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Evolution of antiretroviral phenotypic and genotypic drug resistance in antiretroviral-naive HIV-1-infected children treated with abacavir/lamivudine, zidovudine/lamivudine or abacavir/zidovudine, with or without nelfinavir (the PENTA 5 trial)

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Purpose and methods: To describe the evolution of resistance to zidovudine (ZDV), lamivudine (3TC), abacavir (ABC) and nelfinavir (NFV), 113 previously untreated children in the PENTA 5 trial had resistance assayed at baseline, rebound and/or 24, 48, 72 weeks (VIRCO: phenotyping and genotyping with 'Virtual Phenotype' interpretation).

Results: At baseline, few reverse transcriptase mutations and no primary protease inhibitor mutations were observed. Time to detectable HIV-1 RNA with reduced phenotypic susceptibility to any drug was shortest in the ZDV+3TC arm (overall logrank *P*=0.02). Through a median follow-up of 55 weeks, at their last assessment 11 (28%), 16 (40%) and 13 (32%) children with detectable HIV-1 RNA and a resistance test available had mutations conferring resistance to none, one, or two or more trial drugs, respectively, according to the virtual phenotype. Reduced phenotypic susceptibility to ABC only occurred in the 3TC+ABC arm and required K65R and/or L74V in addition to M184V. NFV-resistant virus was selected slowly through D30N or L90M pathways, and selection of ZDV-resistant virus was rare.

Conclusions: Selection of 3TC-resistant virus was most frequent, followed by NFV and/or ABC; selection of ZDV-resistant virus was rare. Importantly, although *in vitro*, ABC selects for M184V as the first mutation, ABC did not select for M184V when combined with ZDV without 3TC. The most sustained HIV-1 RNA response was in the 3TC+ABC arm, but mutations conferring reduced susceptibility to 3TC and/or ABC evolved more frequently if virological failure occurred with 3TC+ABC than with ZDV+ABC.

Introduction

Treatment with highly active antiretroviral therapy (HAART) has resulted in reductions in mortality and progression of HIV-1 disease in both adults and children [1–3]. However, the selection of drug-resistant mutants may limit efficacy and also decrease future treatment options because of cross-resistance [4–6]. Although virological response to HAART may be broadly similar in adults and children, issues of adherence and pharmacokinetics at different ages may impact on response and on the selection of drug-resistant viruses [7,8]. Since drug options may be more limited for children and because immunological response to HAART is often excellent even in the absence of full virological suppression, paediatricians may continue children on the same regimens rather than switch therapy early.

Most data on resistance have been from adult studies [4–6] and there are few data on selection of drug-resistant viruses in HIV-1-infected children on HAART. The largest study, in previously treated children (PACTG

377), surprisingly showed that virological response was better in those with mutations associated with resistance to nucleoside analogue reverse transcriptase inhibitors (NRTIs) at baseline, but this was restricted to children receiving a non-nucleoside analogue reverse transcriptase inhibitor (NNRTI)-containing regimen [9]. In addition, nevirapine- and lamivudine (3TC)-resistant viruses were selected more slowly in children on four-drug regimens than three-drug regimens in this trial, and ritonavir- and nelfinavir (NFV)-resistant viruses were selected only slowly despite continuing on original regimens in the presence of replicating virus [9].

In this paper we describe the selection of resistance-conferring mutations and the evolution of reduced phenotypic susceptibility in previously untreated HIV-1-infected children participating in the PENTA 5 trial and receiving combinations of zidovudine (ZDV), 3TC, abacavir (ABC) and NFV [10]. To date, no data have been presented on the selection of drug-resistant mutants in adults or children receiving ZDV+ABC or 3TC+ABC as dual NRTI combinations, and this study provides particular insight into resistance patterns associated with these regimens.

Materials and methods

Trial design

One hundred and twenty eight previously untreated children were randomised to ZDV+3TC, ZDV+ABC or 3TC+ABC [10]. Of these, 113 had resistance tests performed on one or more stored samples. Children with early disease (*n*=45) were also randomised to receive NFV or NFV placebo (pNFV) (Part A); remaining children (*n*=68) received open-label NFV (Part B). The protocol was amended during the recruitment phase of the study for a change in NFV dose (from 75–90 to 90–110 mg/kg/day) and schedule (from t.i.d. to b.i.d. dosing) [10]. More than 99% of child-time during the trial was spent taking two or more antiretroviral drugs, and 73% taking triple therapy.

Children were classified as non-responders, rebounders or responders based on their plasma HIV-1 RNA profile during the trial (Table 1). In responders (*n*=58), HIV-1 RNA reached <400 copies/ml before 28 weeks and remained <2000 copies/ml subsequently. In non-responders (*n*=21), HIV-1 RNA was never <400 copies/ml. In rebounders (*n*=34), HIV-1 RNA reached <400 copies/ml before 28 weeks with subsequent rebound to >2000 copies/ml (observed week of rebound ranging 5–66 weeks). Samples post-baseline were assayed for resistance at the time of rebound and at 24, 48 and 72 weeks.

Phenotypic and genotypic resistance testing

Phenotypic resistance was measured using the recombinant virus assay (RVA; antivirogram™, Virco). ABI sequencing was undertaken to detect resistance-conferring mutations, which were then interpreted using the virtual phenotype (VircoGEN™; Virco). Fold-change values presented are the fold-rise (FR) in the 50% inhibitory concentration (IC_{50}) of a particular drug tested with recombinant virus isolates from study samples compared to wild-type reference virus (strain IIIB) [11]. Cut-offs relative to IC_{50} – determining reduced susceptibility by phenotype – were ZDV 4.0, 3TC 4.5, ABC 3.0 and NFV 4.0 (respective values for non-trial drugs: didanosine 3.5, zalcitabine 3.5, stavudine 3.0, nevirapine 8.0, delavirdine 10.0, efavirenz 6.0, indinavir 3.0, ritonavir 3.5, saquinavir 2.5 and amprenavir 2.5 [11]). 'Virtual phenotype' cut-offs were identical except for didanosine 2.0, zalcitabine 3.5, stavudine 1.8 and amprenavir 2.0. Rules-based genotype interpretation was used for 12 viral genomes in which virtual phenotype could not be predicted due to too few matches in the database. The recommended lower limit for HIV-1 RNA for these resistance tests was 2000 copies/ml. All specimens in which either the phenotypic or genotypic assay failed were repeated and reported failure rates correspond to failure of both tests. We have previously shown that assay failure rates were significantly higher in samples from children infected with non-subtype B virus, independent of viral load [12], reflecting PCR-based problems with amplifying a diverse range of viral genomes.

Table 1. Virological response by randomised group

HIV-1 RNA classification	Parts A and B combined			Part A only	
	ZDV+3TC	ZDV+ABC	3TC+ABC	pNFV	NFV
Responder	13 (41%)	19 (49%)	26 (62%)	6 (32%)	11 (46%)
Non-responder	8 (25%)	5 (13%)	8 (19%)	5 (26%)	5 (21%)
Rebounder	11 (34%)	15 (38%)	8 (19%)	8 (42%)	8 (33%)
Total	32 (100%)	39 (100%)	42 (100%)	19 (100%)	24 (100%)

HIV-1 RNA classification based on data during the trial, median follow-up 55 weeks (range 27–88 weeks).

Plasma HIV-1 RNA

Plasma HIV-1 RNA was measured at two central laboratories (Covance in Geneva for European centres, and Indianapolis for Brazil) accredited by the College of American Pathologists Laboratory Accreditation Program. Plasma HIV-1 RNA was measured using the Roche Amplicor Ultrasensitive assay version 1.5 (limit of detection of 50 copies/ml). Any specimen with >40 000 copies/ml on the ultrasensitive assay was re-tested using the standard assay.

Statistical methods

All analyses are by intention-to-treat. Proportions were compared using Fisher exact tests, continuous variables by non-parametric rank-sum tests and t-tests. Time to detectable HIV-1 RNA with the M184V mutation was estimated as the week of the first resistance test with this mutation, censoring at the last resistance test for non-responders and rebounders without M184V and the week of the last HIV-1 RNA measurement in the trial for responders (similarly for time to detectable HIV-1 RNA with other mutations or with any phenotypic- or genotypic-reduced susceptibility). Plasma HIV-1 RNA results were $\log_{10}$ transformed prior to analysis and changes from baseline were compared using normal interval regression [13]. Where applicable, models were adjusted for age, baseline HIV-1 RNA, baseline CD4%, NRTI group and NFV receipt in Part A or B, because of slight imbalances in the randomisation [10].

Results

One hundred and thirteen children had 190 resistance tests performed on one or more stored samples (111 samples at baseline and 79 [from 43 non-responders or rebounders] post-baseline). Overall assay failure rates were 8% (16 tests) and 9% (17 tests) for phenotypic and genotypic assays, respectively (eight failing both) [12].

Baseline resistance

With phenotypic assays, 93 (89%) of 105 children had viruses that were fully susceptible to all 16 drugs tested at baseline; 12 had strains with reduced susceptibility to one (*n*=9) or two (*n*=3) drugs but all were within twofold of the cut-off . No specimen from the 104 children with genotype results had reduced susceptibility to any drug according to the virtual phenotype. However, four (4%) children had the M41L reverse transcriptase (RT) mutation and five (5%) children had at least one secondary mutation associated with NNRTI resistance reflecting non-B subtype polymorphism (A98G, V106I, V179D) [14]. Furthermore, 79 (76%) children had at least one secondary mutation associated with protease inhibitor (PI) resistance (M36I, L10V/I, V77I, K20M/R, A71T/V), the most common being M36I (present in 55% children) [12]. The high prevalence of M36I, in particular, is likely to reflect the high proportion of non-B subtype viruses

Table 2. Selection of primary mutations to trial drugs

	Number of children ever having detectable HIV-1 RNA with specific mutations post-baseline *				
	Parts A and B combined			Part A	
	ZDV+3TC (*n*=32) †	ZDV+ABC (*n*=39) †	3TC+ABC (*n*=42) †	pNFV (*n*=19) †	NFV (*n*=24) †
ZDV mutations					
K70R	2 (6%)	1 (3%)	0	2 (11%)	1 (4%)
T215Y or F	1 (3%)	3 (8%)	0	2 (11%)	1 (4%)
3TC mutations					
M184V	14 (44%)	4 ‡ (10%)	8 (19%)	7 (37%)	7 (29%)
ABC mutations					
L74V	0	0	6 (14%)	3 (16%)	0
K65R	0	0	3 (7%)	0	0
Y115F	0	0	3 (7%)	2 (11%)	0
NFV mutations					
D30N	3 (9%)	1 (3%)	0	1 § (5%)	1 (4%)
N88D	2 (6%)	0	0	1 § (5%)	1 (4%)
L90M	2 (6%)	2 (5%)	1 (2%)	1 § (5%)	2 (8%)

*None of these mutations were present at baseline.
† Total number of children, includes responders (with no samples assayed) in addition to non-responders and rebounders.
‡ All four children took 3TC before M184V was observed.
§ Same child who took NFV before these mutations were observed.
All *P*>0.1 for test of difference in selection of mutation between non-responders and rebounders except for L90M (4/21 vs 1/34: exact *P*=0.06).

(59%) in these therapy-naive children [12], as described elsewhere [15,16].

Eight of the children with samples sent for baseline resistance testing had received *in utero* ZDV monotherapy, reflecting low acceptability of this intervention in some groups and the fact that a number of children were born abroad. Baseline viruses from these children were phenotypically sensitive to all 16 drugs and only one had RT mutations (M41L, D67N, L210W, E44A, V106I, V118I) with FR for ZDV of 3.4 on virtual phenotype. This child was randomised to 3TC+ABC and had HIV-1 RNA <400 copies/ml at 48 weeks.

Results after initiation of ART

Seventy-nine samples were available from 43 (78%) of the 55 children experiencing viral rebound or non-response. Assay failures, insufficient material or failure to reach week 48/72 by October 1999 (when assays were performed) accounted for missing results. Non-responders and rebounders did not differ significantly in the FR at 24 and 48 weeks, or in the proportions with detectable HIV-1 RNA and reduced phenotypic or genotypic susceptibility to any drug, given that samples were only taken at nominal 24 and 48 week assessments (all $P>0.1$). Rebounders and non-responders are, therefore, combined in subsequent analyses.

ART received before resistance testing

PENTA 5 was a pragmatic clinical trial and children could change from randomised therapy according to the protocol for adverse events, lack of HIV-1 RNA response, clinical progression or personal request. Fifty-eight (73%) post-baseline resistance tests were performed before children had changed from randomised therapy and all changes involved only substitution or addition of the drugs used in the trial (ZDV, 3TC, ABC and NFV). Overall, 71 (90%) tests were performed while children were taking randomised NRTIs.

Virological response to therapy

Overall, virological response to therapy was best in the 3TC+ABC arm and poorest in the ZDV+3TC arm (very similar to results from all 128 children in the trial [10]). Thus, at 48 weeks the mean decrease in HIV-1 RNA was 1.72, 2.05 and 2.61 $\log_{10}$ copies/ml in the ZDV+3TC, ZDV+ABC and 3TC+ABC arms, respectively, adjusting for baseline factors and estimated in the absence of NFV (global $P=0.03$). There was a corresponding trend towards a higher proportion of responders being in the ABC-containing arms and in the NFV rather than pNFV arm (Table 1).

There was no evidence that the presence of PI polymorphisms at baseline was associated with poorer virological response, either in all children or restricted to those receiving NFV. If anything, PI polymorphisms at baseline were associated with improved virological response: for example, in those receiving NFV the presence of any PI polymorphism at baseline was associated with a 0.73 greater $\log_{10}$ drop in HIV-1 RNA at 48 weeks (95% CI: 1.46–0.01, $P=0.05$) (see [12] for further details). Similarly, the presence of M41L at baseline was not associated with lack of virological response: of those children randomised to ZDV-containing arms, 2/3 with M41L at baseline had HIV-1 RNA <400 copies/ml at 48 weeks, compared to 30/59 without M41L at baseline.

Thirty-four children (30%, $n=113$) experienced HIV-1 RNA rebound during the trial at median week 31.4 (range 4.4–66.4) with median HIV-1 RNA 4730 copies/ml (range 2006–221 938 copies/ml). Nineteen of these children had samples available for resistance testing at rebound; 15 tests were successful. Five children had samples that were phenotypically sensitive to all drugs, five had reduced susceptibility to one drug (four NRTI, one NFV) and five had reduced susceptibility to two or more drugs (four multiple NRTIs, one NRTI+NFV). Of note, none of the three children receiving 3TC+ABC had fully sensitive virus at rebound, compared with 2/3 and 4/9 in the ZDV+3TC and ZDV+ABC arms, respectively. Similar results were observed for genotypic assays.

Selection of primary mutations

No child had a M184V mutation at baseline, but 26 (23%) children had detectable HIV-1 RNA with M184V during the trial (Table 2). All four children originally randomised to ZDV+ABC with M184V had switched to 3TC prior to genotyping. Comparing the 3TC-containing randomised arms, time to detectable HIV-1 RNA with the M184V mutation was significantly shorter in the ZDV+3TC arm compared with the 3TC+ABC arm (logrank $P=0.03$); with 41 and 14% of children in the ZDV+3TC and 3TC+ABC arms, respectively, estimated to have virus with M184V selected by 48 weeks from initiation of ART. Time to detectable HIV-1 RNA with the M184V mutation did not differ significantly between children in Part A randomised to NFV placebo (on dual therapy) or NFV (on triple therapy) (logrank $P=0.74$).

Selection of other primary mutations to trial drugs are shown in Table 2. Six children had viruses with ABC resistance mutations other than M184V, but these only occurred in the ABC+3TC arm. Seven children had viruses with primary NFV mutations; four and five selected D30N and L90M, respectively, all after exposure to NFV. There was no evidence for a difference in time to detectable HIV-1 RNA with L90M or D30N across NRTI groups (overall $P=0.45$). TAMs

that were not present at baseline were detected in only eight children (four ZDV+3TC, four ZDV+ABC).

Selection of resistant viruses during continued therapy with detectable HIV-1 RNA

Many children remained on their randomised therapy in spite of viral load rebound or non-response. Of 13 children with two or more resistance tests after rebound or non-response (five ZDV+3TC, five ZDV+ABC, three 3TC+ABC), eight had completely concordant genotypic and phenotypic results over time. In the other five, more resistance mutations accumulated or FR increased above cut-offs, or both. In two children (both 3TC+ABC), L74V was observed at week 48 in addition to M184V and K65R present from rebound at weeks 27 and 33, respectively. Three non-responders taking NFV acquired reduced phenotypic susceptibility to NFV between 24 and 48 weeks on a background of reduced susceptibility to 3TC only, with either the selection of L90M or the replacement of D30N+L90M by D30N+N88D (one also acquired reduced phenotypic susceptibility to ABC between 24 [FR=1.0] and 48 weeks [FR=5.5] with only M184V present and without switching from ZDV+3TC+NFV). Overall, there appeared to be increased resistance to NFV at 48 weeks compared with 24 weeks, whereas only two viruses had reduced NFV susceptibility at week 24 (both FR<10); by week 48 there were seven (median FR 15.2, range 4.2–49.3; three with FR>30). None of these 13 children selected ZDV-resistant virus over time and most (9/13) had 3TC-resistant virus present from their first test. After the first resistance test, HIV-1 RNA increased by mean 0.23 $\log_{10}$ copies (SE 0.18) in children in whom susceptibility reduced over time and fell by a mean 0.11 $\log_{10}$ copies (SE 0.09) in those children with no change in susceptibility (t-test P=0.22).

Point mutations associated with reduced phenotypic susceptibility to trial drugs

All but one of the 45 viruses with reduced phenotypic susceptibility to 3TC had the M184V mutation in RT (Figure 1); 13 also had primary NFV mutations (at least one of D30N, N88D/S, L90M in protease). Only one of the 12 viruses with reduced susceptibility to ABC had M184V alone (from a child in the ZDV+3TC arm who never took ABC); nine had M184V plus either K65R or L74V, or both (all 3TC+ABC), and the remaining two had ABC FR close to cut-off (T215Y and no RT mutations, respectively) (Figure 1). Ten of the 12 viruses with reduced phenotypic susceptibility to NFV had the primary NFV mutations D30N (n=7) or L90M (n=3) and two had secondary mutations only (M36I plus K20R or N88S); all 12 also had the M184V mutation. Of note, three additional samples with key NFV mutations (L90M alone [n=1] or L90M+D30N [n=2]) had phenotypic susceptibility below the NFV cut-offs (Figure 1). This may reflect the presence of mixed mutant/wild-type viral populations in these samples.

No more than two TAMS were detected in any sample (two had T215Y+M41L, one had K70R+M41L and one had K70R+D67N). All samples with TAMS had phenotypic susceptibility to ABC below or close to the cut-offs, and only one had reduced susceptibility to ZDV (FR=23.7 with T215Y only). T215 revertants (T215N, T215C, T215S) were not observed in any sample at any time during the trial.

Summary of overall selection of point mutations and reduced phenotypic susceptibility

Table 3 summarises genotype at last follow-up by NRTI group. Of those non-responders and rebounders with a resistance test available, 11 (28%), 16 (40%) and 13 (32%) had mutations conferring resistance to none, one, or two or more trial drugs, respectively, at their last assessment according to the virtual phenotype. Furthermore, in the ZDV+3TC, ZDV+ABC and 3TC+ABC arms 18, 53 and 0%, respectively, had RT wild-type virus at their last assessment (exact P=0.01). Of the 10 non-responders and rebounders with RT and protease wild-type virus at their last sample, only one child (ZDV+3TC) had documented adherence problems, reporting missing between 6 and 10 doses in the last week prior to resistance testing at HIV-1 RNA rebound [17].

Overall, time to detectable HIV-1 RNA with reduced phenotypic susceptibility to any trial drug was significantly shorter in the ZDV+3TC arm (Figure 2, overall logrank P=0.02). However, there was no significant difference between NRTI groups in the time to detectable HIV-1 RNA with reduced phenotypic susceptibility to two or more trial drugs (overall logrank P=0.43). There was no statistical evidence for a difference between NFV and pNFV in the time to detectable HIV-1 RNA with reduced phenotypic susceptibility to any trial drug (P=0.27) or to two trial drugs (P=0.45), although the trend was towards more resistance in the pNFV arm.

Conclusions

We describe the selection of resistance-conferring mutations and the evolution of reduced phenotypic susceptibility in previously untreated children enrolled in a randomised clinical trial comparing different combinations of ZDV, 3TC and ABC with or without NFV. No child took any other drugs by the time resistance assays were performed and most were on therapy as randomised. Although resistance testing could not

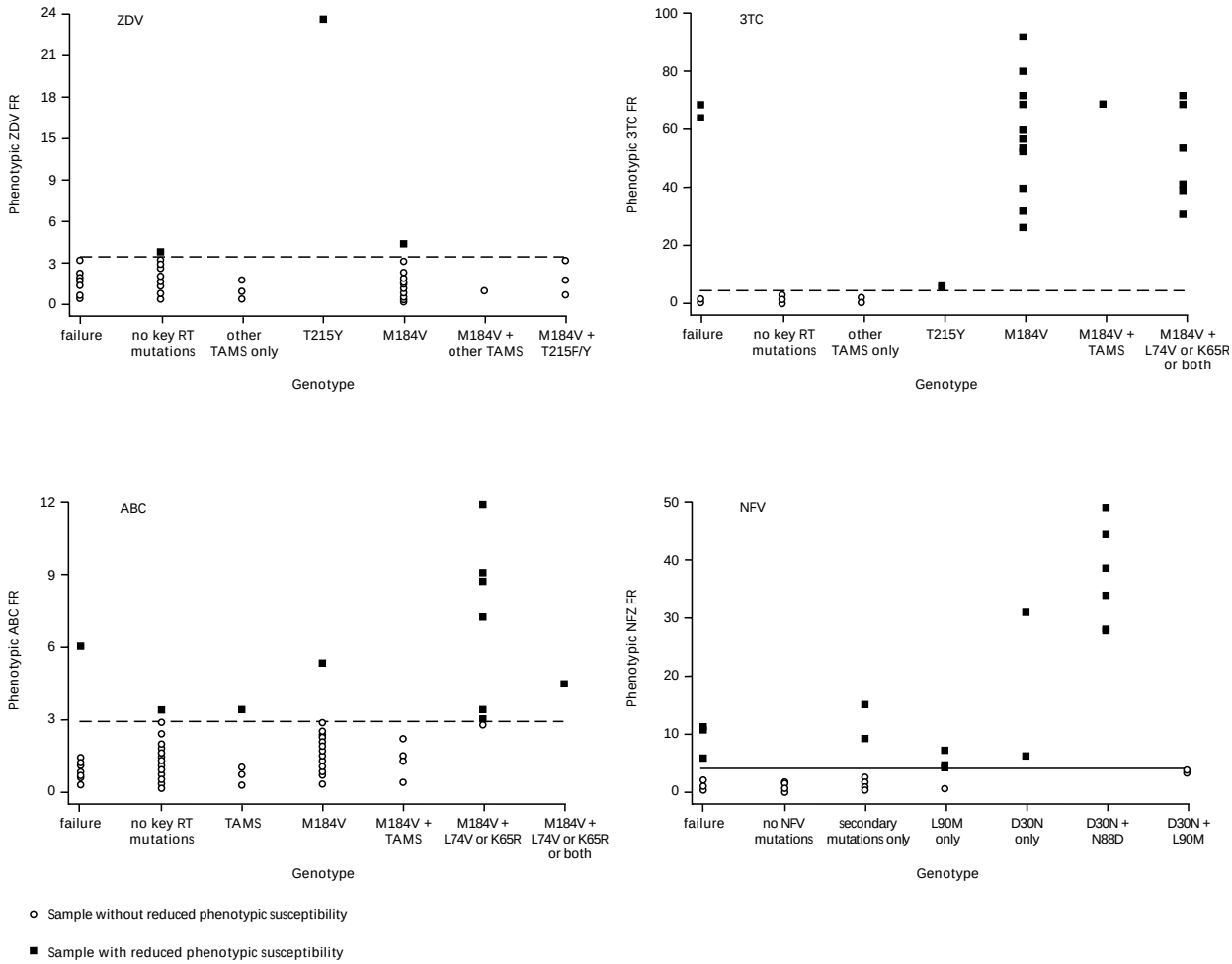
be performed on all samples from children in the trial, for example, if there was insufficient material, samples were sent blind to treatment allocation and baseline characteristics, and therefore should be a representative subset. The only potential for systematic differences between samples with and without resistance results could have been that samples with low plasma HIV-1 RNA and/or from children with non-B clade subtypes were more likely to be assay failures [12]. However, there was no evidence that there were differences in assay failure rates between randomised groups.

Only eight children in the trial had been exposed to ART *in utero* and only one had any evidence of reduced susceptibility to ZDV at baseline, but without T215Y, which is most frequently reported in transmitted ZDV-resistant variants from mother to child [18]. It was therefore not surprising that viruses at baseline were uniformly sensitive or around the phenotypic cut-off values.

Overall, viruses from 33% of children had no evidence of reduced susceptibility at the time of virological rebound, similar to a recent study in adults where 45% were reported to have wild-type virus after rebound on triple ART with indinavir [19]. However, there appeared to be differences according to the dual NRTI combination, with nearly all children experiencing virological failure while still prescribed ZDV+ABC having wild-type virus. A large adherence study carried out as part of PENTA 5 suggested a non-significant trend towards reporting poorer adherence in children in the ZDV+3TC arm [17]. Therefore, poor adherence alone cannot explain the lack of mutants observed at viral rebound in the ZDV+ABC arm.

As observed in adult studies, virological failure in the ZDV+3TC arm was mostly associated with the

Figure 1. Genotype by phenotypic fold-rise in the IC_{50} for trial drugs



Dashed line shows cut-off used to determine reduced phenotypic susceptibility. Includes all viruses with phenotypic and genotypic assay results. Each child can have more than one test.

Table 3. Summary of genotypic patterns at last time point with data

	Parts A and B combined			Part A	
	ZDV+3TC (*n*=32)	ZDV+ABC (*n*=39)	ABC+3TC (*n*=42)	pNFV (*n*=19)	NFV (*n*=24)
Responders (no tests)	13	19	26	6	11
Non-responders and rebounders with no tests (no sample/failed tests)	2/0	3/2	7/1	1/0	2/1
Non-responders and rebounders with test after baseline	17	15	8	12	10
Genotypic pattern (median follow-up 46.3 weeks)					
Wild-type (unchanged from baseline)	3/17 (18%)	8*/15 (53%)	0	2/12 (17%)	3/10 (30%)
M184V only	11/17 (65%)	3†/15 (20%)	2/8 (25%)	3/12 (25%)	5/10 (50%)
M184V + TAMs ‡	3/17 (18%)	1†/15 (7%)	0	1/12 (8%)	2/10 (20%)
TAMs only	0	3/15 (20%)	0	3/12 (25%)	0
M184V + other ABC mutations (K65R, L74V, Y115F)	0	0	6/8 (75%)	3/12 (25%)	0
Primary NFV mutations§ ±reverse transcriptase mutations	3/17 (18%)	3/15 (20%)	1/8 (12%)	1∫/12 (8%)	2/10 (20%)

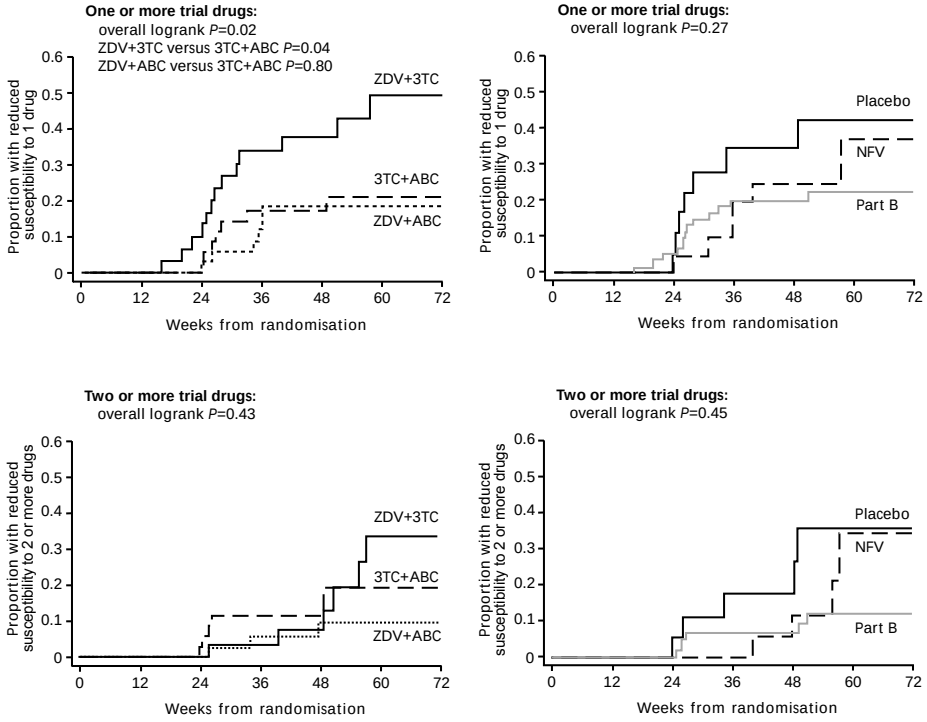
* One virus had a primary mutation (L90M) in protease.
† All four children had switched to 3TC before M184V was observed.
‡ Thymidine analogue mutations, selected by either ZDV or d4T and including M41L, D67N, K70R, T215F/Y and K219Q/E.
§ D30N or L90M.
∫ One child who took NFV before these mutations were observed.

M184V mutation only; and by a median of 55 weeks, only 18% had also selected thymidine analogue mutations (TAMs) (none of these had TAMs at baseline). Overall, a greater number of mutations was observed in the ZDV+3TC arm compared with the ABC-containing arms, which probably reflects increased time spent with non-suppressed viral load in the ZDV+3TC arm [10], as well as the unexpected finding of common wild-type virus rebound in the ZDV+ABC arm. In accordance with adult data, the selection of ZDV-resistant virus was slow [20–23] and in the ZDV+3TC arm may be explained by the M184V mutation delaying the selection of TAMs [24,25].

For the two ABC-containing NRTI combinations, there are few data available in adults and this is the first analysis of such combinations as dual nucleoside components of antiretroviral therapy. At the time PENTA 5 was started, there was concern that the combination 3TC+ABC might not provide a potent or sustainable reduction in plasma HIV-1 RNA, as both 3TC and ABC were assumed to select for the M184V mutation. However, subsequent studies have shown that the presence of M184V alone, while conferring low level resistance to ABC (two- to fourfold) does not significantly diminish the response to ABC compared with wild-type virus [26,27]. Similarly, in general two or more ABC-specific mutations were required for reduced susceptibility to ABC in PENTA 5, in accordance with other studies [28]. The best virological response was in the 3TC+ABC arm [10], but if rebound or non-response occurred, more resistance mutations occurred in the 3TC+ABC arm than in the ZDV+ABC arm. The lack of any of the specific ABC resistance mutations (K65R, L74V, Y115F, M184V) occurring in children failing ABC+ZDV (0%) compared with ABC+3TC (75%) is interesting. However, no mutations of any kind were observed in 47% of the children failing ABC+ZDV compared with none of the children failing 3TC+ABC. In samples with resistance mutations, only TAMs and NFV mutations were observed in the ZDV+ABC arm: in theory the selection of TAMs could have conferred sufficient reduced susceptibility to ABC and ZDV to lead to viral rebound. However, the phenotypic data showing retained ABC susceptibility in the presence of one or two TAMs is consistent with previous findings that cross-resistance to ABC is usually only observed with three or more TAMs [23,26,27]. Finally, it is possible that the presence of M184V as the first mutation in the 3TC+ABC arm may predispose to the further accumulation of ABC mutations within a non-suppressive regimen that does not contain ZDV.

Primary NFV mutations were detected more frequently in the ZDV+3TC arm. The lower dose of NFV used at the start of the trial may have contributed to selection of drug-resistant mutants with this less-potent dual NRTI combination. In our study, there was a suggestion that resistance to NFV, in particular, increased if children remained on drugs in the presence of virological failure. Similarly, NFV-resistant virus was detected only rarely at the time of virological failure in PACTG 377 [9], but was selected subsequently in the presence of continued detectable HIV-1 RNA, consistent with results from studies in adults [29].

Figure 2. Time to detectable plasma HIV-1 RNA with reduced phenotypic susceptibility



Similar results for time to detectable plasma HIV-1 RNA with reduced susceptibility according to the virtual phenotype.

In conclusion, the ZDV+3TC arm showed inferior HIV-1 RNA response and greatest selection of single drug-resistant virus, mostly due to M184V. In addition, over a median of 55 weeks of follow-up around 25% of viruses also had TAMs and/or NFV mutations (a profile similar to the one described in adults). The ABC+3TC arm showed the best HIV-1 RNA response at 48 weeks, but among children with virological non-response or failure, resistance to 3TC and ABC was frequent, whereas resistance to NFV was rare. In the ZDV+ABC arm, TAMs alone (20%) and/or NFV mutations (20%), but no ABC mutations, and no M184V in children who had not switched to 3TC for toxicity, were observed after virological failure, and many viruses remained wild-type. The cause of failure in children on ZDV+ABC without mutations is unclear and requires further research.

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