A randomized controlled trial of genotypic HIV drug resistance testing in HIV-1-infected children: the PERA (PENTA 8) Trial

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Objective: To evaluate the longer-term utility of genotypic resistance testing in HIV-1-infected children with virological failure.

Methods: Children aged 3 months–18 years switching antiretroviral therapy (ART) with HIV-1 RNA >2,000 copies/ml were randomized between genotypic testing (*Virtual* Phenotype™) and no testing at baseline and subsequent virological failures. Children were followed to at least 96 weeks.

Results: One hundred and seventy eligible children, from 24 clinical centres in six countries, were randomized to resistance testing (n=87) or no testing (n=83) between June 2000–July 2003. At baseline, mean HIV-1 RNA and CD4$^+$ T-cell percentage were 4.7 $\log_{10}$ copies/ml and 20%, respectively. Children had taken ART for a mean of 5 years; 24% had received all three classes, 53% nucleoside reverse transcriptase inhibitors (NRTIs)+protease inhibitors (PIs), 9% NRTIs+non-nucleoside reverse transcriptase inhibitors (NNRTIs) and 14% NRTIs only. There was no difference between the arms in the drug classes or the individual PIs/NNRTIs prescribed. However, 49% in the resistance test arm (RT) versus 19% in the no-test arm (NT) continued at least one NRTI from their failing regimen; 56% versus 19% were prescribed didanosine+stavudine as their NRTI backbone. Adjusting for baseline HIV-1 RNA, mean reductions in HIV-1 RNA at 48 weeks were 1.51 $\log_{10}$ copies/ml in the RT arm and 1.23 in the NT arm (P=0.3); the difference between the arms was smaller at week 96 (RT: 1.50, NT: 1.47; P=0.9).

Conclusion: In this first paediatric trial of resistance testing, we observed a substantial difference in NRTI-prescribing behaviour across arms. However statistically significant evidence of a long-term virological or immunological benefit was not observed.

This trial is registered as an International Standard Randomised Controlled Trial, number ISRCTN14367816.

Introduction

Effective antiretroviral therapy (ART) has transformed the prognosis of HIV infection in both adults and children. However, in some patients ART fails to suppress viral replication, resulting in the emergence

of viruses with decreased susceptibility, which, in turn, may cause treatment to fail. Resistance assays have been developed to measure changes in HIV susceptibility to specific drugs (phenotypic assays) and to detect resistance-conferring mutations in the relevant HIV genes (genotypic assays) [1]. The rationale for using resistance testing in clinical practice is to optimize therapy, particularly when drugs are being changed following virological failure. Resistance testing identifies drugs in the current regimen to which the virus has reduced susceptibility; therefore, other components of the regimen may be continued if alternative drug options do not exist. Furthermore, due to cross-resistance within drug classes, resistance testing may guide choices from drugs patients have not received. However, limitations of resistance testing include the fact that only variants present at a frequency of >10–20% can be detected, and lack of drug pressure at the time of testing can lead to archived mutations not being detected.

Several randomized trials in adults have assessed the clinical utility of genotypic [2–7] or phenotypic resistance testing [4,8–10], with or without expert interpretation. These short-term studies (typically 12–26 weeks) reported conflicting results, and at most small virological benefits from resistance testing [11,12]; no direct benefit of expert advice in addition to resistance testing was observed [5]. Despite this, guidelines recommend the use of resistance testing (either genotypic or phenotypic) when treatment change is considered due to virological failure [13–15]. Similar recommendations are also made in paediatric guidelines, although no trial has ever been conducted in children [16,17].

The role of resistance testing in improving virological outcome in children may differ compared with adults. Young children have very high HIV-1 RNA levels [18] and tend to have lower suppression rates on ART than adults [19,20]. Adherence is also often difficult in children who depend on caregivers to give medication. All these factors increase the risk of virological failure and subsequent development of resistance. In addition, paediatric ART options are more limited as fewer drugs are licensed and appropriate formulations are not available for all drugs. Lastly, many paediatricians care for a relatively small number of children and less frequently have the support of virological, immunological or other experts commonly available in large adult treatment centres.

PENTA 8 (PERA – Paediatric Evaluation of Resistance Assays) was designed to evaluate the longer-term utility of genotypic resistance testing in HIV-1 infected children with virological failure.

Materials and methods

Trial design and participants

PERA was an open, randomized, two-arm, parallel-group multicentre trial, which was recruiting HIV-1-infected children aged 3 months –18 years switching ART due to virological failure, with their most recent HIV-1 RNA plasma viral load being >2,000 copies/ml. Children were ineligible if they had been exposed to only two or three nucleoside reverse transcriptase inhibitors (NRTIs) for <2 years, if they had changed ART in the last month before randomization, if a resistance test had previously been performed on the current regimen, or if paediatricians and primary caregivers were unwilling to wait for a resistance test result before switching therapy. The protocol was approved by the ethics committee for each participating centre. All primary caregivers gave written consent to participate, and additional written assent was obtained from children, where appropriate, according to their age and knowledge of their HIV status.

Randomization, which was carried out by the paediatrician faxing/phoning one of two central trials units in the UK and France, was stratified by whether or not the child had had a resistance test on a previous regimen (up to 30 children were allowed into the trial) and centre. Children were randomized between no resistance testing and access to a genotypic resistance test at the time of randomization and at any point during follow-up when the paediatrician considered that the test could influence the choice of drug regimen. For children allocated to no resistance testing, a new ART regimen was prescribed immediately after randomization; children allocated to resistance testing had to wait for the results of the test before switching therapy. If the resistance assay failed, the paediatrician could request a repeat test with a new blood sample or change treatment without the benefit of any resistance test results. Baseline samples for children randomized to the no resistance testing (NT) arm were not genotyped. Follow-up was every 12 weeks until the last randomised child reached 96 weeks.

Resistance assay

Resistance testing was performed by VIRCO (Mechelen, Belgium) using a genotypic test with computer assisted interpretation (VirtualPhenotype™ versions 2.0.00 to 3.2.00). The test report showed key drug-associated mutations and the predicted fold-change in 50% inhibitory concentration for 14 antiretroviral drugs (in addition to lopinavir and tenofovir from November 2001) based on the average fold-change from approximate matches in a genotype-phenotype database. If the predicted fold-change was

less/greater than a cut-off for the normal susceptible range of the drug (that is, the biological cut-off), the test report displayed green ('sensitive')/red ('resistant'), respectively; if the predicted fold change was above the biological cut-off but below the levels of fold-change associated with reduced clinical response to a drug, the test report displayed orange for 'intermediate resistance'. VIRCO introduced tests with biological cut-offs in January 2001; for the 22 children randomized before January 2001, the standard cut-offs used for each drug were ≤4-fold for a virus 'sensitive' to that drug, 4–10-fold for 'intermediate' and >10-fold for 'resistant'. For the purposes of analysis, all 'intermediate' results were considered to be 'resistant'. Expert advice on the resistance test interpretation or on the new antiretroviral regimen was not provided routinely, but paediatricians could consult their local virologist or the virologists in the PENTA Virology Committee for advice. However, expert advice was infrequently sought during the study.

Endpoints and sample size

The primary endpoint was change in plasma HIV-1 RNA viral load between baseline and 48 weeks (regardless of ART changes after baseline). Although local viral load measurements were used throughout the trial and for clinical management, samples at baseline, 48 and 96 weeks from children in centres not using Roche assays were tested centrally using the Roche ultra-sensitive assay (limit of detection ≥50 copies/ml); any sample with HIV-1 RNA >50,000 copies/ml was retested using Roche Amplicor. As viral loads were similar using Roche and locally measured HIV-1 RNA, all analyses are presented based on local HIV-1 RNA as data were more complete and additional time points could be included. Secondary endpoints included the proportion of patients with undetectable viral load (<50 copies/ml) at 48 weeks, change in $CD4^+$ T-cell percentage, antiretroviral treatment prescribed after randomization and progression to new AIDS defining event or death.

It was estimated that 180 children (90 per arm) would give 90% power to detect (at a two-sided significance level of 5%) a difference in mean change from baseline in viral load of 0.3 $\log_{10}$ copies/ml, based on an analysis of covariance of HIV-1 RNA at 48 weeks adjusting for baseline level and allowing for loss to follow-up.

Statistical analysis

All analyses were performed on an 'as randomized' (intention-to-treat) basis. Day 0 was defined as the day of the week 0 visit when a blood sample was taken; this was the day of randomization for 59% of children but ranged from 1 to 32 days after randomization for the remaining 41% of children due to logistical issues. Baseline laboratory and clinical values were those recorded nearest to but before and within 12 weeks of day 0. The closest value to 24, 48, 72 and 96 week visits within equally spaced windows was used to calculate changes from baseline. Missing observations were imputed only for HIV-related deaths when >750,000 copies/ml and 0% were used for HIV-1 RNA and $CD4^+$ T-cell percentage, respectively. Normal interval regression was used to account for viral load values below the lower limit or above the upper limit of the assay [21].

Results

Baseline characteristics

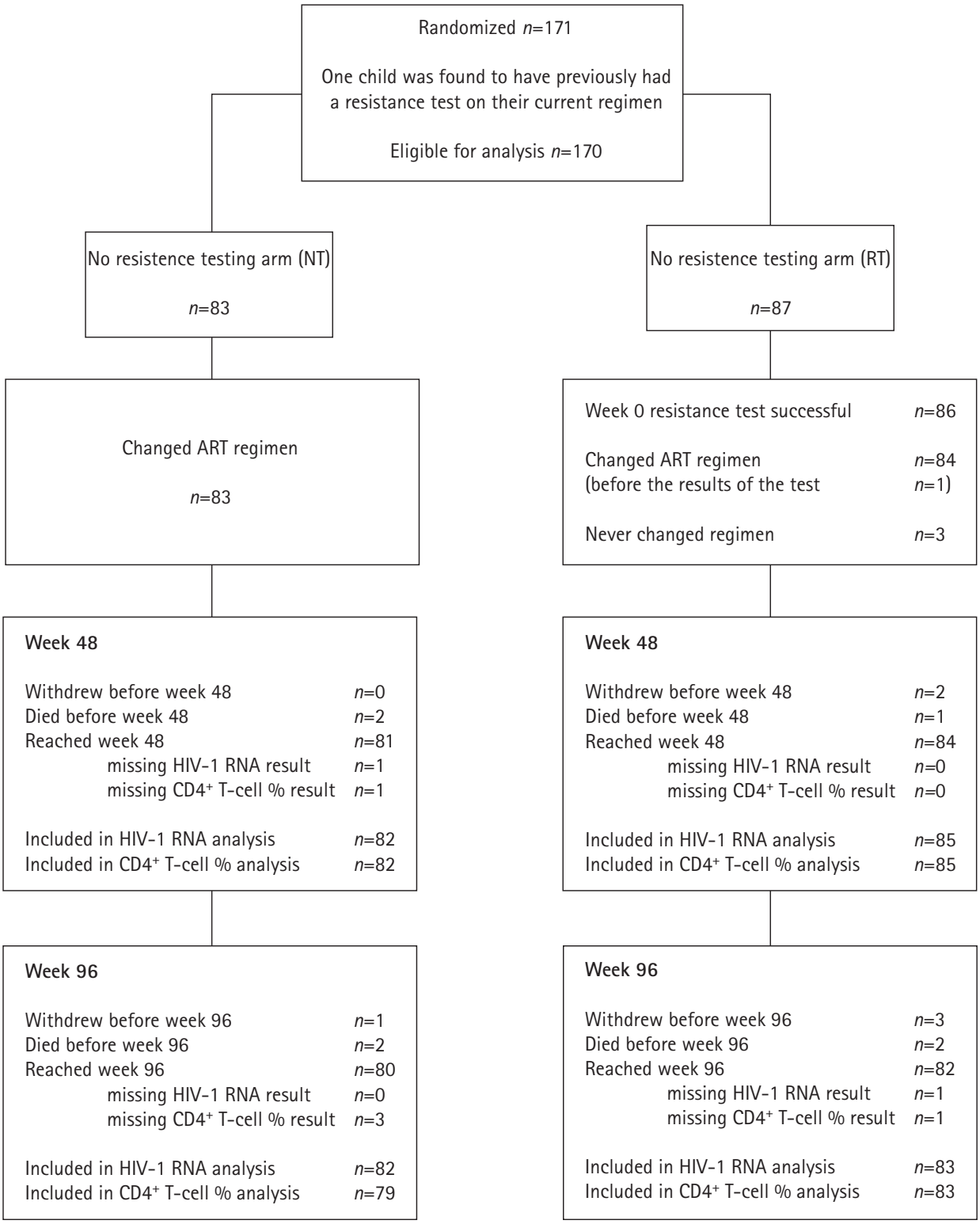
From June 2000 to July 2003, 171 children were randomized, 84 to the no resistance testing arm (NT) and 87 to the resistance testing arm (RT). Children were enrolled from 24 centres in six countries; Italy (68 children), Brazil (64), UK (27), Spain (9), Germany (2) and Portugal (1). Figure 1 shows the flow of participants through the trial. One child was excluded as she was found to have previously had a resistance test on her current regimen, leaving 170 children in the analysis. Two children withdrew consent (weeks 1 and 67) and two children were lost to follow-up (weeks 4 and 53).

Baseline characteristics were reasonably balanced between the two arms (Table 1). One-hundred and sixty-five children (97%) had acquired HIV from mother-to-child transmission; 57 (34%) had HIV-1 RNA ≥100,000 copies/ml. Previous exposure to antiretroviral drugs varied; 24% had received drugs from all three main classes, 23% had not received a protease inhibitor (PI) and 66% had never had a non-nucleoside reverse transcriptase inhibitor (NNRTI). However, 129 children (76%) initiated ART with mono or dual NRTI therapy (82% NT, 70% RT). Children had received, on average, five different antiretroviral drugs over 5.1 years. The percentage of children who had ever received zidovudine, lamivudine, stavudine and didanosine were 88%, 86%, 72% and 55%, respectively; only 11% had ever received abacavir. The most common PI taken previously was nelfinavir (62% of children); only 4% and 1% of children had received amprenavir and lopinavir, respectively; 24% and 12%, had received nevirapine and efavirenz, respectively.

Resistance test results

Eighty-five (98%) children in the RT arm had a successful resistance test at baseline on the first blood sample; in two children the resistance test on the first blood sample was unsuccessful, both had a re-test on a repeat sample and the second test was successful for one child. The median (interquartile range) interval between collection of the blood sample and the receipt

Figure 1. Participant flow



Observed values and imputed values for HIV-related deaths (see Materials and methods) were included in analyses of HIV-1 RNA and CD4+ T-cell percentage. ART, antiretroviral therapy.

Table 1. Baseline characteristics

	NT (*n*=83)	RT (*n*=87)	Total (*n*=170)
Previous resistance test, *n* (%)*	3 (4%)	7 (8%)	10 (6%)
Male, *n* (%)	45 (54%)	48 (55%)	93 (55%)
Age, *n* (%)			
≤6 years	26 (31%)	31 (36%)	57 (34%)
7–10 years	24 (29%)	31 (36%)	55 (32%)
≥11 years	33 (40%)	25 (29%)	58 (34%)
Median (IQR)	9.7 (6.1, 13.6)	9.5 (5.6, 11.3)	9.5 (5.9, 12.2)
Ethnic origin, *n* (%)			
White	54 (65%)	57 (66%)	111 (65%)
Black African	18 (22%)	20 (23%)	38 (22%)
Other	11 (13%)	10 (11%)	21 (12%)
CDC disease stage, *n* (%)			
N	8 (10%)	15 (17%)	23 (14%)
A	14 (17%)	20 (23%)	34 (20%)
B	27 (33%)	28 (32%)	55 (32%)
C	34 (41%)	24 (28%)	58 (34%)
Mean (SD) HIV-1 RNA, $\log_{10}$ copies/ml†	4.7 (0.9)	4.7 (0.9)	4.7 (0.9)
Mean (SD) $CD4^+$ T-cell percentage	21 (11)	20 (9)	20 (10)
Median (IQR) $CD4^+$ T-cell count, cells/mm^3	437 (299–743)	432 (298–756)	433 (257–743)
Previous ART exposure, *n* (%)			
NRTIs only	8 (10%)	15 (17%)	23 (14%)
NRTIs+NNRTIs	6 (7%)	10 (11%)	16 (9%)
NRTIs+PIs	45 (54%)	45 (52%)	90 (53%)
NRTIs+NNRTIs+PIs	24 (29%)	17 (20%)	41 (24%)
Mean (range) number of drugs received			
All three main classes	5.2 (2–10)	4.7 (2–11)	4.9 (2–11)
NRTI	3.5 (2–6)	3.2 (2–5)	3.4 (2–6)
NNRTI	0.4 (0–2)	0.4 (0–3)	0.4 (0–3)
PI	1.3 (0–3)	1.1 (0–4)	1.2 (0–4)
Number (%) ever received drug			
Zidovudine	74 (89)	76 (87)	150 (88)
Didanosine	57 (69)	56 (64)	113 (66)
Zalcitabine	11 (13)	10 (11)	21 (12)
Lamivudine	75 (90)	72 (83)	147 (86)
Stavudine	61 (73)	61 (70)	122 (72)
Abacavir	12 (14)	6 (7)	18 (11)
Nevirapine	20 (24)	21 (24)	41 (24)
Delavirdine	0 (0)	1(1)	1 (1)
Efavirenz	12 (14)	9 (10)	21 (12)
Saquinavir	3 (4)	3 (3)	6 (4)
Ritonavir	25 (30)	30 (34)	55 (32)
Indinavir	15 (18)	11 (13)	26 (15)
Nelfinavir	57 (69)	48 (55)	105 (62)
Amprenavir	5 (6)	1 (1)	6 (4)
Lopinavir	1 (1)	1 (1)	2 (1)
Mean (range) cumulative ART exposure, years			
Any class	5.2 (1–13)	5.0 (0–12)	5.1 (0–13)
NRTI	5.2 (1–13)	5.0 (0–12)	5.1 (0–13)
NNRTI	0.4 (0–5)	0.4 (0–4)	0.4 (0–5)
PI	2.3 (0–6)	1.9 (0–5)	2.1 (0–6)
Initial ART regimen, *n* (%)			
Mono/dual	68 (82%)	61 (70%)	129 (76%)
Triple	15 (18%)	26 (30%)	41 (24%)

*Up to 30 children who had had a resistance test on a previous regimen were allowed into the trial. †One child in the resistence test (RT) arm missing baseline HIV-1 RNA. ART, antiretroviral therapy, CDC, Centers of Disease Control and Prevention; IQR, interquartile range; NNRTI, non- nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NT, no resistence test arm; PI, protease inhibitor.

of the resistance report by the clinician was 21 (16–32) days, which includes the time between collection of the blood sample and receipt by VIRCO (median 8 days).

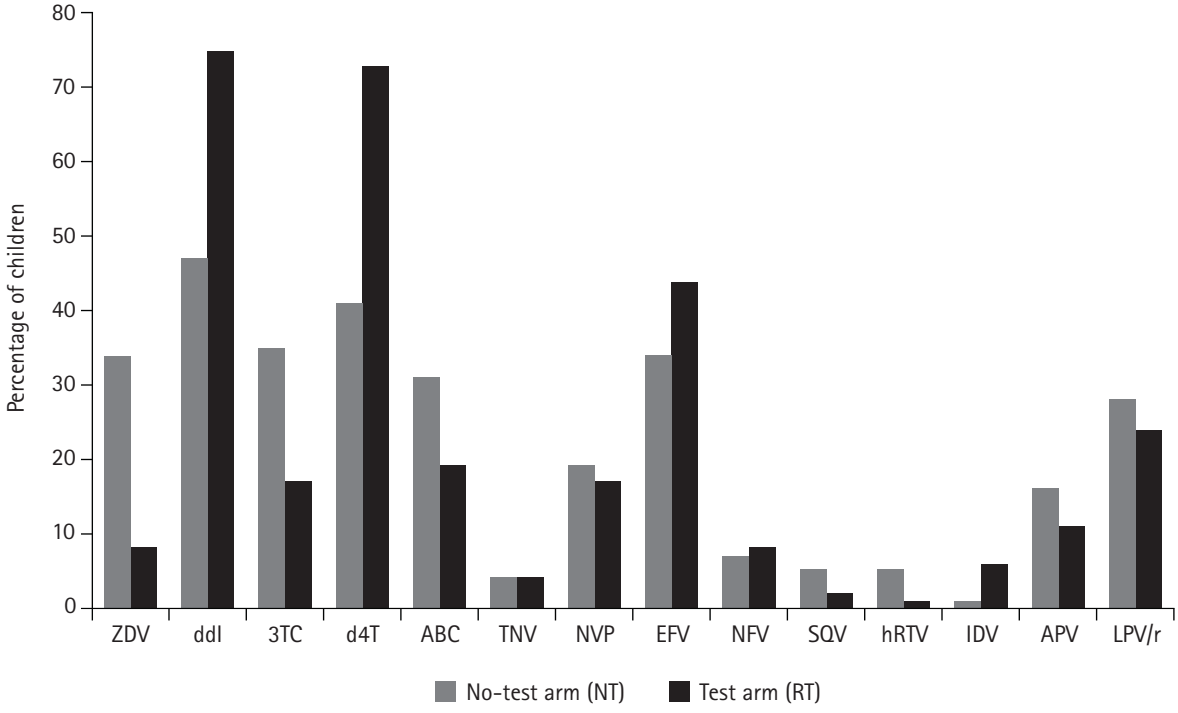
Among the 86 samples successfully genotyped, 98%, 33% and 60% had at least one major mutation associated with reduced susceptibility to NRTIs, NNRTIs and PIs, respectively. The most frequently reported NRTIs mutations included M184V (64% of the 86 samples) as well as the thymidine analogue mutations (TAMs), M41L (63%), T215 (53%), D67N (52%) and L210W (44%); 4 (5%), 16 (19%), 18 (21%), 25 (29%) and 23 (27%) of samples had 0, 1, 2, 3 and >4 TAMs, respectively. Resistance was predicted to zidovudine and lamivudine for 69% and 77% of samples, compared with 19%, 29% and 43% for didanosine, stavudine and abacavir, respectively. Approximately 30% predicted resistance to nevirapine and efavirenz, compared with approximately 50% for nelfinavir, ritonavir and indinavir, and 26% for amprenavir. Of the 86 samples, 40 (47%) were tested for lopinavir susceptibility, and of these 10 (25%) predicted resistance to lopinavir.

Treatment regimen prescribed after randomization

Ninety-four percent of children had started their new regimen by 4 weeks in the NT arm and 94% by 12 weeks in the RT arm (only 27% by 4 weeks due to lag in receiving the resistance test report). Three children in the RT arm did not change regimen (one withdrew consent at week 1, one was lost to follow-up at week 4 and one re-suppressed <400 copies/ml at week 4).

There were no significant differences between the arms in the specific NNRTI or PI drugs in the treatment regimen prescribed after randomization; however, didanosine and stavudine were prescribed significantly more frequently in the RT arm (75% and 73% in RT arm, respectively, versus 47% and 41% in NT arm, respectively), whereas zidovudine, lamivudine and abacavir were prescribed less frequently (Figure 2). Thus, 47 (56%) children in the RT arm were prescribed didanosine and stavudine as their NRTI backbone compared to only 16 (19%) in the NT arm (Table 2). There were no significant differences between the arms in terms of drug classes prescribed; however, children previously exposed to NRTIs+PIs only were predominantly prescribed a regimen containing NNRTIs (35/43 [81%] in the RT arm and 36/45 [80%] in the NT arm), as were children who had been exposed to NRTIs only (11/14 [79%] in RT arm and 6/8 [75%] in NT arm). In contrast, children exposed to NRTIs+NNRTIs only or to all three main

Figure 2. Antiretroviral drugs prescribed after randomization



Excludes three children in the in the RT arm who did not change regimen. Frequency of ritonavir boosting: 5/6 children for saquinavir (SQV), 2/6 children for indinavir (IDV), and 9/22 children for amprenavir (APV). 3TC, lamivudine; ABC, abacavir; ddI, didanosine; d4T, stavudine; EFV, efavirenz; hRTV, ritonavir (high dose); LPV/r, lopinavir/ritonavir; NFV, nelfinavir; NVP, nevirapine; TNV, tenofovir; ZDV, zidovudine.

Table 2. Treatment regimens prescribed after randomization

	NT (*n*=83)	RT (*n*=84)	*P*-value
Number of drugs in regimen, *n* (%)	–	–	0.83[†]
2*	1 (1%)	1 (1%)	–
3	76 (92%)	75 (89%)	–
4	5 (6%)	8 (10%)	–
5	1 (1%)	0 (0%)	–
ART classes, *n* (%)	–	–	0.60[‡]
NRTI+PI	38 (46%)	33 (39%)	–
NRTI+NNRTI	31 (37%)	40 (48%)	–
NRTI+PI+NNRTI	13 (16%)	10 (12%)	–
Two drug regimen*	1 (1%)	1 (1%)	–
NRTI backbone[♠], *n* (%)	–	–	<0.01[‡]
Didanosine, stavudine	16 (19%)	47 (56%)	–
Didanosine, zidovudine	17 (20%)	4 (5%)	–
Lamivudine, stavudine	9 (11%)	5 (6%)	–
Lamivudine, abacavir	9 (11%)	4 (5%)	–
Stavudine, abacavir	6 (7%)	2 (2%)	–
Lamivudine, zidovudine	7 (8%)	0 (0%)	–
Didanosine	3 (4%)	4 (5%)	–
Number of NRTI drugs continued from baseline regimen, *n* (%)	–	–	<0.01[†]
0	67 (81%)	43 (51%)	–
1	16 (19%)	36 (43%)	–
2	0 (0%)	5 (6%)	–
Number of NRTI drugs recycled from previous regimens (excluding baseline), *n* (%)	–	–	<0.01[†]
0	37 (45%)	48 (57%)	–
1	22 (27%)	29 (35%)	–
2	24 (29%)	7 (8%)	–
Number of new NRTI drugs never previously used, *n* (%)	–	–	0.99[†]
0	34 (41%)	31 (37%)	–
1	24 (29%)	31 (37%)	–
2	25 (30%)	22 (26%)	–
Number of drugs in regimen to which the virus was reported as "sensitive", *n* (%)[◆]	–	–	–
0	–	3 (4%)	–
1	–	9 (11%)	–
2[§]	–	22 (26%)	–
3[¶]	–	49 (58%)	–
4[$]	–	1 (1%)	–

*Two children were prescribed two drug regimens (didanosine+efavirenz, lamivudine+tenofovir). [†]Test for trend. [‡]Pearson χ^2 test. [§]*n*=1, [¶]*n*=46, [$]*n*=1 children were prescribed regimen containing all drugs to which the virus was reported as sensitive. [♠]Only nucleoside reverse transcriptase inhibitor (NRTI) backbones with an overall frequency >4% are shown. [◆]Lopinavir and tenofovir were prescribed for 16 children before these drugs had been added as to the resistance reports in November 2001. The table excludes three children in the test arm (RT) who did not change therapy. Low dose ritonavir is not counted as a separate drug. ART, antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitor; NT, no test arm; PI, protease inhibitor; RT, resistance test arm.

classes were predominantly prescribed NRTI+PI regimens (24/27 [89%] in RT arm, 29/30 [97%] in NT arm).

We also assessed how the new regimen was influenced by ART history. There was no significant difference in the number of new (never used before) drugs prescribed between the arms, overall or within class; 183 of the 190 (96%) NNRTIs and PIs prescribed were new drugs, compared with 149/324 (46%) of NRTIs. There were, however, differences in the number of NRTIs continued from the baseline regimen or recycled from previous regimens (Table 2). Fourty-one (49%) children continued one or more NRTIs from the baseline regimen in the RT arm compared to 16 (19%) in the NT arm ($P<0.01$); in contrast, more children in the NT arm recycled NRTIs from previous regimens (46 [55%] in NT compared to 36 [43%] in RT, $P<0.01$). Specific NRTIs most commonly continued in the RT arm were didanosine

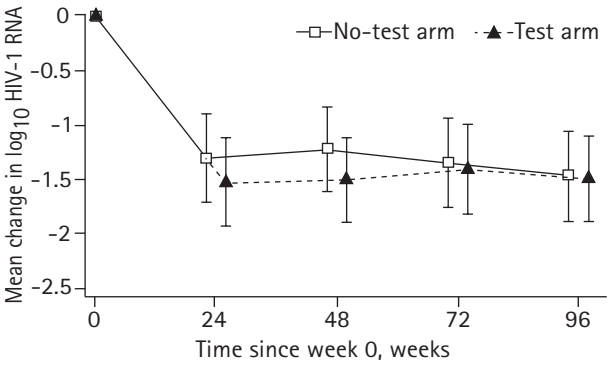
and stavudine, whereas zidovudine and lamivudine were most commonly recycled in the NT arm.

One hundred and fifty-nine clinicians (80 NT, 79 RT) specified at the screening visit the ART regimen that they would have prescribed without a resistance test. This proposed regimen corresponded exactly with the actual regimen prescribed for only 15 (19%) children in the RT arm, compared to 65 (82%) in the NT arm (P<0.001). Clinicians in the RT arm tended to prescribe either only one drug differently (n=36) or all drugs differently (n=29) in the new regimen. For children in the RT arm, 48 out of 84 (57%) clinicians prescribed a regimen containing all drugs to which the virus was reported as 'sensitive' based on the results of the resistance test (not taking into account local availability of drugs; Table 2); 52 (62%) could have prescribed a regimen with at least two NRTIs to which the virus was reported as 'sensitive' and at least one PI or NNRTI to which the virus was reported as 'sensitive' (data not shown).

Resistance tests and changes in antiretroviral regimen after baseline

Thirty-six children in the RT arm had 53 additional resistance tests performed after baseline during 217 person-years of follow-up (24 tests per 100 child-years), all within the first 48 weeks. Four children in the NT arm had local resistance tests after baseline for lack of viral load response or failure. There were a total of 88 (41 NT, 47 RT) second or subsequent changes in ART over 405 person-years; 22 per 100 child-years (20 per 100 child-years in NT, 23 per 100 child-years in RT). There was no difference in the time to first subsequent treatment change between the arms (P (log-rank)=0.6). Although resistance testing was available throughout the trial in the RT arm, only 43% of the subsequent changes in ART in this arm were preceded by a resistance test (within 4 months).

Figure 3. Change in HIV-1 RNA from baseline



NT *n*	83	82	80	82
n (%)<400 c/ml	28 (34%)	28 (34%)	30 (38%)	32 (38%)
n (%)<50 c/ml	20 (24%)	16 (19%)	18 (22%)	17 (21%)
RT *n*	84	85	85	83
n (%)<400 c/ml	32 (38%)	29 (34%)	30 (35%)	31 (37%)
n (%)<50 c/ml	16 (19%)	18 (21%)	14 (16%)	15 (18%)

Bars indicate 95% confidence interval. NT, no test arm; RT, test arm.

Virological, immunological and clinical endpoints

The mean (standard error) reduction in HIV-1 RNA at 48 weeks was 1.23 (0.20) $\log_{10}$ copies/ml in the NT arm compared with 1.51 (0.20) in the RT arm (0.28 in favour of the RT arm, 95% CI: -0.28–0.84, P=0.3). The difference between the arms was reduced by 96 weeks to 0.03 in favour of the RT arm (95% CI: -0.53–0.59, P=0.9; Figure 3). There was also no significant difference between arms in the proportion of children with a viral load <50 or <400 copies/ml at either 48 or 96 weeks (Figure 3). Similar results were obtained adjusting for baseline characteristics.

The mean (standard error) $CD4^+$ T-cell percentage increase at week 48 was 1.7% (0.9) in the NT arm and 3.2% (0.9) in RT, a non-statistically significant difference of 1.6% in favour of the RT arm (95% CI: -0.8–4.0, P=0.2); this difference was maintained to week 96 (NT 0.9% [1.0], RT 3.4% [0.9], 2.5% in favour of the RT arm, 95%CI: -0.1–5.2, P=0.06).

Seven deaths occurred (4 NT, 3 RT), six of which were classified as HIV related (septicaemia [n=4], Kaposi's Sarcoma [n=1] and diarrhoea/failure to thrive [n=1]) and one possibly HIV or treatment related (lactic acidosis). During the study there were 24 (17 NT, 7 RT) new AIDS-defining events in 17 children (11 NT, 6 RT).

Exploratory analyses

In exploratory analyses, we estimated the effect of resistance testing on virological response at 48 weeks adjusted for country of origin, age, calendar year of randomization, number of ART drugs previously received, ART drug classes previously received, duration of previous ART, initiated treatment on mono or dual NRTI therapy, and baseline RNA and $CD4^+$ T-cell count and percentage. A borderline statistically significant interaction was detected for the number of ART drugs previously received (P=0.05), indicating a greater effect of resistance testing the fewer the drugs previously received. For example, the model predicted that resistance testing resulted in a 0.68 $\log_{10}$ copies/ml reduction in HIV-1 RNA for a child exposed to three drugs compared to the NT arm, but a 0.56 $\log_{10}$ copies/ml increase for a child exposed to eight drugs. If this is a genuine effect, one might expect differences in

treatment regimens prescribed between randomized arms to be more pronounced for children with more limited prior ART exposure, but there was no evidence to support this (data not shown). Alternatively, activity of prescribed regimens could vary with the extent of prior ART exposure. No other statistically significant interactions were found. There was also no effect on viral load response in the RT arm of the number of drugs in the new regimen to which to the virus was reported as 'sensitive' (P=0.3).

Discussion

In this first paediatric trial evaluating the clinical utility of resistance testing in children with virological failure, we found no statistically significant evidence of a difference in virological response to antiretroviral regimens chosen by paediatricians with and without the aid of a genotypic test. However, the confidence interval for this comparison was wide (partly due to greater than expected variation in response between children) and included an advantage to the RT arm of up to 0.84 $\log_{10}$ copies/ml. We did find a substantial difference in the NRTIs prescribed suggesting that the resistance test results were influencing the choice of a new regimen. The fact that these prescribing differences did not translate into virological differences is perhaps not surprising given the relatively high exposure to the main NRTIs available to children and the specific choices made. First, on average three different NRTIs had been taken for an average of 5 years, and approximately three-quarters of the children had initiated ART with mono or dual NRTI therapy. Many children were, therefore, likely to have had archived resistance which may not have been readily detectable by resistance testing at baseline (for example, resistance was reported to zidovudine and lamivudine for only 69% and 77% of children in the RT arm although 87% and 83% had been exposed) and may have levelled differences in virological response. This explanation has also been suggested for similar negative findings in adult trials [4]. Indeed, exploratory analyses did suggest that the greatest benefit to resistance testing was in the subgroup of children exposed to fewer ART drugs in the past.

Second, the major impact of resistance testing was towards greater prescribing of didanosine and stavudine in the test arm, which was also reported in adult trials [4,10]. HIV was frequently reported as sensitive to these drugs in the RT arm (81% and 71% respectively), despite a relatively large number of TAMs, and consequently clinicians favoured continuing these drugs from the failing regimen rather than prescribing a new NRTI or recycling NRTIs from previous regimens. The utility of resistance test-guided therapy is largely determined by the interpretation given to sets of mutations and/or estimated fold-resistance values. This is particularly the case for NRTIs, for which small changes in fold resistance may have major implications for response to particular drugs. During the course of our study, VirtualPhenotype™ cut-off values for didanosine and stavudine susceptibilities changed from 4-fold (for both) to 3.5- and 3.0-fold, respectively (a predicted phenotype below these values implied susceptibility). The role of TAMs in predicting reduced susceptibility to didanosine/stavudine was unclear at the time this trial started. More recently, evaluation of virological response in treated patients has led to recognition that short term virological response to didanosine and stavudine begins to be compromised at lower values (>1.3 and >1.1, respectively, for VirtualPhenotype™ predicted fold-change) [22,23]. It seems likely that in this trial the excess use of didanosine and stavudine in the RT arm was guided by a VirtualPhenotype™ interpretation, which overestimated the susceptibility of viruses to these drugs and may therefore explain, in part, the lack of virological benefit observed. Didanosine can also be difficult for children as it has to be taken on an empty stomach and paediatric formulations at that time had to be mixed with antacid and kept refrigerated; poor adherence may also have contributed to the lack of virological difference seen between the arms. Interestingly there was no effect of resistance testing on PI or NNRTI prescribing; children only exposed to two classes were generally prescribed a drug from the class they had never used before and children exposed to all three classes were predominantly prescribed a new PI. As nelfinavir was previously the most commonly received PI, prescribing another PI was likely to be successful without knowing details of specific resistance mutations. Indeed, ART history is an essential adjunct to resistance test results in determining optimal prescribing.

Half the children were prescribed one or more drugs from previous regimens, which may reflect lack of availability of new drugs in appropriate formulations for this relatively ART-experienced paediatric population as well as the delay in introducing new antiretroviral drugs for children. For example, abacavir was the only NRTI available that had not been used by the majority of children in the past, but was only prescribed as part of the new regimen to 25% of children. This may be explained by concerns over the presence of the M184V mutation (64% of samples in the RT arm had M184V and resistance to abacavir was reported for approximately 50%).

Similarly, only two children had been exposed to lopinavir in the past yet only 25% were prescribed the drug as part of their new regimen, although lopinavir was not available for children until 2001 in Europe and 2002 in Brazil. The choice of new regimens in the PERA trial was therefore limited by several factors, which may explain why there was evidence of a better virological response in children exposed to fewer drugs, especially after resistance testing. Furthermore, many children had received sequential previous exposure to mono and/or dual NRTI therapy followed by PIs or NNRTIs. These children were particularly hard to manage, with the selection of potent regimens further limited by the lack of availability of new drugs, reflected by generally low virological response rates (<25% of children with viral load <50 copies/ml at 48 weeks).

HIV resistance testing is a rapidly moving area and changes in genotypic algorithms and phenotypic thresholds mean that all trials have time-limited relevance. The PERA trial started before the recognition of pathways and patterns of resistance was advanced; it is likely that future improvements to the interpretation of resistance mutations (reflected in clinical cut-off values likely to reduce virological benefit) will increase the clinical utility of resistance testing. The continued development of resistance testing as a diagnostic tool, especially simplification of interpretation, is particularly important for paediatricians, many of whom care for small numbers of HIV-1-infected children. For example, seven of the centres participating in PERA care for less than 20 children, and 19 (79%), care for less than 100. Resistance testing was also only routinely performed in about half of the centres at the start of the trial. However, it is possible that the presentation of lack of reduced susceptibility ('sensitive') on the resistance test reports encouraged paediatricians to believe that such drugs could be continued in the new regimen without taking account of potential archived mutations or minority variants.

The PERA trial, in common with some related trials in adults [11,12], was not able to demonstrate a statistically significant long-term clinical benefit from resistance testing to the individual participants. In this fast moving area, further knowledge of resistance testing, which became available during the lifetime of the trial, may have contributed to this negative result. However, we did observe an effect of resistance testing on paediatric ART prescribing, and importantly, additional data generated from this trial will contribute to the very limited knowledge base about resistance testing in children. This should allow resistance testing to be used more effectively in paediatric HIV infection in the future.

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Additional files

The additional file 'The study organization of the Paediatric European Network for the Treatment of AIDS (PENTA)' can be accessed via the Volume 11 Issue 7 contents page, which can be found at http://www.intmedpress.com (by clicking on 'Antiviral Therapy' then 'Journal PDFs').

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