

# Which antiretroviral agents should be included in the second line regimen for antiretroviral treatment in children?

Primary Reviewer: **Beatriz Larru**<sup>1</sup> Secondary Reviewer: **Elizabeth Molyneux**<sup>2</sup> First published online: 11th November 2009

<sup>1</sup> Hospital General Universitario Gregorio Marañón, Madrid, Spain

<sup>2</sup> College of Medicine, University of Blantyre, Malawi

The World Health Organization has produced guidelines for the management of common illnesses in hospitals with limited resources. This series reviews the scientific evidence behind WHO's recommendations. The WHO guidelines, and more reviews are available at [http://www.who.int/child-adolescent-health/publications/CHILD\\_HEALTH/PB.htm](http://www.who.int/child-adolescent-health/publications/CHILD_HEALTH/PB.htm)

**This review addresses the question:** *Which antiretroviral agents should be included in the second line regimen for antiretroviral treatment in children?*

The WHO Pocketbook of Hospital Care for Children recommends that the second-line treatment regimens should include; Abacavir (ABC) plus Didanosine (ddI) plus Protease Inhibitors: Lopinavir/ritonavir (LPV/r) or Nelfinavir (NFV) or Saquinavir/ritonavir (SQV/r) if weight  $\geq 25$  Kg. (page 214)

Introduction:

More than 90% of the 2.3 million HIV-infected children worldwide at the end of 2006 reside in sub-Saharan Africa (SSA)[1]. Without treatment, more than one-third will die during the first year of life and half will not survive to their second birthday[2]. This devastating scenario has mobilised resources to increase access to effective antiretroviral treatment for children in resource poor settings (RPS)[3]. Though far from achieving universal access, programmes in SSA achieve similar outcomes to those in North America and Europe[1, 4].

Children and infants, harbour unique challenges with regard to antiretroviral treatment (ART). Firstly, they have higher viral loads than adolescents and adults, due to an immature immune system that is less able to control viral replication. Secondly, accurate drug dosing is problematic, particularly in small children where lack of pharmacokinetic data and paediatric formulations reduce the availability of effective antiretroviral drugs and predispose to sub-therapeutic drug levels. And finally, the need for a caregiver to administer the drugs to a child makes it hard to achieve more than 90% adherence throughout childhood[5].

These factors make children and infants more susceptible to virological failure with the subsequent risk of developing drug resistance[6]. Providing that there is not a complete viral suppression, the high replication capacity of the HIV favours the development of drug-resistant viruses, especially under the selective pressure of antiretroviral drugs. The mutations that confer resistance to ART can be identified by genotypic or phenotypic assays. The former are preferred in the clinical practise because their faster results, less complicated techniques and lower cost.

The initial optimism after encouraging results from using first-line HAART in children in resource poor settings needs to be

balance against the knowledge acquired from treating the infection in industrialised countries for the last twenty years; namely that failure to first-line drugs is inevitable in a proportion of children[7, 8]. This means that a well considered second-line regimen has to be available so as to optimise the effectiveness of the current treatment options.

The objective of this review is to compile the evidence available to design an adequate second line HAART regimen for children in poor resources settings and compare this with the current WHO Guidelines.

## METHODOLOGY

Articles were identified through PubMed by using the "Clinical Queries" framework. The search strategy used was "HIV AND (child\* OR pedia\* OR paedia\*) AND resistance AND antiretroviral treatment AND (Africa OR resource poor setting)". This yielded 57 articles, of which 48 were selected by their titles. Twenty-nine of these articles dealt with resistance after single dose Nevirapine (NVP) for prevention-to-mother-to-child-transmission (PMTCT) and were not considered. Citations listed in the nineteen articles were hand-searched, abstracts retrieved and read and articles checked for citations using the Cited Reference Tool on the Web of Science. This produced fifteen further articles, including eleven observational studies and four reviews. All these articles were focused on developing countries. Selected articles were restricted to the English language.

A similar strategy was adopted to search the Cochrane Library and a further five reviews were retrieved but were not considered as they only include data from adults patients.

The quality of selected articles was assessed using the Oxford Centre for Evidence-Based Medicine Levels of Evidence framework.

## RESULTS & DISCUSSION

### Risk factors for children failing first line HAART

Antiretroviral treatment in children achieves less viral suppression than in adults and adolescents[5]. In a comprehensive review of ART programmes in children in resource poor settings, Sutcliffe et al. showed that viral suppression was only maintained in 50% of children at 24 months, 47-83% at 36 months and 45% at 42 months[1].

Risk factors associated with virological failure in RPS are similar to those identified in industrialised countries. Gody et al. showed in 52 children in Central African Republic that viral load

at 6 months of ART was inversely related to adherence levels ( $r=0.6970$ ;  $p<0.0001$ )[9]. Adjé-Touré et al. reported in 134 children in Côte d'Ivoire that low CD4 cell count at baseline and previous treatment with dual therapies were associated with the development of drug resistance[10]. Kanya et al. reported increased resistance in 250 children in Uganda with male gender (OR=2.44, 95%CI: 1.20 to 4.93), baseline CD4% $<5$  (OR=2.69, 95%CI: 1.28 to 5.63) and treatment with d4t/3TC/NVP vs ZDV/3TC/EFV (OR=2.46, 95%CI: 1.23 to 4.90)[11]. Puthanakit et al. also described the latter in 107 children in Thailand, where patients who received EFV based regimens had a higher rate of virological success than those who received NVP- based regimens; the mean decrease in HIV RNA levels at week 72 was  $3.1\pm 1.0$  log 10 copies/mL for those who received NVP-based regimens and  $3.6 \pm 0.5$  log 10 copies/mL ( $p=0.007$ )[12].

Socioeconomic constraints in PRS make ART programmes challenging. Janssens et al. found in 212 Cambodian children that being an orphan was a predictor of virological failure ( $p=0.001$ )[13]. Kiboneka et al. also reported that being an orphan was associated with a higher risk of mortality after starting HAART in 770 children in Uganda[4].

### Mutations selected in children failing first-line HAART

NVP and lamivudine (3TC) associated mutations (Y181C and M184V respectively) are most commonly reported; mainly because NVP has been widely used for PMTCT programmes in SSA and most countries include 3TC as a backbone nucleoside reverse-transcriptase inhibitors (NRTIs) in the first-line HAART regimen[14]. Both drugs pose a low genetic barrier to the development of resistance associated mutations. A brief summary of the mutations found in children failing first-line treatment in PRS is shown in Table-1.

The overall frequency of resistant virus among children with virological failure varies by study. Chaix et al. reported that after 11 months of HAART, 23% of children harbour mutations that confer resistance to one or more ARV drugs[15]. This proportion was much higher after 1 year of HAART in Côte d'Ivoire; Adje Toure et al. described 52% of children having at least one resistance mutation in the reverse-transcriptase (RT) and 14% had at least one mutation in the protease (PR). The 1-year cumulative probability for developing any class of drug resistance was 0.44 (95% CI, 0.35, 0.53)[10]. The different definitions of virological failure, previous exposure to HAART and viral subtypes make it difficult to compare results between studies. Nevertheless, all of them have shown that the longer a child remains with virological failure the higher the risk of developing resistance mutations.

In a recent systematic review of clinical trials Gupta et al. have reported this risk to be even higher with nonnucleoside reverse-transcriptase inhibitors (NNRTs); 35.3% of the patients with virological failure harbour the M184V mutation at week 48 and 5.3% the K65R. When boosted protease inhibitors (bPI)-based initial regimens are used, there is less resistance within and across drug classes; 21.0% of those with virological failure had the M184V mutation at week 48 and 0.0% the mutation K65R.[16].

Once resistance has developed to a drug or drug class, resistant quasispecies of the virus remains indefinitely in lymphoid tissues, compromising the efficacy of further treatments. Lwembe et al. have showed this long-term persistence of NNRTI-resistance in 12 vertically HIV-infected Kenyan children who experienced virologic failure after 24 months of ART. They had the K103N mutation detectable after 7 years without

ART[17]. The undesirable effects of single dose NVP as PMTCT on future ART in children is under evaluation, and if proven will complicate further the choice of ART therapies in children[18].

### Outcome of children on second-line HAART

Little evidence is available about second-line regimens after failure of first-line NNRTI-based therapy in children in developing countries. This is because PI-containing regimens, even if administered as a single agent, are considerably more expensive than NNRTIs and, across SSA, there is limited access to laboratory monitoring to detect virologic failure[19-21]. Renaud-Théry et al. reported in a multi-country survey in PRS that only 8% of all the patients receiving ART were less than 15 years old of whom 99% were on first-line therapies and only 1% was receiving second line regimens[22].

Few studies have described the switch to second-line regimens among children receiving ART. One study conducted by Van Grinsven et al. in Rwanda showed that 2 of 315 children were started on second-line after 2 years of HAART initiation[23]. However, according to our literature review, there is no data available for the outcome of children on second-line PI therapies in RPS. Pujades-Rodriguez et al. describe the use of second-line PI regimens in Médecins San Frontières HIV programmes in adults, that show a median CD4 gain of 90 cells/ $\mu$ L at 6 months and 135 cells/ $\mu$ L at 12 months with a higher risk of death among those with severe immune-depression (CD4  $<50$  cells/ $\mu$ L or WHO stage IV)[24].

### Selection of second-line regimens

As Elliot and colleagues mentioned in a recent review of ART in PRS, the choice of second-line drugs depends on the ARV drug used in the first-line regimen, the length of time that patients have had virological failure and the drug options available to use in a second-line regimen[25]. WHO currently recommends NNRTI-based regimens, as first line ART for children so in this review only a child failing with a NVP or EFV based regimen will be considered.

A brief summary of the different pathways and significance of HIV resistance mutations will help explain the choice of further ART.

When viral replication is not suppressed with AZT or d4T containing regimens a group of mutations known as TAMs (Thymidine Analogue Mutations) are commonly selected along one or two pathways: TAM1 (M41I, L210W and T215Y) or TAM 2 (D67N, K70R, T215F and K219Q/E). The type 1 TAMs have been associated with the use of d4T with another ARV drug with low genetic barrier (such as 3TC), and this has a greater impact on the future susceptibility of Tenofovir (TDF) than type 2 TAMs[8, 25].

Another common mutation associated with resistance to 3TC or emtricitabine (FTC) is the M184V mutation. The presence of M184V has been reported to increase susceptibility to AZT or TNF and reduce the accumulation of TAMs, as shown by Averbuch et al. in 55 Ethiopian children infected with the subtype C virus[26]. This observation has led to the recommendation of maintaining a thymidine analogue + 3TC-based regimen in children with incomplete viral suppression. Other options are limited, as this effect may be transient and further mutations may be acquired leading to broad NRTIs resistance[5].

**Table-1: Summary of HIV resistance mutations found in children failing first line-HAART in developing countries**

Author	Country	n	Subtype	First line	Length of ART	RT mutations	PR mutations
Tebit et al.[31]	Burkina Faso	75 [median age=33 y (range 9-61)]	CRF06_cpx (48%) and CRF02-AG (40%)	AZT/d4T + 3TC + EFV (68%)	48 months (random selection of patients on treatment failure)	M184V (57.3%), M41L (37.3%), D67N (34.6%), K70R (14.6%), L210W (34.6%) T215F/Y (48%), K103N (44%), Y181C/I (16%), Y188C/L (10.6%)	M46I (37%), I54V (26%), V82A/T/F (30%), I84V (18.6%), L90M (18.6%)
Gody et al.[9]	Central African Republic	52 (median age= 7.9y (range 1-16y))	CRF11_cpx (38%), CRF01_AE (15%), subtype A1 (12%), CRF02_AG (12%),	3TC + d4T + NVP (65%) *10 children received IND	6 months	M184V (69%), Y181C (64%)	* M46I (10%), V82F (10%), L90M (10%)
Janssens et al.[13]	Cambodia	37 out of 212 (mean age 6y IQR= 4.2-7.9)		3TC + d4T + NVP (69%) d4T + 3TC + EFV (19%) AZT + 3TC + NVP (12%)	16.8 months (IQR= 13.9 - 21.2)	M184V (72.2%), D67N 19.4%), T215Y/F (19.4%), T69D (11.1%), L210W (11.1%)	
Pillay et al. [32]	South Africa	39 children [median age 6y, range 4m-16y] and 26 adults	HIV-1 subtype C	3TC (38%), d4T (65%), ddi (49%), and AZT (31%), EFV (28%) RTV (26%), NFV (10%)	2 year (random selection of patients on treatment failure)	M184V/I (37%), D67N (32%), T215Y/F (25%), K70R (21%), M41L (20%), K219Q/E (14%), K65R (14%), K103N (25%), V106M (20%), G109A (17%)	V82A/T (12%), M46I (11%), L90M (8%) D30N (3%), V32I (1%)
Chiax et al. [15]	Côte d'Ivoire	38 children [median age= 6.35 y (range 1.2 -15y)]	CRF02-AG (95%)	AZT/d4T/3TC/ddi + NFV* (70.5%) or EFV (29.5%)	10.2 months	M184V (42%), K103N (16%)	*90M, 46L, 88S, 54V (38% of children with exposure to NFV)
Puthanakit et al. [12]	Thailand	14 out of 107 (mean age= 7.7y (range, 2.1-13.8))		3TC + d4T + NVP (13 children) / EFV (1 child)	18 months	M184V (100%) K103N (21.4%) Y181C (35.7%) T215F (21.4%) Q151M (14.3%)	
Kamya et al. [11]	Uganda	57 out of 250 (mean age 9.2, SD:4.5) [8 samples for genotypic resistance test]		3TC + d4T + NVP (26%) / EFV (5.6%) or ZDV + 3TC + NVP (13%) / EFV (55%)	12 months	K103N (62.5%), M184V (100%), T215Y (25%)	

(ART: Antiretroviral Treatment; RT: Reverse- Transcriptase; PR: Protease; AZT: Zidovudine, d4T: Estavudine; 3TC: Lamivudine; EFV: Efavirenz; NVP: Nevirapine; RTV: Ritonavir; NFV: Nelfinavir)

Finally, the K65R mutation has been associated with the use of tenofovir (TNF), ddi, ABC and more rarely d4T. Once K65R is selected, the further development of TAMs or L74V mutation (classically associated with ddi or ABC use) is hindered. Susceptibility to ZDV is increased in the presence of K65R (and/or M184V). This supports the recommendation for using a thymidine analogue drug (AZT or d4T) after TNF failure[27].

For all these reasons Elliot et al. recommend that regimens containing AZT + 3TC/FTC may preserve more residual efficacy than regimens containing d4T and TNF[25]. Unfortunately, the Federal Drug Agency (FDA) does not approve the use of TDF in children younger than 10 years old so other NRTIs such as ddi and ABC have to be considered as second line drugs in children[1]. Elliot et al mention that the presence of M184V and TAMs has lead to increased resistance to ABC and, to a lesser degree to ddi[25].

Once resistance to AZT/d4T or 3TC has developed it is likely that further accumulation of TAMs will reduce the susceptibility of ddi and ABC, leaving fewer options for paediatric second-line therapies[28]. A low switch threshold for second-line regimens is advised to preserve further drugs and in older children the role of TNF as part of the second-line regimens should be reconsidered.

Both EFV and NVP have high cross-resistance so once patients fail NNRT-containing regimens it is likely that only a PI-containing regimen will be effective[19]. The concomitant use of low dose RTV with another PI (boosted PI) has shown excellent results in high-income countries and should be recommended as second-line regimens in RPS[25]. LPV/r is the only co formulated PI, it requires no refrigeration and there is pharmacokinetic data in early infancy available. This underscores the reasons for WHO to recommend using LPV/r as

the bPI for second-line regimens. However, no comparative trials between different bPI among children on second-line HAART have been conducted in RPS.

The impact that different HIV-subtypes (clades) might have on the selection of resistance mutations in the RT and PR genotype has been assessed in several studies. Preliminary data suggest that global surveillance should focus on known subtype B drug-resistance mutations, but recent reports have given contradictory results[29, 30]. Gody et al. observed that in children infected with HIV CRF11\_cpx, natural polymorphisms that may reduce the susceptibility to SQV[9]. Tebit et al. in Burkina-Faso also described how NFV in children infected by CRF06\_cpx showed higher level of resistance after selecting M46I and L90M than indinavir or lopinavir, making NFV a poor choice for second-line regimens[31].

## SUMMARY

- Children have a higher risk of developing virological failure than adults and adolescents so close monitoring of antiretroviral treatment and low threshold of switching to second-line regimens should be recommended.
- After failing NNRTIs-based regimens, a bPI-based regimen should be considered.
- After failing AZT / d4T + 3TC regimens, ABC and ddI are the most likely drugs to remain active if the virological failure is detected promptly.
- TNF possesses an antagonist resistance pathway than thymidine analogues and therefore should be considered as a second line in older children.
- LPV/r is a highly efficacious drug in the second-line regimen in children.

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