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The Impact of HIV-1 Subtypes on Virological Response and Emergence of Resistance in the PENTA 5 Trial

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Background & Objectives

Although an increasing proportion of HIV-1 infections in Europe are due to non-subtype B viruses, there are few data on resistance patterns of these viruses and response to antiretroviral therapy (ART).

We considered HIV-1 virological response to ART and genotype in previously untreated children infected with a variety of HIV-1 subtypes in the PENTA 5 trial[1].

PENTA 5 trial design

128 ART-naive children were randomised to ZDV+3TC or ZDV+ABC or 3TC+ABC. 113 had 1 or more stored samples assayed for resistance (n=32, 39 and 42 respectively)[2]. Children with early disease (n=45) were also randomised to receive NFV or NFV placebo (Part A: 24 NFV, 19 NFVp): and children with more advanced disease (n=68) received open label NFV (Part B).

At baseline median CD4% was 22% (IQR 13-29) and HIV-1 RNA was 5.1 $\log_{10}$ copies/ml (IQR 4.5-5.5); 11 children had AIDS at entry.

The children were classified by their plasma HIV-1 RNA profile as:

- **responders**: HIV-1 RNA reached <400 c/ml before 36 weeks and remained <2000 c/ml subsequently
- **non-responders**: HIV-1 RNA always >2000 copies/ml (or first reached <400 c/ml after 36 weeks)
- **rebounders**: HIV-1 RNA reached <400 c/ml before 36 weeks with subsequent rebound to >2000 c/ml

Resistance substudy design

Phenotype and genotypic resistance-conferring mutations were assayed by VIRCO, using the recombinant virus assay(Antivirogram) and ABI sequencing. The recommended lower limit for HIV-1 RNA for these resistance tests was 2000 c/ml. HIV-1 subtype was determined from the pol gene sequence generated by the resistance assay.

All tests which failed initially were repeated, and thus failure rates correspond to failure on two tests. For samples with failed tests, subtype was determined from previous or subsequent assays for the same child.

Plasma HIV-1 from all children was assessed at baseline. Plasma HIV-1 from children classified as non-responders was assayed at 24 and 48 weeks, and plasma HIV-1 from children classified as rebounders was assayed at the time of rebound in addition to 24 and 48 weeks (if after rebound).

Some samples were not analysed due to insufficient material or because the sample was collected after the end of the main study (30 October 1999).

Plasma HIV-1 from 79 children was analysed for resistance-conferring mutations after baseline. All 79 children had remained on trial drugs (two of ZDV, 3TC, ABC) with or without NFV. Fifty-eight (73%) tests were performed while children were still taking therapy as randomised.

References

1. PENTA 5. A randomised trial evaluating three NRTI regimens with and without nelfinavir in previously untreated HIV-infected children: 48 week follow-up from the PENTA 5 trial. Lancet 2002 (in press)

2. Loveday C on behalf of the PENTA Virology Committee. Evolution of drug resistance in antiretroviral therapy-naive children in PENTA 5. Fifth International Workshop on HIV Drug Resistance and Treatment Strategies, 4-8 June 2001, Scottsdale, Arizona. *Antiviral Therapy* 2001; 6 (Suppl 1) Abstract 109

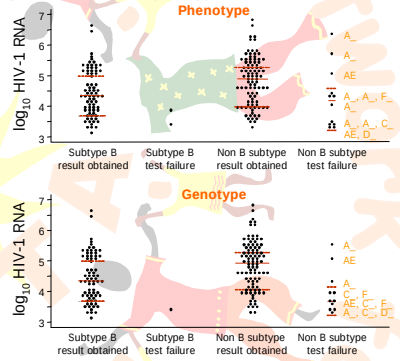
3. Kaye S on behalf of the PENTA Virology Committee. Drug resistance in a trial of nucleoside-analogue and protease inhibitor therapy in children (PENTA 5). Fifth International Congress on Drug Therapy in HIV Infection. *AIDS* 2000; 14 (Suppl 4) Abstract P354

Resistance tests performed

190 tests were performed (111 at baseline) with an overall assay failure rate of 8% (16 tests) and 9% (17 tests) for phenotypic and genotypic repeat testing respectively.

- genotype was not obtained in 5%, 10% and 22% of samples from children from white, black and other ethnic groups (p=0.05) (phenotype p=0.07)
  - 4 of the 5 children for whom HIV-1 subtype could not be inferred because all their resistance tests failed were non-white
- subtype and ethnicity were highly confounded; so failure rates also varied substantially by subtype for genotype (p=0.06) and phenotype (p=0.02)
- however, assay failure rate depended both on HIV-1 RNA and on ethnicity or subtype (Figure 1), and decreased as HIV-1 RNA increased
- overall, there was increased chance of assay failure for non-B subtypes after adjusting for $\log_{10}$ HIV-1 RNA (genotype OR=6.0 [95% CI 1.21-29.8] p=0.03; phenotype OR=17.5 [1.78-172.8] p=0.01)
- the chance of failing resistance tests varied across the non-B subtypes, but there was no statistical evidence for heterogeneity (genotype p=0.93; phenotype p=0.17). The numbers of specific non-B subtypes were small.

Figure 1: HIV-1 RNA by subtype and status of resistance test



Note: solid line denotes within group median, dashed lines IQR

Table 1: Virologic response to ART by subtype

		Change in $\log_{10}$ RNA from randomisation to week 24 Mean (SE)		to week 48 Mean (SE)	
Children with subtype B					
ZDV+3TC		-1.27	(0.37)	-1.78	(0.43)
ZDV+ABC		-1.89	(0.36)	-2.14	(0.42)
3TC+ABC		-2.10	(0.40)	-2.70	(0.46)
Effect of subtype†	N				
AE / AG	5 / 8	-0.06	(0.43)	-0.02	(0.50)
A	16	+0.04	(0.39)	+0.49	(0.44)
B†	44	0		0	
C	17	-0.10	(0.37)	+0.17	(0.42)
D	10	-0.36	(0.45)	-0.30	(0.52)
F / G / H	5 / 2 / 1	-0.01	(0.49)	+0.41	(0.60)
Heterogeneity		p=0.99		p=0.93	

† compared to reference category subtype B; subtype effects assumed common to all NRTI groups

HIV response to ART

The number of children infected with HIV-1 subtypes A, B, C, D, F, G, H, A/E, and A/G were 16 (15%), 44 (41%), 17 (16%), 10 (9%), 5 (5%), 2 (2%), 1 (1%), 5 (5%) and 8 (7%) respectively. Subtype could not be determined for 5 children.

Overall, among the 113 children with resistance tests the mean decrease in HIV-1 RNA was 1.72, 2.05 and 2.61 $\log_{10}$ copies/ml at 48 weeks in the ZDV+3TC, ZDV+ABC and 3TC+ABC groups respectively, adjusting for baseline factors and estimated in the absence of NFV (global p=0.03).

- there was no evidence that HIV-1 subtype was associated with poorer virological response, either in terms of change in HIV-1 RNA to 24 and 48 weeks, or the proportion with HIV-1 RNA below 400 or 50 copies/ml at 24 and 48 weeks, either in all patients (Table 1) or those receiving NFV.
- there was no evidence for a difference between B and non-B subtypes in the relative efficacy of the dual NRTI combinations (p=0.66 and p=0.96 at 24 and 48 weeks respectively).
- there was no evidence that the presence of specific PI polymorphisms at baseline was associated with poorer virological response either in all patients (Table 2) or those receiving NFV.

Patterns of primary mutations

No child had viruses containing primary resistance mutations in protease or reverse transcriptase (RT) at baseline[3].

- the primary PI resistance mutations emerging included L90M, D30M, N88D and N88S. Absolute numbers of mutations were small and no differences were observed between subtype B and non-B viruses:
  - L90M (2/18 versus 3/22 respectively, p=1.00)
  - D30M (1/18 versus 3/22 respectively, p=0.61)
  - N88D (0/18 versus 2/22 respectively, p=0.49)
- M184V was the most prevalent RT resistance mutation, detected in children infected with all HIV-1 subtypes except AG (11/18 versus 15/22 for subtype B versus non-B respectively, p=0.74).
- L74V, 215Y/F, K65R, K70R and V115F mutations were observed at much lower frequencies than M184V. Of note, the development of K65R in 3 patients receiving ABC (all 3TC+ABC) was only observed in subtype B viruses (3/18 versus 0/22; subtype B versus non-B, respectively, p=0.08), although 45%, 43% and 36% children were infected with subtype B in the ZDV+3TC, ZDV+ABC and ABC+3TC groups respectively (p=0.68).

Table 2: Virologic response to ART by baseline PI polymorphisms

		Change in $\log_{10}$ RNA from randomisation to week 24 Mean (SE)		to week 48 Mean (SE)	
Children with no PI polymorphisms at baseline					
ZDV+3TC		-1.05	(0.38)	-1.84	(0.46)
ZDV+ABC		-1.53	(0.38)	-1.96	(0.45)
3TC+ABC		-1.80	(0.39)	-2.54	(0.46)
Effect of polymorphism‡	N				
no PI polymorphisms‡	25	0		0	
M36I without L10V/I	45	-0.64	(0.31)	-0.37	(0.37)
L10V/I without M36I	8	-1.03	(0.51)	-0.17	(0.60)
M36I and L10V/I together	12	-0.08	(0.42)	-0.47	(0.50)
V77I	16	-0.78	(0.36)	-0.91	(0.43)
K20M/R	11	-0.40	(0.41)	-0.27	(0.49)
A71T/V	4	+0.66	(0.64)	+0.39	(0.77)
Heterogeneity		p=0.54		p=0.85	
Interaction between M36I and L10V/I*		p=0.09		p=0.95	

‡ compared to reference category no PI polymorphisms at baseline (multivariable model containing all 5 polymorphisms); subtype effects assumed common to all NRTI groups * no other interactions significant at p<0.20

Patterns of secondary mutations

Of the total 173 samples assayed with a genotype result, 125 (72%) had 1 or more secondary PI mutations at positions 10, 20, 36, 71 or 77 (compared to the consensus sequence for subtype B virus).

- at baseline, 79 of 104 children (76%) had 1 or more of these mutations. Non-B subtypes were more likely to have secondary PI mutations (p<0.001) of which M36I was the most prevalent (82%). Conversely, M36I was least prevalent in subtype B viruses, present in only 7/43 (16%). The most prevalent secondary mutation in subtype B viruses at baseline was V77I, present in 9/43 (21%) of viruses.
- the proportion of strains acquiring these mutations after ART was very low
  - most striking was the emergence of V77I in 4/17 subtype B viruses which were wildtype at this position at baseline (Table 3). However, only 2 of the 4 children had taken NFV before acquiring V77I, both non-responders who also developed other secondary PI mutations during NFV therapy (A71V and M46I respectively). The other 2 children had only ever received placebo NFV and V77I was observed at the time of HIV-1 RNA rebound at weeks 23 and 30 respectively, suggesting that this mutation was already present within the plasma quasispecies at baseline, although at undetectable levels.

Table 3: New secondary PI polymorphisms after baseline in children receiving NFV before resistance testing (n=29)

Subtype	M36I	L10V/I	V77I	K20M/R	A71T/V
AE					
AG			0/1	0/1	0/1
A_		0/6	0/7	0/6	0/6
B_	0/11	0/11	2/13 (15%)	0/13	1/12 (8%)
C_	0/2	0/5	0/5	0/5	0/5
D_	0/1	0/2	0/1	1/2 (50%)	1/2 (50%)
F_		0/1	0/1		0/1
G_				0/1	0/1
Total	0/14 (0%)	0/25 (0%)	2/28 (12%)	1/28 (4%)	2/28 (7%)

Summary

- ★ Virological response to nucleoside analogues with or without NFV was not influenced by virus subtype
- ★ Failure rates for resistance testing may be higher with non-B subtype viruses
- ★ The selection of the K65R mutation by abacavir may be less common in non-B viruses but this requires further studies

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