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Management of paediatric HIV-1 resistance

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Abstract

Purpose of review—Children have higher rates of virological failure than adults, often associated with more extensive resistance and limited second-line options. In order to maintain clinical benefits of highly active antiretroviral therapy (HAART) into adulthood, particularly for children starting at a young age, strategies are needed to limit the emergence of resistance and to offer highly effective subsequent lines of therapy. Similarly, well resourced settings face challenges regarding extensive resistance accumulated over the past decade or more, particularly resulting from suboptimal therapies.

Recent findings—Rates of resistance at failure of nonnucleoside reverse-transcriptase inhibitor based HAART are higher in developing countries than in well resourced settings. In the latter, second-generation protease inhibitors tipranavir and darunavir are promising, with tipranavir now licensed for those above 2 years and darunavir showing good trial results in children above 6 years. However, combination with new classes such as integrase inhibitors (currently in phase I trials) and CCR5 antagonists (no paediatric data yet) will probably be necessary to gain maximal long-term benefits.

Summary—Common goals in paediatric HIV for both resource-rich and resource-limited settings are to limit vertical transmission, minimize emergence of resistant viruses in both mother and child where prevention of mother-to-child transmission fails, and limit resistance in children starting HAART. Optimal sequencing of regimens in the absence of resistance testing is a priority research area. Paediatric studies using newer classes of agents are of paramount importance, as well as expanding access to existing antiretrovirals.

Keywords

antiretroviral; children; HIV; resistance

Introduction

Highly active antiretroviral therapy (HAART) has demonstrated impressive reductions in morbidity and mortality in children [1-5]. According to international guidelines [6-8], the treatment of choice for HIV-1-infected children and adults is a combination of two nucleoside reverse-transcriptase inhibitors (NRTIs) (backbone treatment) and a third agent from a different class, either a nonnucleoside reverse-transcriptase inhibitor (NNRTI) or a ritonavir-boosted protease inhibitor. Although there are currently 22 FDA-approved antiretrovirals (ARVs) available for use in the treatment of HIV-infected adults and

adolescents, only 14 of these drugs are approved for use in children, and 13 of these are available in paediatric formulations (Table 1).

Children, and infants in particular, are a unique population with regards to HIV infection; they are at greater risk of developing resistance for a number of reasons. First, younger children have higher viral loads [9,10] as compared to adolescents and adults. Second, there are challenges to accurate dosing, particularly in smaller children due to formulation limitations increasing the potential for subtherapeutic drug concentrations, viral replication and resistance. In a UK cohort study, children were found to be underdosed for 6–62% of patient-time at risk [11]. Failure to account for ongoing growth and failure to adjust dosing following changes to recommendations were cited as important contributors to the under dosing. Third, there is a complex landscape of psychosocial factors that affect adherence to medication throughout childhood and into adolescence.

Unsurprisingly perhaps, studies have demonstrated only modest virological responses to HAART in children [12]; a recent large multicentre European cohort collaboration study reported a lower probability of virological response in children aged 6–12 years (adjusted hazard ratio 0.87) and 13–17 years (0.78) after starting first-line HAART, compared with adults [13•] (note children <6 years of age were not included in the study). Although the prevalence of resistance at viral failure following first-line therapy has not been directly compared between adults and children, small studies from resource-poor settings suggest that the emergence of resistance at viral failure is similar in children and adults when NNRTI is used as the third agent [14,15•,16,17,18•,19•]. In the context of higher viral failure rates, particularly in younger children [14,15•,19•], this translates into higher resistance in the paediatric population as a whole.

Resistance is a greater challenge in children, as they require longer-term therapy as compared to adults. Any analysis of paediatric HIV-1 resistance needs to address two groups; first, those patients in high-income and middle-income countries where there is access to frequent viral load monitoring, genotypic resistance testing (GRT) and a greater range of ARVs including investigational agents. The second group in the resource-poor world bears the vast majority of global HIV-1 disease burden; these patients generally have no or very limited viral monitoring or GRT, and ARV options are limited to two lines at most (presently not including tenofovir). In this review, we explore current knowledge of resistance in diverse settings, sequencing of regimens including salvage, use of resistance testing, management of transmitted resistance and prevention of mother-to-child transmission (PMTCT) associated resistance.

Resistance following first-line nonnucleoside reverse-transcriptase inhibitor containing highly active antiretroviral therapy

First-line HAART consisting of a thymidine analogue, lamivudine (3TC) and nevirapine (NVP) or efavirenz (EFV) is used in the vast majority of children worldwide, due to availability of cheap generic fixed dose combinations (FDCs). In particular, stavudine (d4T), 3TC and NVP are used, with the advantage of WHO-approved child and infant FDC preparations (Pedimune Junior and Pedimune Baby), which have a higher concentration of NVP in relation to NRTIs compared with their adult counterparts. In Cambodia [18•], high rates of resistance have been found in patients with a median age of 6 who were failing ART (mainly d4T, 3TC and NVP in adult formulations); after 1 year, 76% had M184V conferring high-level resistance to lamivudine, 94% had high-level NNRTI resistance, 11% acquired K65R conferring resistance to all NRTI except zidovudine (AZT), and 11% developed L74V conferring resistance to abacavir (ABC) and didanosine (ddI). Similar resistance to lamivudine and NNRTI has also been seen in Thai and West African studies [15•,16,19•],

although prevalence of K65R and L74V was lower. These findings are similar to those found in adults under similar monitoring conditions [20,21•]. It is becoming apparent that stavudine selects K65R, L74V and Q151M [15•,20,21•], contributing to broad NRTI resistance in a proportion of patients who are not monitored by 3 monthly HIV-1 RNA viral load testing. Notably, the evidence that tenofovir (TDF) loses activity in the face of any of these mutations is weak, and largely based on *in-vitro* phenotypic data rather than clinical response data. Unfortunately, tenofovir is expensive and not currently available as second line in most parts of the developing world. Furthermore, it is not yet licensed for children (phase II trial ongoing), although it is increasingly being used as a salvage drug or as part of Truvada for first-line ART when adult doses can be used in adolescents.

We can conclude therefore that most patients failing NNRTI-based HAART in developing world settings will have high-level resistance to lamivudine and NNRTI. The recommended second-line regimen of ddI/ABC or AZT/boosted protease inhibitor should have durable potency in the majority; those with multinucleoside mutations might gain more durable antiviral impact with TDF and AZT. This is due to the fact that K65R confers hypersusceptibility to AZT and bi-directional antagonism exists between thymidine analogue mutations normally selected by AZT [22] and K65R. The currently approved protease inhibitors for children include lopinavir (LPV), amprenavir (APV), fosamprenavir (fAMP), ritonavir (RTV), nelfinavir (NFV) and tipranavir (TPV). Darunavir (DRV) in children older than 6 years is currently under consideration by the FDA/EMA. As in the case of adults, ritonavir is no longer used alone; instead, it is used to 'boost' other protease inhibitors by virtue of its potent inhibition of CYP4/3A. Boosted atazanavir (ATV) has been used as part of a simplification strategy in protease inhibitor experienced adolescents, but investigators found high rates of virologic failure in those who switched from LPV to ATV [23]. NFV was historically used unboosted, and its use is declining. Difficulties in boosting with separate ritonavir (syrup very unpleasant in taste and 100 mg capsules large) means that in reality only Kaletra (lopinavir and ritonavir combined) is frequently used in preadolescent children.

There are few studies of first-line NNRTI-based HAART in children from settings with viral load monitoring and resistance testing. Mullen *et al.* [24] studied eight children failing NVP-based HAART and six (75%) developing one or more of the K103N, Y181C or G190A mutations. Similarly, Agwu *et al.* [25•] found 80% NNRTI resistance in adolescents on first-line HAART for a median of 102 weeks. These rates are higher than those in adults and probably reflect the delay in switching. In fact, they are similar to resistance rates seen in settings without viral load monitoring, where prolonged virological failure also occurs. Given there is often significant time on a failing regimen, it is important that resistance testing is undertaken as close to the time of switch as possible and that previous resistance test results are also taken into account when deciding on a new regimen.

Resistance following first-line protease inhibitor containing highly active antiretroviral therapy

Resistance profiles at first-line protease inhibitor failure are determined both by the protease inhibitor used, as well as whether ritonavir boosting was used [26]. Certain agents select signature mutations in protease, for example D30N by nelfinavir, I50L by atazanavir and G48V by saquinavir. Other mutations at positions such as 46, 82, 84 and 90 can be selected by multiple protease inhibitors (Table 1).

Unboosted protease inhibitors, especially nelfinavir continue to be used as first-line protease inhibitor, and much of the existing historical data come from children treated with this agent; this is of importance, as choice of HAART regimens need to be considered in the

context of nelfinavir-related resistance, most commonly D30N and L90M. Evidence from adult studies shows that major protease inhibitor mutations are rare following viral failure of ritonavir boosted protease inhibitors, in contrast to unboosted protease inhibitors [26,27•].

Among the 269 HIV-1-infected children enrolled in the ANRS 1278 prospective observational cohort [16] in Côte d'Ivoire, 115 (median age 6.35 years) required treatment and received HAART for at least 6 months. Treatment consisted of two nucleoside analogue reverse-transcriptase inhibitors combined with nelfinavir (70.5%) or efavirenz (29.5%). After a median of 10.2 months of HAART, the 90M, 46L, 88S or 54V mutations were found in 11 (38%) of the 29 children failing on nelfinavir. Second-line therapy for these patients would generally involve switching the third agent to NNRTI or boosted protease inhibitor, in addition to two new NRTI. Alternatively, use of NNRTI and boosted protease inhibitor could be considered.

Saez-Llorens *et al.* [28] studied 100 children with mean age of 5.3 years starting LPV/ritonavir. At 48 weeks, 88 and 58% of naïve and experienced children, respectively, had viral load less than 400 copies/ml. Among the 13 viral failures, M184V was seen in all, with the absence of resistance mutations in protease. Delaugerre *et al.* [29] showed an impressive 100 and 92% of patients with viral load less than 400 and less than 50 copies/ml, respectively, in a small cohort of 21 protease inhibitor naïve children with mean age of 10.3 years after 48 weeks of LPV/r. Persaud *et al.* [30] studied 22 consecutively enrolled infants initiating HAART at a median age of 9.7 weeks of age and treated for up to 96 weeks with AZT or d4T/3TC/boosted LPV. Infants with baseline resistance (mainly to NRTI and NNRTI) were twice as likely to fail boosted LPV therapy, although this result did not reach statistical significance. No protease mutations were seen at viral failure in these patients.

There is evidence from adults that first-line boosted protease inhibitors may lead to lower rates of resistance both to the protease inhibitor itself and to other concomitant ARVs; ACTG 5142 [31•] and a recent meta-analysis demonstrate that M184V, conferring resistance to lamivudine, and K65R are less common in adults treated with boosted protease inhibitor as opposed to NNRTI-based HAART. Agwu *et al.* [25•] reported similar findings in adolescents and if these observations hold true in younger children, first-line boosted protease inhibitors may be beneficial in poorer adherers, given that the plasma half-lives of NRTI and protease inhibitors are similar. Resource-poor settings where drug supplies are erratic and socioeconomic conditions are difficult may also favour such an approach, although this must be balanced against the need for HAART regimens which can be used safely and effectively with anti-TB treatment, and the considerable issues around formulations (Kaletra liquid is expensive, requires refrigeration and is poorly tolerated; Kaletra paediatric tablets are relatively large and cannot be broken due to loss of bioavailability).

Second-line boosted protease inhibitor based therapy following failure of first-line protease inhibitor therapy

There are studies in protease inhibitor experienced children (usually NFV) demonstrating reasonable virological responses to regimens containing LPV/r [32,33], although increasing numbers of previous regimens and resistance mutations reduces response rates to subsequent protease inhibitors. This highlights the potential importance of taking into account GRT and treatment history (including possible in-utero ARV exposure) when deciding on subsequent lines of ART.

Nucleoside reverse-transcriptase inhibitor backbones in first-line highly active antiretroviral therapy

Thymidine analogues have been used widely as part of the public health approach to ART, mainly as a result of cheap fixed-dose combinations. However, these agents may not be ideal in terms of resistance profiles. PENTA 5 was a randomized comparison between ABC/3TC, AZT/3TC and ABC/AZT with or without NFV. Virological response to ABC/3TC was superior to that to ABC/AZT and AZT/3TC, although there was a greater prevalence of resistance to ABC and 3TC with the former regimen [34]. This study also showed no difference in resistance according to subtype [35], although numbers were small. A randomized study into ABC as part of triple nucleoside HAART or with NNRTI is underway in Uganda and Zimbabwe.

Salvage therapies

Salvage has classically applied to patients who have failed multiple lines of HAART. Regardless of the first-line and second-line, in most circumstances, the patient will have been exposed to NRTI, NNRTI as well as protease inhibitor (perhaps unboosted). GRT is important in such circumstances, as long as agents from existing as well as new classes are available. In resource-poor settings, GRT is likely to have limited use in the absence of second-generation protease inhibitors.

Second-generation protease inhibitors

Although second-generation ritonavir-boosted protease inhibitors have been licensed in adults recently, there are few data in children. Salazar *et al.* [36••] conducted a trial of high-dose and low-dose ritonavir-boosted tipranavir in mainly experienced patients with viral loads above 1500 copies/ml, in combination with an optimized background regimen which did not include CCR5 antagonists or integrase inhibitors. Thirteen percentage of the patients received enfurvitide. Nearly half of them were aged 12 and above, with 22% aged 2–6 years and 33% aged 6–12. Median number of previous drugs was seven, and half of the patients exhibited resistance to all protease inhibitors with a similar proportion having a genotypic susceptibility score Genotypic Sensitivity Score (GSS) (the sum of the genotypically sensitive background drugs as determined by a scoring algorithm) of zero. Both oral solutions and capsules were available. At 48 weeks, 39.7% low-dose and 45.6% high-dose TPV/r recipients had viral load less than 400 copies/ml and 34.5 and 35.1%, respectively, achieved viral load less than 50 copies/ml. There was a trend toward improved virological success using the higher dose in older children who also had lower GSS and poorer adherence. Genotypic inhibitory quotient (GIQ), a composite of the TPV mutation score [37] and trough drug levels strongly predicted virological failure, and resistance profiles in those failing therapy were reported to be similar to those found in the adult RESIST studies. The following 21 mutations at 16 positions have been identified as contributing to TPV resistance: 10V, 13V, 20M/R/V, 33F, 35G, 36I, 43T, 46L, 47V, 54A/M/V, 58E, 69K, 74P, 82L/T, 83D and 84V [37].

The other second-generation protease inhibitor, darunavir, was recently studied in a total of 80 heavily treatment-experienced children [38••]. Median age was 14 years with a median of nine previous ARVs. Darunavir/ritonavir was dosed according to weight. Optimized background therapy (OBT) was added on the basis of genotypic resistance testing, although very few were given the fusion inhibitor enfurvitide. After 48 weeks, by intent-to-treat analysis viral load fell below 400 copies/ml in 59% and below 50 copies/ml in 48%. Response rates were reduced by the number of preexisting darunavir-associated mutations (V11I, V32I, L33F, I47V, I50V, I54L, I54M, G73S, L76V, I84V and L89V): viral load

suppression to below 50 copies/ml was achieved in 59% of those with no darunavir resistance mutations at baseline, 47% of those with one or two and none of those with three or more. As with TPV, mean increases in CD4 cell counts were impressive, and darunavir drug exposure levels were comparable to those seen in adults. Results may not be as good as expected, potentially due to difficulties adhering to separately dosed ritonavir (as syrup or 100 mg capsules), the lack of a second highly effective agent in the regimen and the limited use of EFV as part of OBT in these studies.

Dual boosted protease inhibitor therapy

A dual-boosted protease inhibitor strategy has been studied by Kosalaraksa *et al.* [39•] in patients failing standard NRTI/NNRTI regimens in the absence of resistance testing and access to second-generation protease inhibitor. They conducted a study in Thailand in 50 children and used dual boosted protease inhibitor therapy with SQV and LPV together with low-dose ritonavir. At 48 weeks, the median CD4% rise was nine [interquartile range (IQR) 5–16] and median HIV RNA reduction was $-2.8 \log_{10}$ (IQR -3.2 to -1.4), both $P < 0.001$. Thirty-nine (78%) and 32 (64%) children had viral load less than 400 and less than 50 copies/ml. Five children (10%) had viral load failure associated with poor adherence and no major protease inhibitor mutations were seen. Median serum cholesterol and triglyceride increased significantly and mean minimum plasma concentrations ($C_{\min}$) of LPV and SQV were well above the therapeutic level. Robbins *et al.* [40•] have demonstrated the safety of high-dose lopinavir/ritonavir (400/100 mg)/m² in combination with saquinavir in 16 treatment-experienced children with median age of 15 years, albeit with modest virological outcomes. Dual boosted protease inhibitor could be used in patients with extensive NRTI resistance, although further efficacy data are needed to establish whether this strategy offers significant advantage over boosted LPV.

Resistance testing

Genotypic resistance testing following virological failure in adults has been shown to lead to modest virological benefits [41,42]. PENTA 8 [43] was a randomized trial of GRT vs clinician-guided switch in virologically failing children, and this study showed no virological or immunological benefit at 96 weeks. However, this finding should be interpreted with caution, as second-generation protease inhibitors and tenofovir were not available. In the absence of highly potent subsequent lines of therapy, GRT would not be expected to show virological benefit in patients with extensive resistance.

Indirect evidence for the potential utility of GRT comes from retrospective salvage studies where response can be related to the number of protease inhibitor mutations at baseline. In 45 triple class experienced patients, Ramos *et al.* [33] had an ITT response rate of 42% achieving viral load less than 400 copies/ml using LPV/r in combination with OBT. Nonresponders had 7.0 protease inhibitor associated mutations at baseline compared with 4.8 in children achieving virologic suppression ($P = 0.06$). Delaugerre *et al.* [29] found that viral failure with LPV was significantly higher when the children were previously treated with protease inhibitors and when the baseline LPV mutation score exceeded three mutations. GRT could be used in such patients to predict probability of response to boosted protease inhibitor in those with unboosted protease inhibitor experience. In the context of availability of second-generation protease inhibitors, GRT may have a use in deciding which children are treated with TPV vs DRV, as both have a different susceptibility scoring system based on GRT in adults.

Limitations of population GRT include the fact that minority species are not detected below a threshold of 20–30%. Studies suggest that GRT underestimates the prevalence of resistant species by as much as 50% [44–46], although the clinical impact of this is less clear. A

further potential limitation pertains to failure with protease inhibitor. It is increasingly appreciated that mutations in the protease substrate, Gag, can also lead to resistance and/or restoration of replicative capacity [47,48], and have been seen in the absence of protease mutations [49]. Currently, *gag* is not sequenced and, therefore, this information is not available to estimate an isolate's susceptibility to protease inhibitor. Resistance encoded in *gag* may be important following failure of boosted protease inhibitor, where major protease mutations are rarely seen.

Larru *et al.* [50] recently assessed the utility of virtual phenotype resistance testing by performing a retrospective analysis of heavily experienced children with median age 15 years who had at least 6 months follow-up after initiating salvage therapy. The patients with virological response had a mean number of susceptible drugs according to the lower clinical cut-off of 1.7 vs 0.8 ($P=0.03$) in those who did not have a response; for the upper clinical cut-off, the predicted mean number of susceptible drugs was 2.7 vs 1.3 ($P=0.01$). These data reinforces current consensus that patients should be switched for treatment failure when there are at least two agents to which the virus is fully susceptible. However, it should be noted that virtual phenotype suffers similar limitations regarding minority resistant species when based on population sequencing data.

Transmitted resistance

In developing world settings, the prevalence of transmitted resistance is still low [51,52]. However, this seems to be changing in middle-income and high-income countries. Studies from Brazil and Argentina [53,54] have reported primary resistance rates of 27 and 23%, respectively. In USA, Persaud *et al.* [30] reported drug-resistant HIV-1 in five (23.8%) of 21 infants initiating first-line boosted protease inhibitor HAART; four (80.0%) had NNRTI-resistant HIV-1, only one of whom had a history of PMTCP with NVP. The fifth infant had the M184V mutation. In US adolescents, of 55 participants studied by Viani *et al.* [55], major mutations conferring HIV drug resistance were present in 10 (18%). Eight (15%) had NNRTI mutations, two (4%) had NRTI mutations and two (4%) had protease inhibitor mutations. This work suggests that as access to HAART becomes more widespread, the prevalence of transmitted resistance via MTCT or sexual acquisition of HIV infection in teenage years is likely to increase in all parts of the world. Screening at baseline for TDR in well resourced settings should identify such patients for alternative first line. However, in the absence of baseline GRT, rapid allele-specific tests for NNRTI resistance may be needed if NNRTI use as first line is to be continued.

Prevention of resistance and prevention of mother-to-child transmission

Ultimately, management of resistance in children should be aimed at prevention. Effective PMTCT with HAART can reduce vertical transmission to 2% or less (in both well resourced settings [56] and resource-poor settings [57]) using NNRTI-based regimens. Unfortunately, in the latter setting, PMTCT is often still in the form of single-dose nevirapine, despite higher transmission rates and updated WHO guidelines [58].

Resistance in infants exposed to single-dose NVP has been extensively studied, and the prevalence at 4–8 weeks postpartum estimated in a recent meta-analysis at 52.6% [95% confidence interval (CI) 37.7–67.0]. Addition of short course antenatal/intrapartum zidovudine + lamivudine and/or postpartum zidovudine and lamivudine and/or postnatal zidovudine and lamivudine reduced the incidence of resistance to 16.5% (95% CI 8.9–28.3) [59] in the same analysis. HAART during pregnancy offers the best chance of reducing both transmission and resistance in children.

Transmission of HIV infection through breastfeeding is estimated at 10% [60]. Extended prophylaxis with 6 weeks of NVP reduces breast milk associated transmission by around 50% [61••,62••]. In those infants infected in the first 6 weeks of life despite this strategy, the likelihood of NVP resistance has been found to be increased compared with those children who had received sdNVP alone – 92 vs 38% [63] and 84 vs 50% [64•] in two recent studies.

The CHER trial conducted in South Africa [65••] showed that early treatment of infants with boosted protease inhibitors reduced early infant mortality by 76% and HIV progression by 75%. As many of these infants will have been exposed to single-dose nevirapine and have high-level resistance to NNRTI, use of protease inhibitor is appropriate and now recommended by WHO for first-line treatment during infancy following perinatal NVP exposure. Issues remain about how best to use Kaletra in infants, which probably requires higher doses than in adults. The use of boosted protease inhibitor in infants and increasing numbers of patients switching to second line signals an increase in the use of protease inhibitors in resource-poor settings. Although such regimens are currently costly, generic production of combined protease inhibitor/ritonavir is underway. However, more data are needed on the response to boosted protease inhibitor in subtypes prevalent in sub-Saharan Africa, such as A, D and C.

Conclusion

There are specific challenges in treating HIV-infected children, including uncertainty about the best time to start treatment, the need for more paediatric formulations, the lack of pharmacokinetic studies for new drugs and incomplete dosing guidelines. Furthermore, the most appropriate regimen depends on a variety of factors, including the age, appropriate drug formulations the potency, dosing and toxicity.

The combination of these factors makes resistance in children a far more challenging problem. Tenofovir retains activity in a number of resistant strains but is currently not licensed in children due to concerns regarding skeletal toxicity. It is being used in older children, however, due to a lack of alternative agents. Trials using second-generation NNRTI such as etravirine, CCR5 antagonists and integrase inhibitors are urgently needed. Further work on optimal sequencing of regimens is also fundamental to the management of antiretroviral therapy. Future agents targeting viral maturation, those using RNA interference and therapeutic vaccines should also be developed with treatment of paediatric HIV in mind.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

1. Doerholt K, Duong T, Tookey P, et al. Collaborative HIV Paediatric Study. Outcomes for human immunodeficiency virus-1-infected infants in the United Kingdom and Republic of Ireland in the era of effective antiretroviral therapy. *Pediatr Infect Dis J*. 2006; 25:420–426. [PubMed: 16645506]
2. Gibb DM, Duong T, Tookey PA, et al. National Study of HIV in Pregnancy and Childhood Collaborative HIV Paediatric Study. Decline in mortality, AIDS, and hospital admissions in perinatally HIV-1 infected children in the United Kingdom and Ireland. *BMJ*. 2003; 327:1019. Erratum in *BMJ* 2004; 328:686. [PubMed: 14593035]
3. Gortmaker S, Hughes M, Oyomopito R, et al. Impact of introduction of protease inhibitor therapy on reductions in mortality among children and youth infected with HIV-1. *N Engl J Med*. 2001; 345:1522–1528. [PubMed: 11794218]
4. De Martino MM, Tovo PA, Balducci M, et al. Reduction in mortality with availability of antiretroviral therapy for children with perinatal HIV-1 infection. *JAMA*. 2000; 284:190–197. [PubMed: 10889592]
5. Sanchez-Granados J, Ramos JT, Rojo P, et al. Impact of HAART on the survival and disease progression in HIV-1-infected children. *Pediatr Infect Dis J*. 2003; 22:863–867. [PubMed: 14551485]
6. World Health Organization. Antiretroviral Therapy of HIV Infection in Infants and Children in Resource-Limited Settings, Towards Universal Access: Recommendations for a Public Health Approach. 2006 Available at http://www.who.int/hiv/mediacentre/fs_2006guidelines_paediatric/en/index.html
7. Sharland M, Blanche S, Castelli G, et al. PENTA Steering Committee. PENTA guidelines for the use of antiretroviral therapy, 2004. *HIV Med*. 2004; 5(Suppl 2):61–86. [PubMed: 15239717]
8. Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children. Guidelines for the use of antiretroviral agents in pediatric HIV infection. Available at <http://aidsinfo.nih.gov/OrderPublication/OrderPubsBrowseSearchResultsTable.aspx?MenuItem=AIDSinfoTools&ID=115>. [Accessed 12 January 2008]
9. Shearer WT, Quinn TC, LaRussa P, et al. Viral load and disease progression in infants infected with human immunodeficiency virus type 1. Women and Infants Transmission Study Group. *N Engl J Med*. 1997; 336:1337–1342. [PubMed: 9134873]
10. Abrams EJ, Weedon J, Steketee RW, et al. Association of human immunodeficiency virus (HIV) load early in life with disease progression among HIV-infected infants. New York City Perinatal HIV Transmission Collaborative Study Group. *J Infect Dis*. 1998; 178:101–110. [PubMed: 9652428]
11. Menson EN, Walker AS, Sharland M, et al. Collaborative HIV Paediatric Study Steering Committee. Underdosing of antiretrovirals in UK and Irish children with HIV as an example of problems in prescribing medicines to children, 1997–2005: cohort study. *BMJ*. 2006; 332:1183–1187. [PubMed: 16709991]
12. van Rossum AM, Fraaij PL, de Groot R. Efficacy of highly active antiretroviral therapy in HIV-1 infected children. *Lancet Infect Dis*. 2002; 2:93–102. [PubMed: 11901656]
13. Collaboration of Observational HIV Epidemiological Research Europe (COHERE) Study Group. Response to combination antiretroviral therapy: variation by age. *AIDS*. 2008; 22:1463–1473. [PubMed: 18614870] [This paper importantly showed that children do not respond as well as adults to first-line HAART.]
14. Puthanakit T, Oberdorfer A, Akarathum N, et al. Efficacy of highly active antiretroviral therapy in HIV-infected children participating in Thailand's National Access to Antiretroviral Program. *Clin Infect Dis*. 2005; 41:100–107. [PubMed: 15937769]
15. Adjé-Touré C, Hanson DL, Talla-Nzussouo N, et al. Virologic and immunologic response to antiretroviral therapy and predictors of HIV type 1 drug resistance in children receiving treatment in Abidjan, Côte d'Ivoire. *AIDS Res Hum Retroviruses*. 2008; 24:911–917. [PubMed: 18593341] [This work provided data on resistance following first-line failure in a setting without regular viral load monitoring.]
16. Chaix ML, Rouet F, Kouakoussui KA, et al. Genotypic human immunodeficiency virus type 1 drug resistance in highly active antiretroviral therapy-treated children in Abidjan, Côte d'Ivoire. *Pediatr Infect Dis J*. 2005; 24:1072–1076. [PubMed: 16371868]

17. Pillay V, Pillay C, Kantor R, et al. HIV type 1 subtype C drug resistance among pediatric and adult South African patients failing antiretroviral therapy. *AIDS Res Hum Retroviruses*. 2008; 24:1449–1154. [PubMed: 19000027]
18. Janssens B, Raleigh B, Soeung S, et al. Effectiveness of highly active antiretroviral therapy in HIV-positive children: evaluation at 12 months in a routine program in Cambodia. *Pediatrics*. 2007; 120:e1134–e1140. [PubMed: 17954553] [This study provided valuable resistance data on children aged more than 13 years using generic adult stavudine/lamivudine and nevirapine.]
19. Gody J-C, Charpentier C, Mbitikon O, Si-Mohamed A. High prevalence of antiretroviral drug resistance mutations and HIV-1 non-B subtype strains from children receiving antiretroviral therapy regimen according to the 2006 revised WHO recommendations. *J Acquir Immune Defic Syndr*. 2008; 49:566–569. [PubMed: 19202461] [This study demonstrated low virological response rates and high rates of resistance at only 6 months following initiation of HAART.]
20. Sungkanuparph S, Manosuthi W, Kiertiburanakul S, et al. Options for a second-line antiretroviral regimen for HIV type 1–infected patients whose initial regimen of a fixed-dose combination of stavudine, lamivudine, and nevirapine fails. *Clin Infect Dis*. 2007; 44:447–452. [PubMed: 17205457]
21. Marconi VC, Sunpath H, Lu Z, et al. South Africa Resistance Cohort Study Team. Prevalence of HIV-1 drug resistance after failure of a first highly active antiretroviral therapy regimen in KwaZulu Natal, South Africa. *Clin Infect Dis*. 2008; 46:1589–1597. [PubMed: 18419495] [This paper, although looking at adults, reported resistance data on a large number of patients in a resource-poor setting.]
22. Parikh UM, Bachelier L, Koontz D, Mellors JW. The K65R mutation in human immunodeficiency virus type 1 reverse transcriptase exhibits bidirectional phenotypic antagonism with thymidine analog mutations. *J Virol*. 2006; 80:4971–4977. [PubMed: 16641288]
23. Macassa E, Delaugerre C, Teglas JP, et al. Change to a once-daily combination including boosted atazanavir in HIV-1-infected children. *Pediatr Infect Dis J*. 2006; 25:809–814. [PubMed: 16940839]
24. Mullen J, Leech S, O'Shea S, et al. Antiretroviral drug resistance among HIV-1 infected children failing treatment. *J Med Virol*. 2002; 68:299–304. [PubMed: 12226814]
25. Agwu A, Lindsey JC, Ferguson K, et al. Pediatric AIDS Clinical Trials Group 381 Study Team. Analyses of HIV-1 drug-resistance profiles among infected adolescents experiencing delayed antiretroviral treatment switch after initial nonsuppressive highly active antiretroviral therapy. *AIDS Patient Care STDS*. 2008; 22:545–552. [PubMed: 18479228] [This study highlights the resistance implications associated with delayed switch after viral failure in adolescents.]
26. Bartlett JA, Buda JJ, von Scheele B, et al. Minimizing resistance consequences after virologic failure on initial combination therapy: a systematic overview. *J Acquir Immune Defic Syndr*. 2006; 41:323–331. [PubMed: 16540933]
27. Gupta R, Hill A, Sawyer AW, Pillay D. Emergence of drug resistance in HIV type 1-infected patients after receipt of first-line highly active antiretroviral therapy: a systematic review of clinical trials. *Clin Infect Dis*. 2008; 47:712–722. [PubMed: 18662137] [This meta-analysis using data from 8000 patients compares resistance outcomes following boosted protease inhibitor vs NNRTI-based HAART, showing that resistance to both the third agent and NRTI is lower when boosted protease inhibitor are used.]
28. Sáez-Llorens X, Violari A, Deetz CO, et al. Forty-eight-week evaluation of lopinavir/ritonavir, a new protease inhibitor, in human immunodeficiency virus-infected children. *Pediatr Infect Dis J*. 2003; 22:216–224. [PubMed: 12634581]
29. Delaugerre C, Teglas JP, Treluyer JM, et al. Predictive factors of virologic success in HIV-1-infected children treated with lopinavir/ritonavir. *J Acquir Immune Defic Syndr*. 2004; 37:1269–1275. [PubMed: 15385734]
30. Persaud D, Palumbo P, Ziemniak C, et al. Pediatric AIDS Clinical Trials Group P1030 Team. Early archiving and predominance of nonnucleoside reverse transcriptase inhibitor-resistant HIV-1 among recently infected infants born in the United States. *J Infect Dis*. 2007; 195:1402–1410. [PubMed: 17436219]
31. Riddler SA, Haubrich R, DiRienzo AG, et al. AIDS Clinical Trials Group Study A5142 Team. Class-sparing regimens for initial treatment of HIV-1 infection. *N Engl J Med*. 2008; 358:2095–

2106. [PubMed: 18480202] [This randomized trial showed that NNRTI-based and protease inhibitor based regimens were highly efficacious; although viral failure was more common in patients using boosted protease inhibitor, prevalence of resistance was lower in this group.]
32. Resino S, Bellon JM, Ramos JT, et al. Salvage lopinavir–ritonavir therapy in human immunodeficiency virus-infected children. *Pediatr Infect Dis J*. 2004; 23:923–930. [PubMed: 15602192]
 33. Ramos JT, De Jose MI, Duenas J, et al. Safety and antiviral response at 12 months of lopinavir/ritonavir therapy in human immunodeficiency virus-1-infected children experienced with three classes of antiretrovirals. *Pediatr Infect Dis J*. 2005; 24:867–873. [PubMed: 16220083]
 34. Gibb DM, Walker AS, Kaye S, et al. Evolution of antiretroviral phenotypic and genotypic drug resistance in antiretroviral-naïve HIV-1-infected children treated with abacavir/lamivudine, zidovudine/lamivudine or abacavir/zidovudine, with or without nelfinavir (the PENTA 5 trial). *Antivir Ther*. 2002; 7:293–303. [PubMed: 12553485]
 35. Pillay D, Walker AS, Gibb DM, et al. Impact of human immunodeficiency virus type 1 subtypes on virologic response and emergence of drug resistance among children in the Paediatric European Network for Treatment of AIDS (PENTA) 5 trial. *J Infect Dis*. 2002; 186:617–625. [PubMed: 12195348]
 - 36•. Salazar JC, Cahn P, Yogev R, et al. PACTG 1051/BI Study Team. Efficacy, safety and tolerability of tipranavir coadministered with ritonavir in HIV-1-infected children and adolescents. *AIDS*. 2008; 22:1789–1798. [PubMed: 18753862] [An important study using the second-generation protease inhibitor tipranavir in children.]
 37. Baxter JD, Schapiro JM, Boucher CA, et al. Genotypic changes in human immunodeficiency virus type 1 protease associated with reduced susceptibility and virologic response to the protease inhibitor tipranavir. *J Virol*. 2006; 80:10794–10801. [PubMed: 16928764]
 - 38•. Blanche, S.; Bologna, R.; Cahn, P., et al. 48-week safety and efficacy of darunavir/ritonavir in treatment-experienced children and adolescents in DELPHI. Proceedings of the 48th Interscience Conference on Antimicrobial Agents and Chemotherapy; American Society of Microbiology; Washington. 25–28 October 2008; abstract H-894. [An important salvage study using the second-generation protease inhibitor darunavir in children.]
 - 39•. Kosalaraksa P, Bunupuradah T, Engchanil C, et al. HIV-NAT 017 Study Team. Double boosted protease inhibitors, saquinavir, and lopinavir/ritonavir, in nucleoside pretreated children at 48 weeks. *Pediatr Infect Dis J*. 2008; 27:623–628. [PubMed: 18520443] [An interesting study into double boosted protease inhibitor in children.]
 - 40•. Robbins BL, Capparelli EV, Chadwick EG, et al. PACTG 1038 Team. Pharmacokinetics of high-dose lopinavir–ritonavir with and without saquinavir or nonnucleoside reverse transcriptase inhibitors in human immunodeficiency virus-infected pediatric and adolescent patients previously treated with protease inhibitors. *Antimicrob Agents Chemother*. 2008; 52:3276–3283. [PubMed: 18625762] [Important PK data for LPV as part of a single or dual boosted protease inhibitor strategy.]
 41. Durant J, Clevenbergh P, Halfon P, et al. Drug-resistance genotyping in HIV-1 therapy: the VIRADAPT randomised controlled trial. *Lancet*. 1999; 353:2195–2199. [PubMed: 10392984]
 42. Clevenbergh P, Durant J, Halfon P, et al. Persisting long-term benefit of genotype-guided treatment for HIV-infected patients failing HAART. *Viradap study: week 48 follow-up*. *Antivir Ther*. 2000; 5:65–70. [PubMed: 10846595]
 43. Green H, Gibb DM, Compagnucci A, et al. Paediatric European Network for the Treatment of AIDS. A randomized controlled trial of genotypic HIV drug resistance testing in HIV-1-infected children: the PERA (PENTA 8) trial. *Antivir Ther*. 2006; 11:857–867. [PubMed: 17302248]
 44. Metzner KJ, Bonhoeffer S, Fischer M, et al. The Swiss HIV Cohort Study. Emergence of minor populations of human immunodeficiency virus type 1 carrying the M184V and L90M mutations in subjects undergoing structured treatment interruptions. *J Infect Dis*. 2003; 188:1433–1443. [PubMed: 14624368]
 45. Metzner KJ, Rauch P, Walter H, et al. Detection of minor populations of drug-resistant HIV-1 in acute seroconverters. *AIDS*. 2005; 19:1819–1825. [PubMed: 16227789]

46. Charpentier C, Dwyer DE, Mammano F, et al. Role of minority populations of human immunodeficiency virus type 1 in the evolution of viral resistance to protease inhibitors. *J Virol*. 2004; 78:4234–4247. [PubMed: 15047838]
47. Myint LM, Matsuda Z, Matsuda Y, et al. Gag noncleavage site mutations contribute to full recovery of viral fitness in protease inhibitor-resistant human immunodeficiency virus type 1. *Antimicrob Agents Chemother*. 2004; 48:444–452. [PubMed: 14742193]
48. Gatanaga H, Suzuki Y, Tsang H, et al. Amino acid substitutions in Gag protein at noncleavage sites are indispensable for the development of a high multitude of HIV-1 resistance against protease inhibitors. *J Biol Chem*. 2002; 277:5952–5961. [PubMed: 11741936]
49. Nijhuis M, van Maarseveen NM, Lastere S, et al. A novel substrate-based HIV-1 protease inhibitor drug resistance mechanism. *PLoS Med*. 2007; 4:e36. [PubMed: 17227139]
50. Larru B, de Mendoza C, Bellón JM, et al. Predictive factors of virological success to salvage regimens containing protease inhibitors in HIV-1 infected children. *BMC Infect Dis*. 2007; 7:55. [PubMed: 17559687]
51. Soundararajan L, Karunaianandham R, Jauvin V, et al. Characterization of HIV-1 isolates from antiretroviral drug-naïve children in southern India. *AIDS Res Hum Retroviruses*. 2007; 23:1119–1126. [PubMed: 17919107] [Important data on transmitted resistance in the Indian subcontinent.]
52. Kanya MR, Mayanja-Kizza H, Kambugu A, et al. Predictors of long-term viral failure among Ugandan children and adults treated with antiretroviral therapy. *J Acquir Immune Defic Syndr*. 2007; 46:187–193. [PubMed: 17693883]
53. Pedrosa C, Queiroz AT, Alcântara LC, et al. High prevalence of primary antiretroviral resistance among HIV-1-infected adults and children in Bahia, a northeast state of Brazil. *J Acquir Immune Defic Syndr*. 2007; 45:251–253. [PubMed: 17527096]
54. Vignoles M, Barboni G, Agosti MR, et al. High frequency of primary mutations associated with antiretroviral drug resistance in recently diagnosed HIV-infected children. *Antivir Ther*. 2007; 12:1133–1137. [PubMed: 18018772] [This study shows that baseline resistance is a concern among children in middle-income areas.]
55. Viani RM, Peralta L, Aldrovandi G, et al. Adolescent Medicine Trials Network for HIV/AIDS Interventions. Prevalence of primary HIV-1 drug resistance among recently infected adolescents: a multicenter adolescent medicine trials network for HIV/AIDS interventions study. *J Infect Dis*. 2006; 194:1505–1509. [PubMed: 17083034]
56. Thorne C, Newell ML. Prevention of mother-to-child transmission of HIV infection. *Curr Opin Infect Dis*. 2004; 17:247–252. [PubMed: 15166829]
57. Ekouevi DK, Coffie PA, Becquet R, et al. Antiretroviral therapy in pregnant women with advanced HIV disease and pregnancy outcomes in Abidjan, Côte d'Ivoire. *AIDS*. 2008; 22:1815–1820. [PubMed: 18753864]
58. World Health Organization. Report of the WHO Technical Reference Group, Paediatric HIV/ART Care Guideline Group Meeting; WHO Headquarters; Geneva, Switzerland. 10–11 April 2008; Available at <http://www.who.int/hiv/topics/paediatric/en/index.html>. [Accessed 10 November 2008]
59. Arrivé E, Newell ML, Ekouevi DK, et al. Ghent Group on HIV in Women and Children. Prevalence of resistance to nevirapine in mothers and children after single-dose exposure to prevent vertical transmission of HIV-1: a meta-analysis. *Int J Epidemiol*. 2007; 36:1009–1021. [PubMed: 17533166] [A very important meta-analysis of studies into PMTCT with single-dose NVP vs NVP combined with one or more NRTI.]
60. Nduati R, John G, Mbori-Ngacha D. Effect of breastfeeding and formula feeding on transmission of HIV-1: a randomized clinical trial. *JAMA*. 2000; 283:1167–1174. [PubMed: 10703779]
61. Kumwenda NI, Hoover DR, Mofenson LM, et al. Extended antiretroviral prophylaxis to reduce breast-milk HIV-1 transmission. *N Engl J Med*. 2008; 359:119–129. [PubMed: 18525035] [This study showed that extended prophylaxis substantially reduced transmission associated with breast-feeding in African patients.]
62. Six Week Extended-Dose Nevirapine (SWEN) Study Team. Bedri A, Gudetta B, et al. Extended-dose nevirapine to 6 weeks of age for infants to prevent HIV transmission via breastfeeding in

- Ethiopia, India, and Uganda: an analysis of three randomised controlled trials. *Lancet*. 2008; 372:300–313. [PubMed: 18657709] [This study showed that extended prophylaxis substantially reduced transmission associated with breast-feeding in subtype A, C and D infected patients.]
63. Moorthy A, Gupta A, Bhosale R, et al. Nevirapine resistance and breast-milk HIV transmission: effects of single and extended-dose nevirapine prophylaxis in subtype C HIV-infected infants. *PLoS ONE*. 2009; 4:e4096. [PubMed: 19119321]
- 64•. Church JD, Omer SB, Guay LA, et al. Analysis of nevirapine (NVP) resistance in Ugandan infants who were HIV infected despite receiving single-dose (SD) NVP versus SD NVP plus daily NVP up to 6 weeks of age to prevent HIV vertical transmission. *J Infect Dis*. 2008; 198:1075–1082. [PubMed: 18684096] [This study demonstrates that extended NVP prophylaxis in infants is associated with increased NVP resistance in those who become infected.]
- 65••. Violarì A, Cotton MF, Gibb DM, et al. Early antiretroviral therapy and mortality among HIV-infected infants. *N Engl J Med*. 2008; 359:2233–2244. [PubMed: 19020325] [This very important trial of early vs deferred HAART in infants showed a mortality benefit for the former group. WHO guidelines were changed as a result of this study.]

Table 1

Antiretrovirals currently licensed in adults, use in children and associated resistance mutations

Drug	FDA licensed in children?	Age restriction	Rifampicin co-administration	Important mutations leading to reduced susceptibility
Zidovudine (AZT)	Yes	No	Yes	RT: M41L, D67N, K70R, L210W, T215Y, K219Q/E, Q151M, T69insetions
Stavudine (D4T)	Yes	No	Yes	RT: M41L, D67N, K70R, L210W, T215Y, K219Q/E, K65R, Q151M, T69insetions
Abacavir (ABC)	Yes	Yes	Yes	RT: L74V, K65R, Y115F, M184V
Lamivudine (3TC)	Yes	No	Yes	RT: M184V/I
Didanosine (ddI)	Yes	No	Yes	RT: L74V, K65R
Tenofovir (TDF)	Yes	Over 12 years	Yes	RT: K65R, K70E
Efavirenz (EFV)	Yes	Over 3 years	No	RT: L100I, K103N, V106M/A, V108I, Y181C/I, Y188C, G190S/A, P225H
Nevirapine (NVP)	Yes	No	No	RT: L100I, K103N, V106M/A, V108I, Y181C/I, Y188C/L, H, G190S/A
Etravirine (ETV)	No	NA	NA	RT: V90I, A98G, L100I, K101E/P, V106I, V179D/F, Y181C/I/V, G190A/S
Lopinavir (LPV)	Yes	No	No	PR: V32I, I47V/A, V82A/F/T/S
Saquinavir (SQV)	No	NA	No	PR: G48V
Atazanavir (ATV)	No	Over 6 years	No	PR: I50L, I84V, N88S
Indinavir (IDV)	No	NA	No	PR: M46I/L, V82A/F/T/S,

Drug	FDA licensed in children?	Age restriction	Rifampicin co-administration	Important mutations leading to reduced susceptibility
				I84V
Nelfinavir (NFV)	No	No	No	PR: D30N, L90M
Fosamprenavir (fAMP)	Yes	Over 2 years	No	PR: I50V, I84V
Tipranavir (TPV)	Yes	Over 2 years	No	PR: L33F, V82L/T, I84V
Darunavir (DRV)	No	NA	No	PR: I50V, I54M/L, I84V
Raltegravir (RGV)	No	NA	NA	INT: Q148H/K/R, N155H
Maraviroc (MVC)	No	NA	NA	ENV: complex envelope mutations
Enfuvirtide (ENF)	Yes	Over 6 years	Yes	GP41: G36D/S, A37V, V38A/M/E, Q39R, Q40H, N42T, N43D

NA, not applicable.