucleotide mixture was present in the sequences of both DNA strands. Sequences from both DNA strands were obtained for the entire region analyzed for 214 (93%) of 229 samples.

Statistical analysis. Genotyping data were transmitted electronically to a central data management center and were entered into a central database. Statistical analysis was performed at the ACTG Statistical Data Analysis Center (Boston). Results presented in table 2 are based on the Wilcoxon test. The Wei-Johnson statistical technique [14] was used to combine the dependent tests at weeks 8, 12, 24, 36, and 48. Multifactorial simulation "hotspot" analysis was done as described elsewhere [15].

Table 2. Comparison of mean change in virus load among children who did and did not have primary drug resistance mutations at baseline.

Children, week	Reduction in virus load[a]		95% CI[b]	P[c]
	Mutation(s) present	Mutations(s) absent		
All				
8	1.7 (91)	1.0 (29)	0.3–1.2	.002
12	1.6 (88)	1.2 (31)	0–0.9	.048
24	1.7 (75)	1.1 (24)	0.2–1.2	.005
36	1.8 (71)	1.2 (21)	0.1–1.1	.024
48	1.9 (71)	1.3 (23)	0.1–1.1	.014
Received Nvp[d]				
8	1.8 (51)	1.0 (21)	0.4–1.4	<.001
12	1.6 (50)	1.0 (23)	0.2–1.2	.011
24	1.7 (45)	1.0 (17)	0.08–1.2	.014
36	1.7 (43)	1.1 (17)	0.1–1.1	.026
48	1.7 (41)	1.3 (18)	0.03–0.9	.100
Did not receive Nvp[e]				
8	1.5 (40)	1.1 (8)	−0.3 to 1.3	.226
12	1.5 (38)	1.5 (8)	−0.8 to 0.9	.933
24	1.8 (30)	1.1 (7)	−0.3 to 1.6	.129
36	1.9 (28)	1.8 (4)	−1.0 to 1.3	.739
48	2.1 (30)	1.4 (5)	−0.4 to 1.5	.178

NOTE. Data are no. of mutations (no. of children), unless otherwise indicated. Nvp, nevirapine.

[a] Mean decrease in $\log_{10}$ RNA copies/mL from baseline at each time point. Mean baseline virus loads for children with or without ⩾1 primary resistance mutation at baseline were 4.6 and 4.4 $\log_{10}$ RNA copies/mL, respectively.

[b] 95% Confidence intervals (CIs) for difference of means.

[c] Two-sided P values.

[d] Study arms A, C, and D.

[e] Study arms B and Q.

GenBank accession numbers. GenBank accession numbers for the HIV-1 sequences analyzed in this report are as follows: baseline sequences, AF357605–AF357739; failure sequences, AF357740–AF357798; and late failure sequences, AF357799–357833.

Results

PACTG 377 Patient Cohort

In total, 181 children aged 4 months to 17 years were enrolled in PACTG 377 from December 1997 through September 1998. Previous treatment with Zdv, ddC, or ddI was acceptable: 35% had previous treatment with ddI, 60% with Zdv/ddI, and 5% with other drug combinations. Children were randomized to the study arms shown in table 1. In PACTG 377, satisfactory initial virologic suppression was defined as plasma HIV-1 RNA level ⩽400 copies/mL or a decrease in RNA that was both ⩾2 $\log_{10}$ copies/mL below baseline and <10,000 copies/mL on ⩾ 2 of the 3 RNA determinations done at weeks 8, 12, and 16 [13]. Subsequent virologic failure was defined as an RNA value >10,000 copies/mL that also was a 0.75 $\log_{10}$ increase above the RNA nadir (the average of the $\log_{10}$ transformation of the 2 lowest RNA determinations at weeks 8, 12, and 16) [13]. In PACTG 377, children were divided into failure and nonfailure groups on the basis of their virologic response to their PACTG

377 treatment regimen. The nonfailure group included children who had satisfactory initial virologic suppression with sustained virologic suppression for the duration of the study period (to week 48). The failure group included children who did not achieve satisfactory initial virologic suppression or who had subsequent virologic failure.

PACTG 377 Patient Subcohort in Resistance Analysis

HIV-1 genotyping was done by using plasma samples collected at 3 different time points: baseline, failure, and late failure, as defined below. All available samples from each time point were analyzed. Of the 181 children in PACTG 377, 141 had ⩾1 plasma sample available for resistance studies. This included 71 children in the nonfailure group and 70 children in the failure group. In total, 229 samples were analyzed, including 135 baseline samples, 59 failure samples, and 35 late failure samples. The subcohort of patients analyzed in this report ($n = 141$) were of similar age and had similar baseline virus loads, baseline CD4 cell counts, and previous treatment histories, compared with those in 181 children enrolled in PACTG 377. This also was the case for the individual study arms.

Analysis of Baseline Samples

In PACTG 377, plasma samples were collected before the initiation of antiretroviral study treatment (baseline samples). Baseline samples were available for genotypic analysis from 135 of 141 children, including samples from 71 (75%) of 95 children in the nonfailure group and 64 (76%) of 84 children in the failure group. Protease and RT sequences obtained from genotypic analysis of baseline samples were analyzed for the presence of primary drug resistance mutations associated with PACTG 377 study drugs (3TC, d4T, Nvp, Nfv, and Rtv) and for nonstudy drugs that the children might have received before enrollment in the trial (Zdv, ddI, and ddC). We observed a high rate of primary drug resistance mutations at baseline in both the failure and nonfailure groups. However, there was no significant difference between the rate of mutations associated with resistance to nonstudy drugs or study drugs in the failure versus nonfailure groups. Analysis of specific mutations is described below.

Baseline mutations associated with resistance to nonstudy drugs. At study entry, many children had baseline mutations associated with resistance to nonstudy drugs (table 1), which is consistent with previous exposure to nucleoside RT inhibitors. Of note, most of these mutations were associated with Zdv resistance mutations (Zdv^R), which also are recognized increasingly as playing a role in resistance to d4T. Furthermore, 30 (47%) of 64 children in the failure group and 39 (55%) of 71 children in the nonfailure group had ⩾2 Zdv^R mutations. There was no significant difference between the number of baseline Zdv^R mutations in the failure group and that in the nonfailure groups ($P = .39$, Fisher's exact text). Furthermore, the presence of any single Zdv^R mutation or of ⩾2 Zdv^R mutations at baseline was not predictive of virologic failure. Mutations associated with ddI^R or ddC^R also were detected at baseline but were not associated with virologic failure in this cohort. Specifically, the presence of the M184V mutation, which also confers resistance to the study drug 3TC, was not predictive of virologic failure in the 3TC-containing treatment arms (see below).

Baseline mutations associated with resistance to study drugs. Primary mutations associated with resistance to drugs included in the study also were detected at baseline (table 1). Of these 43 mutations, 37 (86%) were associated with resistance to 3TC (E44D, V118I, and M184V). The M184V mutation, which may have been selected during previous ddI or ddC treatment, was identified in only 4 (3%) of 135 children. In contrast, the E44D and V118I mutations were identified at baseline in 10 (7%) and 23 (17%) of 135 children, respectively. The latter 2 mutations probably were selected for by previous treatment with Zdv in combination with ddI [16]. Those mutations (E44D and V118I) have been associated with low-to-moderate cross-resistance to 3TC when Zdv^R mutations also are present [16]. Of the 79 children who received a 3TC-containing regimen in PACTG 377, 16 had the E44D and/or V118I mutation and ⩾1 Zdv^R mutation at baseline. There was no significant difference in the frequency of this mutation pattern at baseline among the failure and nonfailure groups among children who received 3TC (69% and 51%, respectively; $P = .26$, Fisher's exact test). In addition to the mutations described above, we identified 4 children with Nvp^R mutations (K103N, V108I, and G190A) at baseline and 2 children with the L90M mutation associated with Nfv^R. The protease polymorphisms V77I and M36I were detected in many of the baseline samples. However, there was no significant difference between the frequency of these polymorphisms in the failure group and those in the nonfailure group, or among any of the treatment arms (A, B, C, or D, pairwise comparisons).

Relationship of baseline genotype to virus load. We first examined whether the presence of individual primary drug resistance mutations at baseline correlated with virus load at time of virologic failure. The only finding of statistical significance was that the 8 children who had the V118I mutation at baseline had slightly lower mean virus loads at failure than did the 54 children who did not have this mutation (4.5 $\log_{10}$ and 4.0 $\log_{10}$ RNA copies/mL, respectively; $P = .04$). The clinical significance of this finding is not clear. We found no significant difference in the mean virus load at failure between children who had the E44D and/or V118I mutation in the context of ⩾1 Zdv^R mutation and those who did not have those mutations at baseline.

We next compared the reduction in virus load during the treatment period among children who did and did not have ⩾1 primary drug resistance mutation at baseline. Ten children for whom study treatment was changed or discontinued before week 8 were excluded from this analysis. Those children were withdrawn from the study because of drug intolerance or for

other clinical reasons (virologic data were not available until study week 12). At each time point, a linear regression model with baseline RNA adjustment was used for analyzing the reduction of virus load ($\log_{10}$ RNA; table 2). We also analyzed virus loads over time, combining results from weeks 8, 12, 24, 36, and 48. Results for the 2-sided combined test were highly significant ($P < .001$). Our analysis indicates that the reduction in virus load over the course of the 48-week study was consistently greater for children who had ⩾1 primary resistance mutation at baseline than for those who did not. This effect also was observed in children who received Nvp-containing regimens in PACTG 377 but not in children who did not receive Nvp (table 2).

Analysis of Failure Samples

We next examined whether resistance mutations were selected in children who experienced virologic failure. We analyzed samples collected at week 12 from children who failed to attain satisfactory initial virologic suppression and samples collected at later times from children who initially attained virologic suppression but had subsequent rebound in HIV-1 load. Of the 70 children, virologic failure time (time of sample collection) occurred at week 12 for 59 (84%), at week 24 for 3 (4%), at week 36 for 7 (10%), and at week 48 for 1 (1%) (median, 12 weeks; range, 12–48 weeks).

Mutations associated with resistance to nonstudy drugs that were identified at the time of virologic failure included those associated with Zdv^R and ddI^R or ddC^R. There was no significant difference between the frequency of any of these mutations in the baseline and failure samples. In contrast, 47 (81%) of 58 children had ⩾1 primary resistance mutation to a study drug at the time of virologic failure versus 16 (25%) of 64 children at baseline. That difference was highly significant ($P < .0001$, Fisher's exact test). The majority of these mutations were associated with $3TC^R$ (V118I and M184V) and Nvp^R (K103N, V106A, Y181C, Y188L, and G190A). In contrast, mutations associated with Nfv^R (D30N and V82A) and Rtv^R (V82A) were detected rarely at the failure time point (table 1). When we analyzed the 52 children who had both baseline and failure samples genotyped, 3 mutations were present at significantly higher rates in the failure samples (table 3), the K103N and Y181C mutations associated with Nvp^R and the M184V mutation associated with $3TC^R$. A significantly higher rate of these mutations in failure samples was substantiated further by using multifactorial simulation hotspot analysis of the entire set of data and comparing the rate of each mutation at the baseline versus failure time points ($P < .0001$ for K103N, $P = .002$ for Y181C, and $P = .0006$ for M184V). No primary Nvp^R mutations were detected at virologic failure among children in study arm B, which lacked Nvp. Primary $3TC^R$ mutations were detected at virologic failure in 4 children in study arms A and C, which lacked 3TC. Two of these children had the V118I mutation, and 2 had the M184V mutation. All 4 children received ddI before study entry. These mutations were detected at baseline in 2 of the 3 children for whom a baseline sample was available.

Table 3. Comparison of numbers of children ($n = 52$) with the K103N, Y181C, or M184V mutations in baseline and failure samples.

Mutation	At baseline	After treatment failure	P[a]
K103N	1 (2)	17 (33)	<.0001
Y181C	0 (0)	11 (21)	.0005
M184V	3 (6)	18 (35)	.0004

NOTE. Data are no. (%) of children.
[a] P values were obtained by a nonparametric resampling method.

We next analyzed whether children who received 3-drug Nvp-containing regimens (study arms A and C) were more likely to develop Nvp^R mutations than those who received the 4-drug regimen (study arm D). Of the children who received 3-drug regimens, 27 (84%) of 32 had ⩾1 primary Nvp^R mutation at the time of virologic failure. In contrast, only 2 (29%) of 7 children who received the 4-drug regimen (study arm D) had those mutations ($P = .007$, Fisher's exact test). We performed a similar analysis to compare the rate of selection of primary $3TC^R$ mutations in children who received 3-drug 3TC-containing regimens (study arms B + Q) versus the 4-drug 3TC-containing regimen (study arm D). The number of children in these 2 groups who had ⩾1 $3TC^R$ mutation at the time of virologic failure was not statistically significant ($P = .32$, Fisher's exact test).

Analysis of Late Failure Samples

An amendment added to PACTG 377 allowed children in the failure group to remain on their initial study regimen, provided that their HIV-1 RNA levels remained <100,000 copies/mL. We evaluated whether continued treatment after an initial virologic failure led to increased selection of drug resistance mutations. In most cases, children who were maintained on their study regimen after an initial virologic failure were still receiving their initial study regimen at week 48. For those children, we analyzed samples collected at week 48. For children whose treatment regimen was changed or discontinued before week 48, samples were collected within 1 week of a discontinuation or change in study therapy. In a few cases, PCR amplification was unsuccessful because of a low virus load. In those cases, a sample collected no more than 4 weeks earlier, while the child was still on the study regimen, was analyzed.

Late failure samples were available and analyzed from 35 children (table 1). Of these 35 children, 27 also had baseline samples and samples from their initial virologic failure analyzed. For the 27 children, the mean time between collection

of baseline samples and the late failure sample was 40 weeks (range, 20–48 weeks), and the mean time between collection of samples from the initial virologic failure and late failure was 26.8 weeks (range, 9–41.7 weeks). We found that 11 (40.7%) of these children developed ⩾1 new primary mutation at the late failure time point (not present in baseline or initial failure samples). Those mutations were associated with $3TC^R$ (M184V [$n = 3$]), Nvp^R (K103N [$n = 3$], Y181C and Y188L [$n = 1$ each] and G190A [$n = 2$]), Rtv^R (V82A [$n = 1$]), and Nfv^R (D30N and V82A [$n = 1$ each], and L90M [$n = 4$]). Four children had ⩾2 new primary mutations at late failure. We saw no association between the presence of primary mutations at the time of virologic failure and the development of additional primary mutations at late failure; however, the number of children in that analysis was small.

Discussion

We describe the analysis of HIV-1 drug resistance mutations among a large cohort of children enrolled in the PACTG 377 protocol. Many of these children had been treated with antiretroviral nucleoside RT inhibitors (nRTIs) before enrollment in the trial. Consistent with treatment histories of children in this cohort, we found a high rate of primary mutations associated with resistance to the nRTIs, Zdv, ddI, and ddC. However, none of the baseline mutations were associated with a higher rate of virologic failure. Of interest, many of the children had the RT mutations E44D and V118I at study entry. Those mutations were probably selected during previous treatment with Zdv and ddI and are associated with low-to-moderate $3TC^R$ when Zdv^R mutations also are present [16]. Sixteen of the children who were randomized to one of the 3TC-containing study arms had either E44D or V118I in combination with ⩾1 Zdv^R mutation at baseline. However, children with these baseline mutation patterns did not experience a significantly higher rate of virologic failure than did children who lacked these mutations.

Previous studies have demonstrated that HIV-1 with drug resistance mutations may have reduced replicative capacity when the drug is absent [17–19]. For example, decreased fitness has been observed for HIV-1 with the M184V mutation [20–22]. We found that children who had any primary resistance mutation at baseline had consistently greater reductions in virus loads over the course of the 48-week study than did children without these mutations. The majority of primary resistance mutations present at baseline were Zdv^R mutations. This effect was observed in children who received Nvp-containing regimens in PACTG 377 but was not seen in the children who did not receive Nvp. This suggests that Nvp hypersusceptibility may play a role in the greater reduction in virus load seen in children with baseline resistance mutations. Alternatively, the presence of drug resistance mutations in HIV-1 may lower the replicative capacity of the virus. This finding suggests that the presence of drug resistance mutations may actually improve the virologic response to therapy in children who are under treatment with other antiretroviral drugs. This interesting finding is not completely unexpected and merits further investigation.

Our analysis of HIV-1 from samples collected at the time of virologic failure revealed a high rate of selection of HIV-1 with primary Nvp^R and $3TC^R$ mutations. An important observation from this study was that the rate of primary Nvp^R mutations at the time of virologic failure was significantly higher for children receiving 3-drug versus 4-drug Nvp-containing regimens. This suggests that the addition of a fourth drug to the treatment regimen may provide additional potency and therefore slow selection of Nvp^R variants. This may explain the better virologic response in the 4-drug arm (study arm D) in PACTG 377. Continued treatment after virologic failure also was associated with selection of additional primary drug resistance mutations associated with resistance to RT inhibitors. In several cases, children developed resistance to ⩾2 of their study drugs.

Mutations associated with resistance to the protease inhibitors, Nfv and Rtv, were detected rarely at the time of virologic failure. Only 2 children had the D30N mutation at the failure time point, and none had the L90M mutation. Furthermore, additional protease inhibitor mutations (D30N, L90M, or V82A) were detected in only 5 (19%) of 27 children who were maintained on their initial study regimen after an initial virologic failure. In contrast, mutations associated with resistance to protease inhibitors have been observed frequently at the time of virologic failure in adults receiving Nfv [23, 24]. Furthermore, in a phase I study of Nfv in a pediatric cohort [25], 8 (73%) of 11 children who experienced virologic failure after an initial decrease in virus load from baseline developed the D30N mutation, and 1 had a new L90M mutation [26]. In that phase I study, many patients, including 5 of the 9 with new protease inhibitor mutations, did not have new nRTIs added to their treatment regimens when Nfv therapy was begun. It is not clear why mutations associated with resistance to protease inhibitors were so infrequent in the PACTG 377 cohort, but all patients in this study received 2 or 3 new RT inhibitors with the protease inhibitors.

Our findings demonstrate that genotypic resistance is frequent in children receiving highly active regimens with less than complete viral suppression. In this cohort, resistance was more frequent among children receiving 3-drug regimens than in those receiving 4-drug regimens. The relative and possibly interacting roles of adherence, regimen potency, and pharmacokinetics in selection and emergence of drug-resistant HIV-1 in children remain to be determined.

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