Lamivudine/abacavir maintains virological superiority over zidovudine/lamivudine and zidovudine/abacavir beyond 5 years in children

Paediatric European Network for the Treatment of AIDS (PENTA)

Objective: To describe the long-term efficacy over 5 years of regimens including combinations of abacavir, lamivudine and/or zidovudine in previously untreated children in the PENTA 5 trial.

Design: PENTA 5 was a 48-week randomised controlled trial comparing three dual nucleoside reverse transcriptase inhibitor (NRTI) combinations as part of first triple antiretroviral therapy (ART).

Methods: 128 ART-naïve children were randomised to zidovudine\lamivudine (n = 36), zidovudine\abacavir (45) or lamivudine\abacavir (47). Asymptomatic children (n = 55) were also randomised to nelfinavir or placebo; all other children received open-label nelfinavir. Analyses are intent-to-treat and adjusted for minor baseline imbalances and receipt of nelfinavir/placebo.

Results: Median follow-up was 5.8 years. By 5 years, 17 (47%), 28 (64%) and 18 (39%) children had changed their randomised NRTIs in the zidovudine\lamivudine, zidovudine\abacavir and lamivudine\abacavir groups respectively, but 18%, 50% and 50% of these changes were either early single drug substitutions for toxicity or switches with viral suppression (HIV-1 RNA <400 copies/ml; e.g. to simplify regimen delivery). At 5 years, 55%/32% zidovudine\lamivudine, 50%/25% zidovudine\abacavir and 79%/63% lamivudine\abacavir had HIV-1 RNA $<400/<50$ copies/ml respectively ($p = 0.03/p = 0.003$). Mean increase in height-for-age 0.42, 0.68, 1.05 ($p = 0.02$); weight-for-age 0.03, 0.13, 0.75 ($p = 0.02$). Reverse transcriptase resistance mutations emerging on therapy differed between the groups: zidovudine\lamivudine (M41L, D67N, K70R, M184V, L210W, T215Y); zidovudine\abacavir (M41L, D67N, K70R, L210W, T215F/Y, K219Q); lamivudine\abacavir (K65R, L74V, Y115F, M184V).

Conclusions: Five year data demonstrate that lamivudine\abacavir is more effective in terms of HIV-1 RNA suppression and growth changes, with lower rates of switching with detectable HIV-1 RNA than zidovudine\lamivudine or zidovudine\abacavir, and should be preferred as first-line NRTI backbone.

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Introduction

Introduction of effective antiretroviral therapy (ART) has significantly reduced mortality and morbidity in adults and children [1,2]. However, virological response to ART has typically been poorer in children [3,4] compared to adults [5]. As most HIV-infected children are vertically infected, start therapy at relatively young ages and will need to take ART lifelong, poorer virological response and the potential for subsequent emergence of resistance is a cause for concern.

A large number of randomized trials provide a robust evidence base for the treatment of adults with combination ART: although the majority are of short duration (48 weeks), the number reporting long-term

From the MRC Clinical Trials Unit, London, UK.
Correspondence to H. Green, MRC Clinical Trials Unit, 222 Euston Road, London, NW1 2DA, UK.
Tel: +44 20 7670 4733; fax: +44 20 7670 4818; e-mail: hjg@ctu.mrc.ac.uk
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follow-up (3 years and beyond) is increasing [5–8]. In contrast, randomized trials comparing different ART combinations in previously untreated children are few [9–11]. Differences in available formulations, variable pharmacokinetics and robustness of dosing recommendations, as well as reliance on caregivers to give medications may all lead to differing relative efficacy in adults and children both short and long term. The PENTA 5 trial has previously reported that at both 24 and 48 weeks after initiation of ART, regimens including abacavir as one of the nucleoside analogue reverse transcriptase inhibitors (NRTIs) were more effective than zidovudine/lamivudine in reducing in $\log_{10}$ HIV-1 RNA and suppressing HIV-1 RNA < 400 copies/ml [10]. All regimens were generally well tolerated and the incidence of suspected hypersensitivity to abacavir (3%) was similar to that observed in adults. Here we consider long-term efficacy over 5 years of regimens including combinations of abacavir, lamivudine and/or zidovudine in previously untreated children in the PENTA 5 trial.

Methods

PENTA 5 trial design

PENTA 5 was a 48-week randomized controlled trial comparing three dual NRTI combinations, with or without nelfinavir, as first-line ART [10]. One-hundred and twenty-eight ART-naive children were randomized between January 1998 and April 1999 from 34 centres in nine countries, to zidovudine/lamivudine (n = 36) or zidovudine/abacavir (n = 45) or lamivudine/abacavir (n = 47). Asymptomatic children (n = 55) were also randomized to receive nelfinavir or nelfinavir placebo in a factorial design (Part A); children with more advanced disease (n = 73) received open-label nelfinavir (Part B). Therefore 103 of the 128 children initiated ART with three drugs; the remaining 25 started dual NRTI therapy only. Children in Part A were unblinded to nefinavir/placebo allocation when the last child enrolled reached 24 weeks of follow-up.

Long-term follow-up

One child was lost to follow-up after 3 days, and one died from sepsis in the first month after starting lamivudine/abacavir/nefinavir. All other children were followed beyond 48 weeks (Fig. 1). Ethics committees for each centre approved long-term follow-up and primary caregivers and children, where appropriate, gave written consent. All CD4 cell counts and percentages, HIV-1 RNA measurements, local resistance test results, ART received, AIDS events and growth measurements were collected annually; additional toxicity data was not collected. Results from centralized resistance testing up to 48 weeks have been reported elsewhere [12]; a trial sample was also requested for centralized viral load and resistance testing at 3 years.

Statistical methods

All analyses are intention-to-treat (ignoring changes to randomized treatment) based on the 126 children followed after 48 weeks. Baseline values were those before and nearest to randomization (within 4 weeks). Changes from baseline were calculated from the closest value to nominal assessment years, within a quarter-year window either side. We calculated changes in HIV-1 RNA using normal interval regression [13], replacing values below the lower limit of quantification with the interval in which the true value could lie (e.g. for values < 50 copies/ml, the interval [0,50] copies/ml was used). Proportions were compared using exact tests. Because of minor imbalances in baseline characteristics and receipt of nelfinavir in the NRTI groups, all analyses were adjusted for age, HIV-1 RNA and CD4% at baseline; plus allocation to nelfinavir in Part A or Part B, or placebo in Part A [10]. Adjusted analyses of proportions used logistic regression with Wald tests. Generalized Estimating Equations were used for global tests of differences between randomized groups over the entire study period (1–5 years), also adjusted for minor baseline imbalances [14]. Significance tests compared all three randomized groups, i.e., testing the hypothesis that the effect of at least one treatment group on outcome is different from the other groups. Analyses were also repeated restricted to children allocated to nelfinavir at trial entry (i.e., initiating ART with three drugs), and similar results were obtained. CD4 cell counts, height and weight were expressed as Z scores with reference to healthy uninfected children [15,16].

Results

One-hundred and twenty-six children were followed after 48 weeks (n = 36/zidovudine/lamivudine, n = 44/zidovudine/abacavir, n = 46/lamivudine/abacavir) (Fig. 1). At randomization, their median age was 5.4 years (range, 0.3–16.7 years), median CD4% was 22% [interquartile range (IQR), 13–29%], mean HIV-1 RNA was 5.1 $\log_{10}$ copies/ml (SD 0.8); 11 children (9%) had had an AIDS-defining event.

Follow-up and clinical events

Median follow-up to 15 November 2005 was 5.8 years (range, 3.1–7.8 years). Two of the 126 children followed beyond 1 year were lost to follow-up at 3.1 and 3.9 years after randomization respectively, and a further 16 children were last seen alive between 4 and 5 years. Six children had new AIDS events within 5 years of randomization (four before and two after 48 weeks); there were no recurrent AIDS events. One child died at 3.1 years following Hodgkin's lymphoma, leaving 94%, 93%, 96% alive without a new or recurrent AIDS event at 5 years in the zidovudine/lamivudine, zidovudine/abacavir and lamivudine/abacavir groups respectively (Kaplan–Meier proportions, $P = 0.87$, log-rank test).

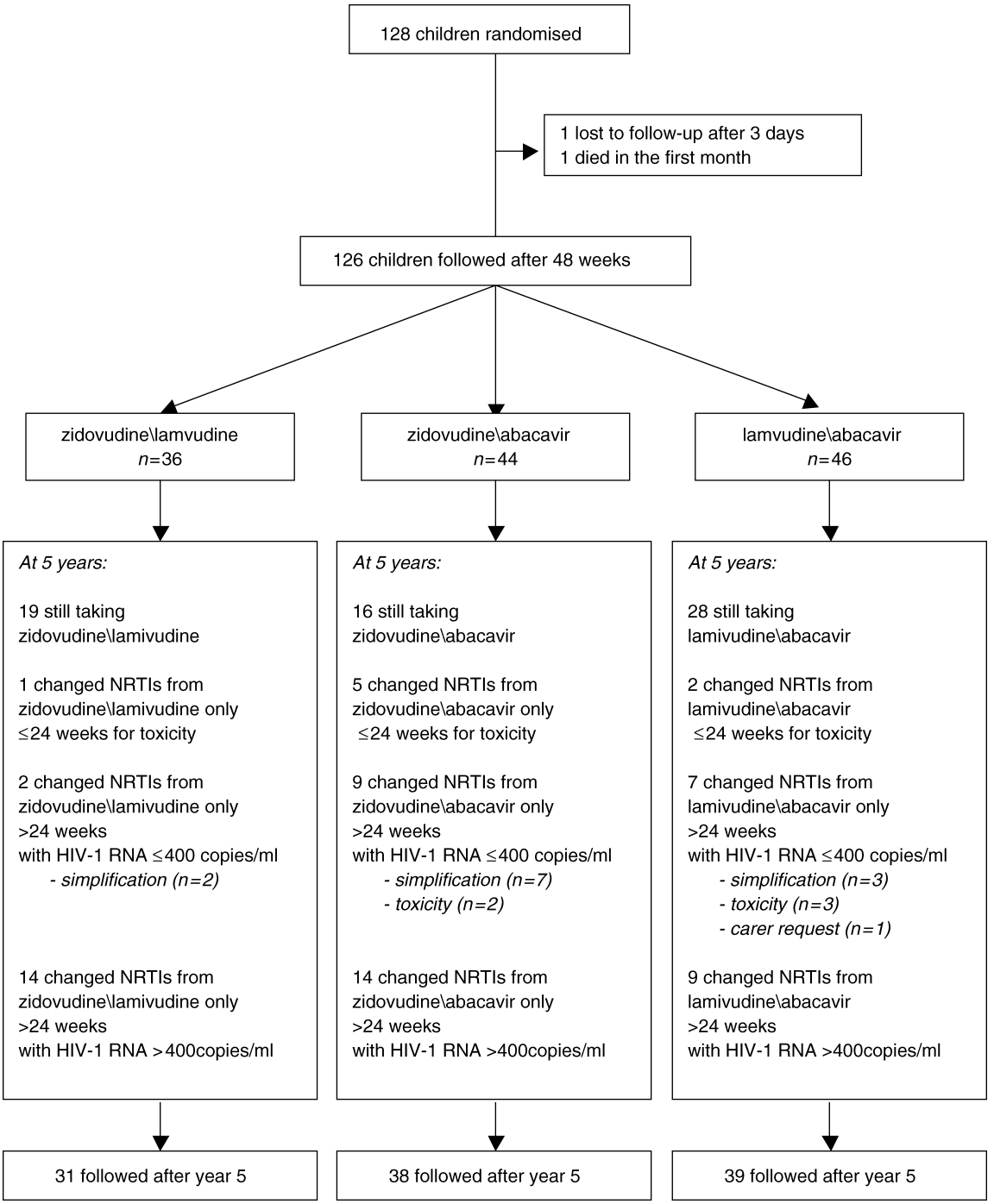

Fig. 1. Trial profile for follow-up and treatment changes from randomized NRTIs at 5 years (counting children adding another NRTI [such as moving to trizivir (zidovudine/lamivudine/abacavir)] as a change from dual randomized NRTIs).

Antiretroviral treatment to 5 years

Up to 5 years, children in the zidovudine/lamivudine and zidovudine/abacavir groups had been exposed to a median (range) of four (three to nine) and four (two to six) drugs compared to only three (two to seven) in the lamivudine/abacavir group ($P = 0.13$, Kruskal–Wallis test). At 5 years, 37 (29%) children had switched to second-line therapy (three or more new drugs compared to the original regimen, n = 29) or were off ART (n = 8) [14 (39%), 14 (32%) and 9 (20%) respectively; $P = 0.15$, exact test].

As expected, the proportion of child-time spent taking the randomized antiretroviral drugs decreased over time. Between 0 to 2.5 years after randomization, approximately 85% of child-time was spent taking the two NRTI drugs exactly as randomized in all groups.

However, between 2.5 to 5 years, the proportion still taking randomized NRTIs was lower in the zidovudine groups (61%, 54%) than the lamivudine/abacavir group (69%). Other non-randomized NRTIs were also taken more in the zidovudine groups during this time: didanosine and stavudine in the zidovudine/lamivudine and the lamivudine/abacavir groups (26%, 27% and 14%, 13% respectively), and lamivudine, didanosine and stavudine in the zidovudine/abacavir group (Fig. 2). The proportion of child-time spent taking nelfinavir decreased over time in all groups; between 2.5 and 5 years, the proportion of child-time spent taking lopinavir, efavirenz and nevirapine was 11%, 14% and 12% in the zidovudine/lamivudine group respectively, 2%, 13% and 6% in the zidovudine/abacavir group and 0%, 16% and 4% in the lamivudine/abacavir group.

By 5 years, 17 (47%), 28 (64%) and 18 (39%) children were taking NRTIs other than randomized in the zidovudine/lamivudine, zidovudine/abacavir and lamivudine/abacavir groups respectively ($P = 0.06$, exact test) (Fig. 1), but 18% (3/17), 50% (14/28) and 50% (9/18) of these changes were either early single drug substitutions for toxicity (< 24 weeks after randomization) or switches in children with viral suppression (plasma HIV-1 RNA viral load < 400 copies/ml) for simplification, toxicity or child/carer request. Ten of the 12 switches for simplification were to triple NRTI (zidovudine/lamivudine/abacavir). Overall, excluding early single drug switches for toxicity, 17 (47%) children randomized to zidovudine/lamivudine, 16 (36%) to zidovudine/abacavir and 10 (22%) to lamivudine/abacavir had substituted or added one or more drugs (i.e., including changes to

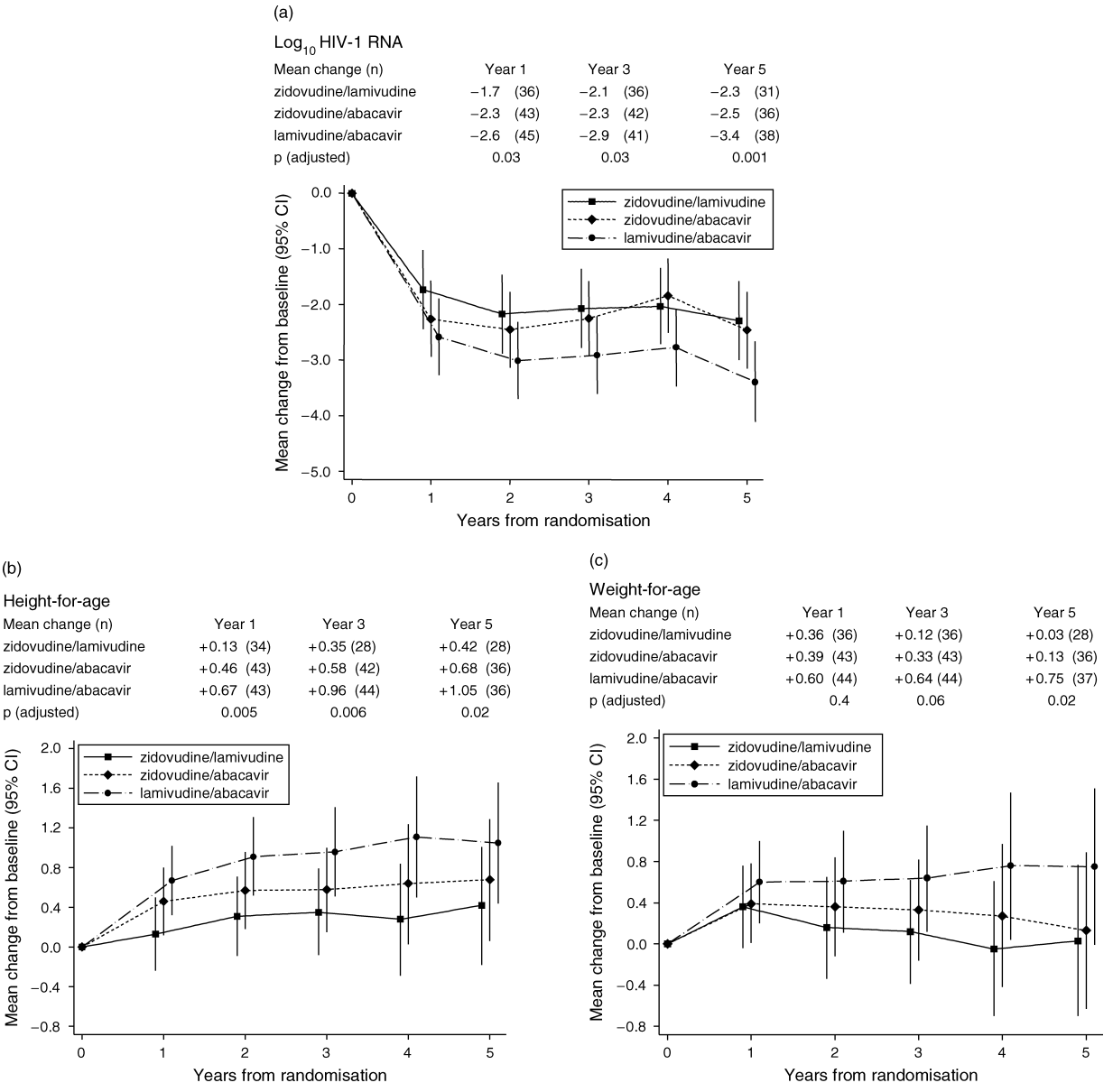

Fig. 2. HIV-1 RNA and growth changes to 5 years.

non-NRTIs) with HIV-1 RNA >400 copies/ml by 5 years ($P=0.04$, log-rank test).

HIV-1 RNA, CD4% and growth at and to 5 years

The mean (SE) reduction in HIV-1 RNA from baseline to 5 years was 2.3 (0.36) and 2.5 (0.35) $\log_{10}$ copies/ml in the zidovudine/lamivudine and zidovudine/abacavir groups compared to 3.4 (0.37) in the lamivudine/abacavir group ($P=0.001$ at 5 years, global $P<0.001$) (Fig. 2). There was no evidence that this difference between randomized groups increased or decreased over time (heterogeneity $P=0.5$). Of the 105 (83%) children with HIV-1 RNA measured at 5 years, suppression was greatest in the lamivudine/abacavir arm: 55%, 50% and 79% had HIV-1 RNA <400 copies/ml in the three NRTI groups respectively ($P=0.03$ at 5 years, global $P=0.003$), with 32%, 25% and 63% <50 copies/ml ($P=0.003$ at 5 years, global $P=0.006$) (Table 1). Similar results were seen restricting the analysis to children allocated to nelfinavir at trial entry (i.e., initiating ART with three drugs) (Table 1). There was no evidence that the difference in HIV-1 RNA supression between the randomized groups varied over time (heterogeneity $P=0.4$ <400 copies/ml, $P=0.1$ <50 copies/ml). Similar results were obtained when the analysis was restricted to the selected subgroup of children remaining on randomized NRTIs (on treatment analysis, at 5 years, 53%, 60% and 76% had HIV-1 RNA <400 copies/ml, and 27%, 27% and 67% had HIV-1 RNA <50 copies/ml in the three NRTI groups respectively).

Increases in height-for-age and weight-for-age were significantly greater in the lamivudine/abacavir group (global $P=0.001$ and $P=0.04$ respectively) (Fig. 2). Further there was a trend towards an increasing benefit from lamivudine/abacavir in weight-for-age compared to the other randomized groups over time (heterogeneity $P=0.09$), but no variation in effects on height-for-age (heterogeneity $P=0.6$). Of the 102 (81%) children with CD4 cell count measured at 5 years, mean (SE) increase in CD4% was 12% (2%) in the zidovudine/lamivudine group, 9% (2%) in the zidovudine/abacavir group and 12% (2%) in the lamivudine/abacavir group ($P=0.2$), which were similar to increases from baseline to 1 year (8%, 7% and 7% respectively, $P=0.5$) and to 3 years (9%, 7% and 9% respectively, $P=0.5$). However, whilst CD4% varies less with age than CD4 absolute cell count, younger children still tend to have higher percentages and children in the lamivudine/abacavir group were younger. Adjusting more fully for age imbalances using age-adjusted CD4 z-score [15], there was a trend towards greater increases in the lamivudine/abacavir group at 5 years [mean (SE) increase 1.0 (1.4), 1.4 (1.3) and 2.4 (1.4) in the three NRTI groups respectively], but this was not statistically significant ($P=0.5$).

Children randomized to dual NRTI

Twenty-four children (7 zidovudine/lamivudine, 11 zidovudine/abacavir, 6 lamivudine/abacavir) were randomized to nelfinavir placebo in Part A and thus initiated ART with only two drugs. At 5 years, 7 (29%) children were still taking randomized dual NRTI therapy; none were taking zidovudine/lamivudine, 3/11 (27%) were taking zidovudine/abacavir, and 4/6 (83%) were taking lamivudine/abacavir. Four of the 7 children remaining on dual therapy had HIV-1 RNA <400 copies/ml through to 5 years (1/3 zidovudine/abacavir, 3/4 lamivudine/abacavir), and the remaining three had HIV-1 RNA <4000 copies/ml. Of the 17 children who moved from dual NRTI treatment, 12 started triple therapy, one child switched from zidovudine/lamivudine to lamivudine/stavudine at 1 year (last seen at 5 years) and four children stopped ART (one child subsequently started triple therapy 2 years after stopping; three children were still off treatment 3, 4 and 6.5 years after stopping).

Table 1. HIV-1 RNA suppression over time. All analyses are intention-to-treat, i.e., ignoring changes to randomized treatment. *P*-values are adjusted for baseline characteristics and test the hypothesis that HIV-1 RNA suppression in at least one treatment group is different from that in the other groups, at each year or over 1–5 years.

	HIV-1 RNA <400 copies/ml [n/N (%)][a]				HIV-1 RNA <50 copies/ml [n/N (%)]				HIV-1 RNA <50 copies/ml; initiated ART with three drugs [n/N (%)]			
Year	ZDV/3TC (n=36)	ZDV/ABC (n=44)	3TC/ABC (n=46)	*P*	ZDV/3TC (n=36)	ZDV/ABC (n=44)	3TC/ABC (n=46)	*P*	ZDV/3TC (n=29)	ZDV/ABC (n=33)	ZDV/ABC (n=38)	*P*
1	17/36 (47)	27/43 (63)	31/45 (69)	0.2	12/36 (33)	19/43 (44)	24/45 (53)	0.2	11/29 (38)	13/33 (39)	22/37 (59)	0.2
2	19/36 (53)	21/41 (51)	32/44 (73)	0.05	9/36 (25)	10/41 (24)	20/44 (45)	0.03	8/29 (28)	9/31 (29)	18/36 (50)	0.06
3	19/36 (53)	20/42 (48)	30/41 (73)	0.02	14/36 (39)	14/42 (33)	19/41 (46)	0.5	12/29 (41)	11/31 (35)	17/33 (52)	0.4
4	18/31 (58)	16/43 (48)	28/36 (78)	0.01	15/31 (48)	8/33 (24)	16/36 (44)	0.06	11/29 (38)	13/33 (39)	22/37 (59)	0.1
5	17/31 (55)	18/36 (50)	30/38 (79)	0.03	10/31 (32)	9/36 (25)	24/38 (63)	0.003	8/24 (33)	7/27 (26)	22/32 (69)	0.002
	Overall difference between randomised groups, years1–5			0.003	Overall difference between randomised groups, years1–5			0.006	Overall difference between randomised groups, years1–5			0.006
	Difference between randomised groups varies over 5 years			0.4	Difference between randomised groups varies over 5 years			0.1	Difference between randomised groups varies over 5 years			0.2

[a]Conservatively assuming that children with HIV-1 RNA recorded as below a limit of detection greater than 50 (e.g., 400) are not below 50 copies/ml (a total of 57 of 569 tests, 10%). ART, Antiretroviral therapy; ZDV, zidovudine; 3TC, lamivudine; ABC, abacavir.

Resistance

Sixteen children had one or more resistance tests after 1 year whilst still on their randomized NRTI, having either never achieved HIV-1 RNA <400 copies/ml ($n=3$) or rebounded after initial suppression ($n=13$, HIV-1 RNA <400 copies/ml and then >2000 copies/ml, confirmed). All four children who had received zidovudine/lamivudine (two also received nelfinavir) developed M184V alone by 1 year and all subsequently developed thymidine analogue mutations (TAM) [M41L ($n=4$), T215Y ($n=2$), D67N ($n=1$), K70R ($n=1$), L210W ($n=1$)] whilst maintaining M184V. In contrast four of the six children who received zidovudine/abacavir (all six also received nelfinavir) maintained wild-type virus despite ongoing viral replication and without documented ART interruption (last resistance test at 3–4.5 years, with latest HIV-1 RNA 2343–13210 copies/ml); the other two children had wild-type virus at year 1 but developed TAM at 3–3.5 years (D67N, K70R and K219Q; M41L, D67N, L210W, T215F/Y). Two of the six children who had received lamivudine/abacavir had only the M184V mutation by 3 and 5 years respectively (both received nelfinavir). The remaining four children had 'non-TAM' mutations by year 1 [L74V ($n=4$), M184V ($n=4$), K65R ($n=3$), Y115F ($n=1$), one also received nelfinavir], which were maintained in two children, lost in one child without other mutations (HIV-1 RNA 70,841 copies/ml, no documented interruptions in ART, also received nelfinavir) and replaced by TAM at 4.5 years in one child (D67N, K70R, K219Q, HIV-1 RNA 2106 copies/ml, no documented change in ART). Overall, the majority of children who received nelfinavir developed nelfinavir mutations by the first test and kept them or developed more over time.

Discussion

There are very few randomized trials of combination ART in chronically HIV-infected, previously untreated children. Indeed, apart from the ongoing PENPACT 1 trial addressing questions about initial ART and switching strategies [11], PENTA 5 is the only such post monotherapy randomized trial to report from well-resourced countries. PENTA 5 was also the first trial in adults or children to report on the use of lamivudine/abacavir as part of a triple therapy regimen. Here we have demonstrated long-term sustained virological superiority of lamivudine/abacavir compared with either zidovudine/lamivudine or zidovudine/abacavir beyond 5 years. Further, the benefits from this regimen in terms of growth identified over the short-term appear to persist and even increase over time. Although nelfinavir is no longer a preferred first-line option in children, we do not consider there to be strong *a priori* reasons for qualitatively different results with the main choices available to paediatricians today (efavirenz, nevirapine, kaletra).

Only one child died and two developed AIDS over a median of nearly 5 years additional follow-up after week 48, all these events occurring within the first 3 years. The single death (from lymphoma) may not have been preventable with ART in any case. In accordance with the week 48 results [10], significant differences between the NRTI groups in terms of increases in CD4 percent were not apparent at 5 years; although adjustment for natural variation in absolute CD4 counts with age [15] suggested greater CD4 cell gains may have occurred in the lamivudine/abacavir group in line with changes in HIV-1 RNA and growth. Thus overall, in spite of differences in virological and growth outcomes, children appear to do well clinically and immunologically on all regimens.

Suppression of HIV-1 viral load was sustained at similar levels between 1 and 5 years and overall less than one-third of children switched to second-line therapy (three or more new drugs compared to the original regimen), lowest in the lamivudine/abacavir group. Further, in the lamivudine/abacavir group, only 22% had ever substituted or added one or more drugs at a time of incomplete viral suppression (HIV-1 RNA >400 copies/ml) when resistance potentially could have arisen. There were also clear trends to longer use of this combination as dual NRTI and less use of other non-trial PIs and NNRTIs in this group. This is encouraging; also considering that nelfinavir is a relatively low potency PI with a high pill burden and relatively low acceptability by self-report from carers and children in PENTA 5 [17]. Few data on rates of drug substitutions and switch to second-line therapy have been reported in paediatric cohort studies, although anecdotally rates appear to be lower than in adults, most likely due to a combination of more limited drug choices for children, innate conservatism among parents and paediatricians and the fact that children frequently maintain clinical and immunological benefit in the face of virological failure. In this study, more substitutions occurred for non-failure and non-toxicity reasons, reflecting clinical practice and particularly efforts to simplify therapy, which are often not captured in short-term trials.

The extended period of randomized treatment despite continuing viral replication in some children allowed a detailed exploration of resistance evolution according to NRTIs received in 16 children. These data suggest that the order and pattern of resistance may be dependent on the combinations of NRTI utilized, which in turn can potentially determine cross resistance patterns to other reverse transcriptase inhibitors. The most striking differences were observed between children on zidovudine/lamivudine and lamivudine/abacavir. In the former, the initial emergence of M184V was followed by thymidine analogue mutations, generally of the TAM-1 pathway. This is now a well-recognized pattern within clinical practice [18]. By contrast resistance in the

lamivudine/abacavir group was characterized by the initial appearance of M184V plus K65R and/or L74V. It has previously been observed that the poor fitness of viruses containing both 65 and 74 mutations explains the absence of such mutational patterns in clinical databases [19,20], and that these mutations are not found on the same genome [21]. However, we observed the co-existence of these mutations in three children receiving this combination, as also described by Lanier *et al*. [22], although by consensus sequencing of plasma virus. It is interesting to speculate whether the co-existence of M184V in all three cases facilitated ongoing replication, although previous studies demonstrate the initial emergence of M184V and K65R on different genome [23]. Nevertheless, the relative fitness disadvantage of such viruses may explain the modest viral load rebound observed for these children.

It is important to speculate on the relative risks and benefits of a lamivudine/labacavir combination; to what degree should the potential for emergence of such resistance patterns be counterbalanced by the clear virological advantages and improved growth with this combination for children, as demonstrated in this study? Phenotypic assessment of viruses containing K65R does indeed demonstrate extensive cross resistance to all nucleoside/nucleotide analogues other than zidovudine [24]. However, more data regarding *in vivo* activity of non-zidovudine nucleoside analogues in the face of this mutation are required before developing evidence-based drug sequencing strategies. At the present time, the relative advantages of being infected with a virus containing extensive TAMs with M184V against one containing K65R, L74V and M184V remain unclear, and we consider that treatment regimens be guided by virological and clinical efficacy data.

Historically zidovudine has been preferred as a first-line NRTI. However abacavir has been added to the list of NRTI recommended for first-line therapy in the revised (2006) WHO guidelines [25] and is commonly used in Europe [11]. Of all antiretrovirals, abacavir is one of the NRTIs with least effect on mitochondrial DNA [26,27], and, unlike zidovudine, has little haematologic toxicity which may be important in settings where malaria is common. In addition, toxicity rates to abacavir are likely to be lower in African compared with Caucasian children because of polymorphisms leading to less abacavir hypersensitivity in Africans, although clinical vigilance for the presence of hypersensitivity remains paramount. The rates of adverse reactions to abacavir were considerably lower than to nevirapine in African adults in a recently reported double-blind substudy of DART, the Nevirapine Or Abacavir (NORA) trial [28]. Of additional value for adherence, abacavir and lamivudine can be given once daily; the PENTA 13 trial showed equivalent pharmacokinetics and continued viral load suppression in children over 3 years of age after switching from twice to once daily lamivudine and/or abacavir [29]. Further lamivudine and abacavir are low volume, reasonably pleasant tasting liquids, whereas zidovudine liquid has higher volume and requires storage in brown glass containers because of light sensitivity. Simplifying ART for children and carers is an important objective in HIV management, and may be of particular value for children approaching teenage years. If fixed dose combination 'baby tablets' of abacavir/lamivudine could be made, this could add an important potent simple once-daily alternative to the inconvenient single liquid formulation NRTI drugs currently available for children in resource-limited settings. Five-year data suggest that lamivudine/abacavir is more effective in terms of virological response and increase in height and weight than zidovudine/lamivudine or zidovudine/abacavir and should be preferred as first-line NRTI backbone in triple therapy regimens.

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Writing Committee

H. Green, D.M. Gibb, A.S. Walker, D. Pillay, K. Butler, F. Candeias, G. Castelli-Gattinara, A. Compagnucci, M. Della Negra, A. de Rossi, C. Feiterna-Sperling, C. Giaquinto, L. Harper, J. Levy, Y. Saidi, U. Wintergerst.

Medical Research Council Clinical Trials Unit, UK

A. Babiker, L. Buck, J. Darbyshire, L. Farrelly, D.M. Gibb, H. Green, L. Harper, D. Johnson, P. Kelleher, A. Newberry, A. Poland, G. Wait, A.S. Walker.

INSERM SC10, France

J.P. Aboulker, A. Compagnucci, M. Debré, V. Eliette, S. Girard, S. Leonardo, Y. Saidi

Executive Committee for PENTA 5

J.P. Aboulker, A. Babiker, A. Compagnucci, J. Darbyshire, M. Debré, M. Gersten (Agouron), C. Giaquinto (chairperson), D.M. Gibb, W. Snowdon (GlaxoSmithKline)

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PENTA 5 DSMC

C. Hill (chairperson), P. Lepage, A. Pozniak, S. Vella. Secretary R. Withnall

Participating Centres

*Pharmacists, **Virologists

Belgium: Hôpital Saint Pierre, Brussels: J. Levy, M. Hainaut, A. Peltier, S. Carlier*, G. Zissis**.

Brazil: Instituto de Infectologia 'Emilio Ribas', São Paolo: M. Della Negra, W. Queiroz; **Fleury Laboratories, São Paolo**: L.P. Feitosa**, D. Oliveira**.

France: Centre Hospitalier Universitaire, Nantes: F. Mechinaud, V. Reliquet, F. Ballerau*, A. Lepelletier*, S. Billaudel**, V. Ferre**.

Germany: Virchow-Klinikum, Humboldt-Universität zu Berlin: I. Grosch-Wörner, R. Weigel, K. Seel, C. Feiterna-Sperling, D. Ohlendorf*, G. Rieße*, C Müller**; **Universitäts-Kinderklinik Düsseldorf:** V. Wahn, T. Niehues, J. Ndagijimana, G. Horneff, S. Gudowius, N. Vente**; **Universitäts-Kinderklinik Eppendorf, Hamburg**: R. Ganschow, G. Englert. **Universitat zu Köln**: T. Simon, R. Vossen*, H. Pfister**; **Universitätskinderkliniken, Munich**: U. Wintergerst, G. Notheis, G. Strotmann, S. Schlieben.

Ireland: Our Lady's Hospital for Sick Children, Dublin: K. Butler, E. Hayes, M. O'Mara, J. Fanning*, F. Goggins*, S. Moriarty*, M. Byrne.

Italy: Ospedale Regionale di Bolzano: L. Battisti; **Spedali Civili, Brescia**: M. Duse, S. Timpano, E. Uberti, P. Crispino, P. Carrara, F. Fomia, R. Schumacher, A. Manca**; **Ospedale Meyer, Florence**: L. Galli, M. de Martino; **Istituto G Gaslini, Genova**: F. Fioredda, E. Pontali, R. Rosso; **Ospedale Civile, Modena**: M. Cellini, C. Baraldi, M. Portolani**, M. Meacci**, P. Pietrosemoli**; **Università di Napoli 'Federico II'**: A. Guarino, M.I. Spagnuola, R. Berni Canani, V. Giacomet, P. Laccetti**, M. Gobbo**; **Università di Padova**: C. Giaquinto, V. Giacomet, R. D'Elia, O. Rampon, V. Balasso, A. de Rossi**, M. Zanchetta**; **IRCCS Policlinico San Matteo, Pavia**: D. Caselli, A. Maccabruni, E. Cattaneo**, V. Landini**; **Ospedale Bambino Gesù, Rome**: G. Castelli-Gattinara, S. Bernardi, A. Krzysztofiak, C. Tancredi, P. Rossi**, L Pansani**; **Università degli Studi di Torino**: E. Palomba, C. Gabiano; **Ospedale S. Chiara, Trento**: A. Mazza, G. Rossetti**; **Ospedale S. Bortolo, Vicenza**: R. Nicolin, A. Timillero.

Portugal: Hospital de Dona Estefania, Lisbon: L. Rosado, F. Candeias, G. Santos, M.L. Ramos Ribeiro, M.C. Almeida, M.H. Lourenço**, R. Antunes**

Spain: **Instituto de Salud Carlos III, Madrid**: M.J. Mellado Peña**, P.M. Fontenlos, ML Carillo de Albornoz*, P. Martinez Santos; **Hospital Son Dureta, Palma de Mallorca**: L. Ciria Calavia, J. Dueñas Morales, J. Serra Devecchi*, O. Delgado, N. Matamoros**.

UK: Bristol Royal Hospital for Sick Children, Bristol: A. Foot, H. Kershaw, C. Kelly*; **PHL Regional Virus Laboratory, Bristol**: O. Caul**; **Ninewells Hospital and Medical School, Dundee**: W. Tarnow-Mordi, J. Petrie, A. McDowell*, P. McIntyre**, K. Appleyard**; **Ealing Hospital, Middlesex**: K. Sloper, V. Shah, K. Cheema*, A. Aali**; **Royal Edinburgh Hospital for Sick Children**, J. Mok, R. Russell, A. Brewster*, N. Richardson*; **City Hospital, Edinburgh**: S. Burns**; **Great Ormond St Hospital for Children NHS Trust, London**: D. Gibb, V. Novelli. N. Klein, L. McGee, S. Ewen, J. Flynn, V. Yeung*; **King's College Hospital, London**: C. Ball, K. Himid, D. Nayagam, D. Graham, S. Hawkins, A. Barrie*, K. Stringer*, S. Jones*, N. Weerasooriya*, M. Zuckerman**, P. Bracken**; **Newham General Hospital, London**: D. Gibb, E. Cooper, T. Fisher, R. Barrie, S. Liebeschuetz, S. Wong, U. Patel* (deceased); **Royal Free Hospital, London**: V. Van Someren, K. Moshal, S. McKenna, L. Perry*, T. Gundlach**; **St Bartholemew's Hospital, London**: J. Norman**; **St George's Hospital, London**: M. Sharland, M Richardson, S. Donaghy, S. Storey, Z. Mitchla*, C. Wells*, J. Booth** (deceased), A. Shipp** D. Butcher**; **St Mary's Hospital, London**: G. Tudor-Williams, H. Lyall, J. White, S. Head, C. Walsh, C. Hanley, S. Campbell, S. Lambers*, K. O'Hara*; C. Stainsby**; **St Thomas' Hospital, London**: G. Du Mont, R Cross, T. Solanki*, S. Swanton*, S. O'Shea**, A. Tilsey**; **University College London Medical School**: S. Kaye**; **Children's Hospital, Sheffield**: A. Finn, S. Choo, R. Lakshman, J. Hobbs, L. Barr*; **Sheffield Public Health Laboratory**, Sheffield G Bell**, A. Siddens**.

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