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"HIV infection and Infancy:
From diagnosis to treatment and their setting-related issues"

Direttore della Scuola: Ch.mo Prof. Giuseppe Basso

Supervisori: Ch.mo Prof. Carlo Giaquinto
Ch.ma Prof.ssa Diana Gibb

Dottorando: Martina Penazzato

To Raffaella

and

to those little changes

which can make a big difference

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Preface

I started this doctoral program after completing my medical degree and specialty training in infectious diseases with a primary focus on HIV/AIDS, tuberculosis and tropical medicine. While still a Resident in infectious diseases I was involved in the implementation of an HIV care program in Uganda. This experience allowed me to enrich my clinical knowledge of uncommon presentations but more importantly increased my awareness of the enormous challenges faced in terms of providing treatment and care of infectious disease in Sub-Saharan Africa.

Working in this resource-limited setting led me to contextualize my infectious disease interest in the broader picture of restricted access to care and the challenges of the provision of life-saving interventions. This interest was consolidated by the work I conducted for the WHO where I acquired solid grounding on the new standards for the development of evidence-based guidelines.

These experiences taught me how clinical research can be translated into public health improvements in high as well as in low and middle-income countries. Analytical research skills became an essential element of my work which I needed to further develop to enable me to produce, evaluate, interpret and apply scientific evidence to inform clinical and public health actions.

In this context, the link with the Paediatric European Network for Treatment of AIDS (PENTA) and the Medical Research Council Clinical Trials Unit has been critical as it has given me the opportunity to be exposed and involved in high quality research in the field of paediatric HIV. It has also been an inspiring environment where to learn from the expertise of senior colleagues and to acquire new skills receiving facilitation in the planning and development of my research.

My time spent in the field, and the exposure to colleagues working in similar settings, made me realize how in many areas of the world infants are one of the most vulnerable populations, particularly in Sub-Saharan Africa where HIV is one of the leading causes of death in those aged under 12 months. Without intervention, mortality in HIV-infected children reaches 50% by 2 years of age and is therefore an issue, which requires urgent response.

The research presented here was planned and developed to evaluate the robustness of the

evidence currently available on the management of HIV infected infants and young children, highlighting the existing gaps in knowledge, by conducting a systematic review. Data produced from routine clinical practice in several European countries were analysed to investigate optimal management of HIV-infected infants. A significant part of this research was then focused on studying and discussing the operational challenges of treating HIV-infected infants in different settings with the hope of informing a more effective policy development.

The aim of my thesis is to describe my research activity during the PhD project, to highlight the different approaches used and present my contribution to the topic as a coherent body of work. The thesis is divided into 8 chapters, of which four are results chapters containing selected publications and study proposals. Each project can be read as a stand-alone piece; inevitably, concepts and definitions are often repeated in different chapters and their content is not uniformly linked and standardised.

The introduction in Chapter 1 describes: the natural history of HIV infection in infants and young children highlighting the differences across high and low/middle income countries; the standards for diagnosis of HIV infection in early life; the specific features of antiretroviral therapy in early childhood; and finally the global policy context.

The main overall objectives are presented in Chapter 2 and described in more detail within the different projects. Similarly the methodologies applied are summarised in Chapter 3 and defined in detail in each independent piece of work.

In consideration of the four different research approaches which were used to develop the project, the main findings of my research are presented within four separate results chapters: systematic literature review (Chapter 4), clinical research (Chapter 5), operational research (Chapter 6), translation of evidence into policies (Chapter 7).

A conclusive part, Chapter 8, summarises the research work, presents the key messages of the thesis and highlights the potential directions for future work.

Additional work and publications produced such as non-systematic reviews or papers developed on collateral topics are also included within the appropriate Appendices.

In conclusion, this research project was developed with the hope that it would contribute toward a concrete improvement in the clinical management of HIV-infected infants, including early diagnosis and the initiation of a prompt and optimal treatment without restriction of geographical, economical or cultural setting.

Summary

The research work here presented was conducted with the aim of assessing knowledge gaps in managing HIV-infected infants and young children as well as informing the implementation and policy development for low and middle-income countries.

The present thesis, which includes a summary of the competencies acquired at the University of Padova as well as at the Medical Research Council Clinical Trial Unit and the London School of Hygiene and Tropical Medicine, reports the original research which I carried out during the program and outlines the research activities in which I was involved as coordinator or collaborator.

This project has been developed through four different intertwining components: a systematic revision of the literature; a primary data analysis of clinical data from European cohorts of HIV infected infants; three different projects of operational research informing the implementation of international standards of care in low and middle-income countries; and finally the systematic application of standard methodologies to translate evidence into policies.

The systematic revision of the literature highlighted how while there is now conclusive evidence in favour of early initiation of ART in infants, the superiority of LPV/r-based regimens as a first-line therapy is still matter of debate. Moreover, the need of additional evidence from ongoing trials to inform alternative treatment strategies such as interruptions or reuse of NNRTIs when virological suppression is achieved with LPV/r-based regimens was reinforced.

The analysis conducted on European HIV-infected infants starting treatment by the first year of age showed early treatment being effective and durable even outside trial settings. However, did not confirm the superiority of LPV-based regimen observed in trials, but showed a better viro-immunological response in those infants starting ART with a 4 drugs NVP-based regimen.

The implementation of early therapy in HIV-infected infants was shown to increase significantly the number of children eligible for treatment in a Ugandan cohort taken as example to explore the impact of new WHO guidelines at programmatic level. It was also observed that increase in eligibility occurs particularly at enrolment and that delays in initiation are considerable highlighting the need of a prompt diagnosis and rapid pre-ART counselling.

Early infant diagnosis is increasingly being recognised as a building block for treatment and care in infants. A study protocol was therefore developed to investigate the cost implications of different testing strategies and inform the budget allocation at the country level.

HIV drug resistant strains circulation represents a further challenge for the implementation of treatment, particularly in children in low-middle income countries where treatment options are limited. A surveillance study protocol was developed to investigate at a population level the prevalence of resistant viruses transmitted from the mother to the baby and inform national guidelines development.
Finally the use of standardised method to translate evidence into policies allowed investigating the implications of recommending globally in infants and young children the use of LPV/r-based regimen as first line therapy regardless of their exposure to PMTCT interventions. Significant challenges in acceptability and feasibility make the revision of recommendations difficult until new formulations will become available.

The aim of my future work will be to apply these skills to facilitate and promote research to guide the development of international policies for life-saving public health actions in the field of infectious diseases.

Riassunto

Il progetto di ricerca di seguito presentato si pone l'obiettivo di analizzare ed espandere le attuali conoscenze sulla gestione dei lattanti con infezione da HIV e di contribuire ad informare l'implementazione e lo sviluppo di adeguate raccomandazioni per i paesi a risorse limitate.
La presente tesi raccoglie una sintesi delle competenze e dell'esperienza maturata presso l'Universita' di Padova ed il Dipartimento di Pediatria, presso l'unita' di clinical trial del Medical Research Council di Londra e presso la London School of Hygiene and Tropical Medicine attraverso la partecipazione a corsi avanzati in epidemiologia e biostatistica. Questo scritto illustra le attivita' di ricerca in cui sono stata coinvolta o come principale coordinatore o come stretto collaboratore.

Questo lavoro si basa su quattro componenti fortemente interconnesse: 1) la revisione sistematica della letteratura disponibile sull'argomento; 2) l'analisi di dati osservazionali provenienti da diverse coorti europee di bambini con infezione da HIV che hanno iniziato la terapia entro il primo anno di vita; 3) tre differenti progetti di ricerca operazionale finalizzati ad informare la fase di implementazione degli standard di cure internazionali nei paesi a risorse limitate; 4) infine una parte dove l'evidenza raccolta viene utilizzata in maniera sistematica per sviluppare raccomandazioni internazionali finalizzate all'ottimizzazione della gestione della terapia nei bambini con infezione da HIV sotto l'anno di vita.
La revisione sistematica della letteratura da un lato ha messo in luce come esista già una solida evidenza a favore dell'inizio precoce della terapia anitretrovirale nei bambini sotto l'anno di vita, ma dall'altro lato ha mostrato anche che la superiorità dei regimi di prima linea contenenti Lopinavir/ritonavir è ancora oggetto di discussione. Inoltre, si è osservato come siano necessari ulteriori dati in merito all'utilizzo di strategie nuove come quelle che prevedono l'interruzione strutturate della terapia o il riutilizzo di NNRTI una volta che la soppressione virologica è raggiunta con un regime contenente Lopinavir/ritonavir.
L'analisi condotta su dati europei provenienti da bambini che hanno iniziato la terapia antiretrovirale nel primo anno di vita, ha confermato come una risposta efficace e duratura sia possibile anche al di fuori di trial clinici. Tuttavia, non ha evidenziato alcuna superiorita' terapeutica dei regimi contenenti Lopinavir/ritonavir rispetto a quelli contenenti Nevirapina ed

ha invece suggerito che un regime a quattro farmaci contenente Nevirapina sarebbe correlato ad una migliore risposta viro-immunologica .

L'implementazione della terapia precoce per tutti i bambini HIV-infetti sotto l'anno di vita ha dimostrato di aumentare significativamente il numero dei pazienti eleggibili per l'inizio della terapia in una coorte Ugandese presa come esempio per esplorare l'impatto che le nuove linee guida WHO stanno avendo a livello programmatico. E stato inoltre osservato che l'aumento del numero dei pazienti eleggibili avviene in particolare al momento dell'arruolamento e che esistono ancora considerevoli ritardi nell'inizio della terapia, sottolineando la necessità di una diagnosi precoce e di un rapido counselling pre-terapia.

La diagnosi virologica nei bambini sotto i 18 mesi è sempre più considerata come l'elemento fondamentale per lo scaling-up della terapia e delle cure nei bambini con infezione da HIV. Un protocollo di studio è stato pertanto messo a punto allo scopo di studiare le implicazioni economiche di differenti strategie diagnostiche ed informare l'ottimale utilizzazione del budget che ciascun paese ha a disposizione.

La diffusione di sottotipi virali resistenti agli attuali farmaci antiretrovirali disponibili è un'altra delle difficoltà incontrate durante la fase di implementazione ed ottimizzazione delle cure dei bambini infetti da HIV nei paesi a risorse limitate. Uno studio di sorveglianza è stato pertanto messo a punto per studiare la frequenza con cui ceppi resistenti vengono trasmessi dalla madre al bambino allo scopo di informare lo sviluppo delle linee guida nazionali.

Infine, l'utilizzo di una metodologia standardizzata per lo sviluppo di policy basate sull'evidenza ha permesso di indagare in profondità le implicazioni legate ad una revisione delle linee guida che raccomandi a livello globale l'utilizzo di un regime di prima linea contenete Lopinavir/ritonavir. Si sono riscontrate delle barriere significative in termini di accettabilità e fattibilità tali da non raccomandare attualmente una modifica delle linee guida, almeno fino a quando non saranno disponibili formulazioni più appropriate.

Il mio futuro lavoro sarà orientato verso l'utilizzo delle capacità acquisite per condurre e promuovere attività di ricerca che possano guidare lo sviluppo di adeguate politiche sanitarie internazionali nel campo delle malattie infettive.

Abbreviations

AIDS	acquired immunodeficiency syndrome
ART	antiretroviral therapy
cART	combined antiretroviral therapy
CBA	cost-benefits analysis
CEA	cost-effectiveness analysis
CUA	cost-utility analysis
DSMB	data safety monitoring board
EID	early infant diagnosis
EFV	efavirez
FEM	fixed effect model
FDCs	fixed drug combinations
HIC	high-income countries
HIV	human immunodeficiency virus
HIVDR	HIV drug resistance
LIC	low-income countries
LPV/r	ritonavir boosted lopinavir
MIC	middle-income countries
MTCT	mother-to-child-transmission
NNRTI	non-nucleoside reverse-transcriptase inhibitors
NRTI	nucleoside reverse-transcriptase inhibitors
NVP	nevirapine
PI	protease inhibitors
PMTCT	prevention of mother to child transmission
RCT	randomised control trial
REM	random effect model
RTV	ritonavir
TB	tuberculosis
Sd-NVP	single dose nevirapine
WHO	World Health Organisation
QALYs	quality-adjusted life years
DALYs	disability-adjusted life years

1. Background

The number of children under the age of 15 years living with HIV increased from 1.6 million (range, 1.4 million-2.1 million) in 2001 to 3.4 million (range 3.0 million-3.8 million) in 2010, and over 1000 newly infected infants continue to be born daily [1]. In the absence of combination antiretroviral therapy (cART), over 50% of HIV-infected infants progress to AIDS and death by 2 years of age. The introduction of cART has dramatically changed the natural history of HIV infection in children [2, 3], and now, in well-resourced countries, over 90% HIV-infected children reach the age of 10 years [4]. However, of the 2.02 million (1.8 –2.3) children estimated to need antiretroviral therapy in 2010, only 23% (20–25%) had access versus 51% of adults (48–54%)[5].

The global paediatric HIV epidemic involves two distinct populations with different sets of challenges. In resource-limited settings, where over 90% of new infections in children under 15 years of age occur every day[6] barriers to treatment as fundamental as drug access still exist [7] and HIV remains a major cause of maternal and child morbidity and mortality in such countries, particularly in Sub-Saharan Africa [1]. Even where antiretrovirals are available in these settings, limited availability of diagnostic testing and the use of regimens that fail to suppress plasma viral load can lead to the emergence of drug resistance [7]. Conversely, in countries where antiretroviral therapy is widely available, HIV MTCT has largely already been reduced, and children who acquire HIV survive into adolescence and adulthood [8] facing issues associated with long-term antiretroviral use, including drug resistance and toxicity [9].

1.1 Natural history of HIV infection in young children

The natural history of HIV infection in children differs significantly from that observed in adults. Early cohort studies in Europe have shown that prior to the use of antiretrovirals, 20-25% of children infected with HIV progressed rapidly to AIDS or die during infancy (Rapid Progressors). After one year of age progression to AIDS or death was slower at 5% per year and around 50% of children survived to 10 years of age (Slow Progressors). Although with fluctuating symptoms, most surviving children had moderate symptoms and less than 1% were Long Term Non Progressors [10].

Reasons to explain these different patterns are still not completely understood, but infants who progress very rapidly tend to be born to mothers with more advanced HIV disease, lower CD4 counts and higher viral loads. Unfortunately it is very difficult to predict which infants with HIV will progress rapidly [30] as in contrast with adults, CD4 and viral load are not reliable prognostic markers of progression, particularly in infants and the youngest children [11-13].

Survival in resource-limited countries is decreased by the exposure to a high risk of intercurrent infections often on a background of poor nutrition. A large meta-analysis with data from 7 randomised African mother-to-child transmission intervention trials demonstrated the overall mortality was 11% (378 / 3468 children). At 1 year of age, 35% of HIV infected and 5% of uninfected infants had died; and by 2 years of age, 53% and 8%, respectively. Mortality varied by geographical region, and was associated, with maternal death, low maternal CD4 count and infant HIV infection. Mortality was significantly lower for those with late infection (i.e. via breast feeding after 4 weeks of age) than those with early perinatal infection [14].

The Cross Continents Collaboration for Kids (3Cs4kids) study, which combined longitudinal data from approximately 2500 untreated HIV-infected children enrolled in African cohorts, evaluated the prognostic value of selected laboratory and growth markers for 12-month risk of mortality [13]. There were insufficient studies focusing on sub-Saharan African infants to include children <12 months of age in this analysis, which evaluated risk factors for mortality in children >1 years of age only. Nevertheless, prognosis for children with a given CD4 count or percentage was shown to be poorer at younger ages, and the predictive value of both markers improved with age, similar to findings for European/US children [11]. Moreover, both CD4 percentage and count were shown to be less effective in discriminating between low and high mortality risk for children in resource-limited, compared to well-resourced, settings. Similar findings were also reported by a recent cohort analysis conducted on more than 1700 children in Ivory Coast [15].

1.2 Diagnosis of HIV infection in infants and young children

Reduction of early mortality requires access to early infant diagnosis (EID); standard diagnostic antibody tests cannot diagnose HIV under the age of 12-18 months as maternal antibodies cannot be distinguished from those of an infected infant.

Virological assays, especially HIV-1 NAATs (nucleic acids amplification tests), such as HIV-1 DNA PCR assays, represent the gold standard for infants and children younger than 18 months [16]

as characterized by 99.6% specificity and 100% sensitivity from 4 weeks of age [16]. With such testing, the diagnosis of HIV-1 infection (as well as the presumptive exclusion of HIV-1 infection) can be established within the first few weeks of life among non-breastfed infants.

According the most recent WHO guidelines on diagnosis [17] any virological test (NAAT or Agp24 on liquid or DBS sample) can be used to undertake EID. In addition, in order to maximize specificity a confirmatory test should be performed for those with first positive results and a third test should be considered in case of discordant results.

The use of dried blood spots (DBS) or filter paper-based HIV- DNA or HIV RNA testing and p24 antigen assays offers significant logistical and financial advantages for resource-limited settings [17]. DBS can be transported without need for refrigeration and have helped to widen coverage [18].

Expanding access to HIV counseling and testing is increasingly recognized as the building block to the scale-up of HIV prevention, treatment and care. Significant progress has been made in scaling up the coverage of early infant diagnosis in 2010. In 65 low and middle-income countries providing data, 28% [24–30%] of infants were reported to have been tested for HIV within the first two months of birth, versus 6% [5–7%] in 2009 [5]. However, early infant diagnosis by virological testing is currently unavailable, unaffordable or unfeasible in many settings.

1.3 Antiretroviral Treatment

Careful management of HIV-infected infants and children is of great importance given the need for treatment during periods of significant growth and development, as well as the requirement for life-long therapy. Paediatric therapies need to have a good safety profile, potent antiviral activity and compatibility with other drugs. In addition, adequate central nervous system penetration is critical in decreasing the incidence of HIV-associated encephalopathy, which can manifest as developmental delay [19, 20]. Palatability and once-daily dosing, as well as paediatric-suitable formulations, are all important additional considerations in addressing adherence challenges and, in turn, limiting the emergence of drug resistance [19, 21].

Early antiretroviral therapy in infancy regardless of clinical and immunological condition was shown to reduce mortality and disease progression in South African children enrolled in the

CHER trial [22]. Initiating HAART before the age of 3 months had been reported to maintain CD4 counts and percentages at protective levels despite low rates of HIV RNA viral load suppression [23-27]. Several small studies have confirmed that even though the level of viral replication in perinatally infected infants is very high, early initiation of ART can result in sustained viral suppression and normalization of immunologic responses to non-HIV antigen [25, 28, 29]. However, there are potential problems with treatment of asymptomatic infants. The rates of virologic failure reported seem to be higher with therapy started earlier rather than later. In studies addressing the effect of early therapy, the proportion of infants with viral levels remaining below quantification after 12–24 months of therapy has been lower than observed in older children and adults, ranging from 18%–62% [4, 30, 31].

Although early initiation of cART has been shown to be beneficial for young children, lifelong treatment is problematic, given the limited availability of appropriate drugs, long-term cART toxicity, and difficulties with adherence, risk of viral resistance, and cost of such a strategy. Although 25 antiretroviral drugs are licensed worldwide for the treatment of HIV-infected adults, currently only 6 drugs (D4T, 3TC, AZT, NVP, ABC, LPV/r) are registered and available in appropriate infant formulations and treatment options are limited, especially in countries where expensive drugs remain unavailable.

The optimization of first line regimens in these infants and young children is therefore critical. Drug choice, affected by efficacy, safety and tolerability profile, needs further investigation. In the past, the choice between NNRTI- and boosted PI-based regimens as first-line therapy has been limited by the lack of head-to-head comparisons between these regimens in controlled clinical trials.

Currently, while robust evidence is available to support the superiority of a PI-based regimen as a first-line treatment in NNRTI-exposed infants, [32] two recent trials [33, 34] have investigated the effectiveness of different 1st-line cART regimens in children unexposed to NNRTI as part of prevention of mother-to-child transmission (PMTCT) interventions, presenting contradictory results.

1.3.1 The Role of NNRTIs

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) have played a key role in the management of paediatric HIV infection, providing important components of antiretroviral therapy in both resource-rich and resource-limited settings [35].

For infants and children under 3 years old with unknown or no prior exposure to antiretrovirals, nevirapine plus two NRTIs is currently recommended as first-line regimen [21]. A second NNRTI, Efavirenz (EFV), is licensed for children >3 years, but the appropriate dose for children under 3 years has yet to be determined as drug levels are widely variable in this age-group; pharmacokinetic studies are ongoing in the IMPAACT P1070 study. Meanwhile EFV is not recommended in this age group.

A rare but potentially life-threatening risk of hepato-toxicity is associated in adults with the use of NVP [9] and this makes the drug less suitable for treating children who are on other hepatotoxic medications. However, in children NVP can more frequently be associated with rash which has been observed to be mostly mild and transient, particularly in the youngest children [36].

Small studies in children have suggested that co-administration of rifampicin may not result in lowered NNRTI concentrations in paediatric populations, and that prescribing the maximum dose of nevirapine in children aged less than 3 years should be a sufficient approach to avoid sub-therapeutic drug levels[37-39]. The use of NNRTIs is therefore still key in the management of tuberculosis and HIV co-infection.

The cost of components of NVP-based therapy is significantly lower than other classes; generic and fixed-dose combinations are also available [21]. In addition, NNRTIs are generally considered to have better tolerability profiles than ritonavir-boosted PIs, with fewer metabolic complications such as dyslipidaemia, fat redistribution and insulin resistance [40, 41].

Unfortunately, NVP presents a low genetic barrier for resistance and the possibility of extensive class cross-resistance, which precludes sequential use of efavirez [42, 43].

1.3.2 The Role of PIs

The only protease inhibitor currently available to administer to infants and young children is lopinavir/ritonavir and still little is known about LPV/r safety and tolerability. The use of LPV/r oral solution has been approved by the FDA for use in infants 14 days and older, but current

guidelines do not recommend use in preterm babies in the immediate postnatal period[44] due to a reported fatal case. Studies in the U.S. [45] report that a high dose of 300/75 mg/m^2/dose of LPV/r is considered appropriate for most infants <6 months of age, however, lower LPV concentrations have been reported in the first months of life and there may be a rational to explore the effect of higher doses of LPV/r in very young infants, in whom absorption of the drug maybe limited.

LPV/r-based regimens show a better safety profile in the short-term with minor gastrointestinal symptoms, which are usually transient. However, difficulties with formulations (LPV/r is only available as a liquid with a short shelf-life or as a relatively large, non breakable tablet), as well as long-term complications are associated with LPV/r: a higher prevalence of lipodystrophy [41] and metabolic outcomes including hypercholesterolemia [42] has been described in older children. These complications are poorly known in infants and young children, but should be considered in the context of life-long treatment.

The use of a PI during concomitant anti-tuberculosis treatment remains challenging. The co-administration of LPV/r with rifampicin is not recommended as results in a 90% to 99% reduction in the trough concentration (C_{min}) of lopinavir. Administering double doses of co-formulated LPV/r [46] resulted in inadequate lopinavir concentrations but a study conducted in South African patients (aged 6 months-3 years) demonstrated that an adjusted dose regimen of LPV/r (increasing ritonavir to make the LPV:ritonavir ratio of 1:1) may be effective in achieving adequate concentration [47]; this is however based on a total of only 15 children.

Robust evidence has shown that LPV/r, similarly to other boosted PIs, has a high genetic barrier to drug resistance resulting in good durability and potency [48, 49]. In the PENPACT1[33] trial PI-resistance was very uncommon with virological failure and PI seemed to be protective against the development of NRTI-resistance, which was more common (10% more) in those initiating treatment with an NNRTI-based regimen, particularly in those switching at higher viral load levels.

However, it is unfortunately acknowledged that the use of LPV/r in a 1st-line regimen may compromise the potential to construct a potent 2nd-line regimen, particularly in children who have been exposed to sdNVP in resource-limited settings.

1.3.3 Treatment strategies

An adequate balance between efficacy and mid/long –term toxicity of an ARV regimen is often hard to achieve in the context of treatment sequencing over a long-life treatment approach, particularly when adequate formulations are limited to first- and second-line according to the public health approach in Africa, where most HIV-infected infants live.

The potential benefits of a two-class, four drug induction-*maintenance strategy* using an NNRTI-based regimen may have advantages and preserve future therapeutic alternatives, minimizing the exposure to potentially toxic drugs and decreasing the risk of adverse events or resistance selection [50]. Evaluating this approach, the ongoing ARROW (AntiRetroviral Research fOr Watoto) trial is a randomised trial of monitoring strategy and first-line ART in 1207 children 3 months to 12 years in Zimbabwe and Uganda. An induction maintenance approach starting with 4 drugs from 2 classes (NNRTI+3NRTI) for the first 32 weeks, reducing to 3 drugs is compared with standard 3-drug ART regimen from initiation, using a NRTI backbone of 3TC+ABC, which is itself more potent than ZDV+3TC (PENTA 5 trial). About 370 children less than 2 years old have also been enrolled in this trial and results are awaited to inform global treatment strategies in infants.

The safety and effectiveness of a *PI-sparing strategy* have also been investigated in infants starting treatment early by the NEVEREST trial (NEVirapinE REsistance STudy) [51] which was designed with the aim of testing if NVP prophylaxis-exposed children could switch to NVP after initial suppression on a LPV/r-based regimen. HIV-infected children under the age of two years exposed to NVP prophylaxis and who met immunologic and clinical criteria for antiretroviral therapy were started on LPV/r, D4T and 3TC. This study provides proof of concept that re-use of NVP following successful suppression on LPV/r-based therapy is possible for HIV-infected children exposed to NVP prophylaxis. However further research is necessary to determine the circumstances and interventions required to safely re-use NNRTIs and additional studies are ongoing (MONOD, NEVEREST-3).

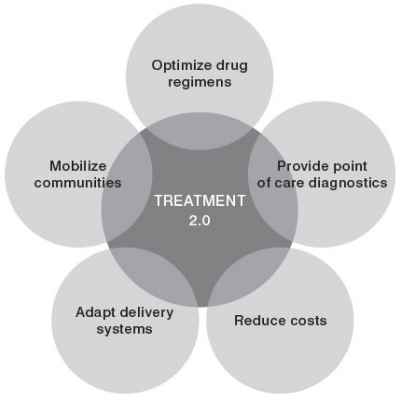
Whether *treatment interruption* within strategies where therapy is begun in early infancy and stopped after a defined period of treatment (e.g., 1–2 years) allowing the child to be protected during the period at greatest risk for HIV disease progression and mortality, and restarting therapy when the child meets standard age-related criteria, is under evaluation in clinical trials in South Africa and Kenya, with results expected in early 2012. Currently, planned treatment

interruption (PTI) is not recommended in children outside of a clinical trial setting; However, PTIs may have a role in the future management of paediatric HIV as reassuring data have been reported by PENTA 11 (TICCH - Treatment Interruption in Children with Chronic HIV-infection [52]) trial, where a CD4-guided PTI in chronic HIV has been piloted; no deaths or serious clinical events occurred in the PTI group, while more minor clinical events (but not infections) have been recorded. Although CD4 levels and HIV RNA suppression were inferior at 72 weeks in the PTI group, some PTI children were off ART at 72 weeks and CD4 recovery after PTI was significantly better in younger children. In addition further reassurance comes from the high proportion of children, in the PTI group, with suppressed viral load after 24 weeks back on ART, among whom no evidence of increased resistance was observed than in the continuous treatment group was found[52]. Follow-up of children for five years post-trial completion is ongoing: complete CD4 recovery in the PTI group was observed compared with the continuous group and neurodevelopmental testing a one and two years post trial end showed no differences between the arms; more detailed immunological studies are ongoing (Compagnucci et al., 3rd HIV Paediatrics Workshop Rome 2011).

1.4 Global and Policy context

The area of optimizing diagnosis and treatment of HIV infected infants and children is of particular interest because although access to cART for children has expanded considerably over the last few years, children still lag behind adults in terms of coverage. A better understanding of the most effective and most appropriate treatment regimens for infants is urgently needed to inform global policy decisions.

WHO currently recommends starting cART with an NNRTI-based first line regimen for HIV-infected infants who have not been previously exposed to NNRTI for PMTCT [21] and is now discussing whether to revise guideline based on recent data on efficacy of LPV/r-based regimen for any infant starting treatment. The decision-making process is occurring in the context of a new phase for HIV care and treatment scale up evolving from the '3 by 5' initiative. Despite the achievements of the past decade in reducing HIV-related illness and death, the global response to HIV is dealing



with huge financial and technical challenges in achieving universal access. A strategy called "Treatment 2.0" has been developed to meet these challenges by improving the efficiency and impact of HIV care and treatment programmes in resource-limited countries [53]. Key pillars are simplification, innovation, efficiency, effectiveness and cost-effectiveness, accessibility, affordability, decentralisation and integration, equity, and community mobilization.

A global plan 'Countdown to Zero' has been developed by UNAIDS and the US President's Emergency Plan for AIDS Relief (PEPFAR) and was launched in June 2011 [54] making a commitment by 2015 to eliminate new HIV infections among children and to keep their mothers alive, in an effort to save millions of lives across the developing world, particularly in Africa. A more energetic expansion of PMTCT programmes is being rolled out, with the aim of having a significant impact on the number of infants acquiring the HIV infection. A shift to more efficacious regimens for PMTCT is expected, to reduce infections as well as to reduce the development of drug resistance compromising treatment options for infants.

1.4.1 Evidence based guidelines

Evidence-informed dissemination and implementation strategies are increasingly recognised as a core part of the development of recommendations. Every year, WHO develops a large number of recommendations aimed at many different target audiences, including the general public, healthcare professionals, managers working in health facilities (eg, hospitals) or regions (eg, districts), and public policymakers in member states. These recommendations address a wide range of clinical, public health, and health policy topics related to achieving health goals. Evidence of the effects of alternative policies, programmes, and services is essential for well-informed decisions.

The production of systematic reviews is an important approach to assess the evidence of effectiveness. The risk of bias in selecting studies and interpreting their results is significantly reduced as well as the risk of focusing on a limited subset of relevant evidence. Systematic reviews provide a critical appraisal of the available evidence and place individual studies or subgroups of studies in the context of all the relevant evidence. However, judgments are still needed about the quality and, especially for public health topics, the applicability in different contexts [55]

A comprehensive evaluation of the effects of an intervention requires information about needs, factors that could affect whether effectiveness will be realised in the field, such as the available resources, costs, and the values of those who will be affected by the recommendations. Moving from evidence to recommendations requires judgments, particularly judgments about goals and about the balance between the desirable and undesirable consequences of choosing one option over another.

Processes for developing recommendations have typically relied heavily on expert opinion, and not on representatives of those who will have to live with the recommendations; more recently approaches (like systematic reviews) have emerged as central to the development of recommendations [10, 56, 57].

The GRADE framework has become particularly instrumental to develop recommendation in the context of international organisation such as the World Health Organisation.

1.4.2 Economic evaluation to inform public health action

Resource scarcity and the need to optimise the health budget use in various settings, make the economic evaluation an essential tool for evaluating the most effective health intervention for a given resource in order to guide public health action.

"Economic evaluation is the comparative analysis of alternative courses of action in terms of both their costs and consequences". In the context of health, diagnostics or treatment/prevention interventions are evaluated on the basis of health benefits obtained by the patients comparing the costs that are required for different courses of action [58].

The existing 4 main approaches are:

a) *Cost-benefits analysis* when alternative strategies are compared and a net benefit is calculated from evaluating both costs and health consequences in monetary unit.
b) *Cost-minimisation analysis* when costs required by two strategies are compared assuming the health consequences are equivalent.
c) *Cost-effectiveness analysis* when costs are related to a common health effect which may differ in magnitude between alternative strategies (ie. Life-years gained by $ spent)
d) *Cost-utility analysis* when health outcomes are evaluated in terms of: quality-adjusted life-years (*QALYs)* or disability-adjusted life years (*DALY)s.*

Clinical trials, particularly in the field of HIV and when conducted in resource-limited settings, have included an economic component to assess concomitantly benefits gained and costs required. However, trial settings are considered to be a highly-selected environment and a sensitivity analysis is often needed to generalise findings to a “real life” setting [59]. In settings were health systems are struggling, resources are very limited and the socio-economical structure in the society suffers because of conflicts or environmental barriers, the expected course of action can be significantly affected. For instance even in countries where early infant diagnosis is performed efficiently the subsequent lost to follow up at different stages in the cascade is considerable, such that only about a third of infants being tested successfully start treatment [60]. The evaluation of effectiveness, when performing economic evaluation needs therefore to account for distortion from the standard practice and its potential effect on health outcomes gained.

In the current context of limited resources and active ART scaling up economic evaluations to assess the cost-effectiveness of different interventions in the field of paediatric HIV are essential.

2. Objectives

The research activities here reported were developed to:

1. Evaluate the robustness of the evidence available on when antiretroviral treatment should be started in HIV-infected infants and young children, which ART regimen should be considered optimal for this population and whether alternative strategies to long-life treatment could be introduced to maximize effectiveness and minimize toxicity.

2. Investigate the virological and immunological response, the length of first line regimen and treatment interruption in European HIV-infected infants starting early treatment.

3. Explore the programmatic implications of early initiation of ART in HIV-infected infants and young children (less than 2 years) diagnosed early in life by:

3.1 Quantifying the burden on treatment programmes in resource limited settings;

3.2 Exploring the challenges and the cost-effectiveness of different strategies for early infant diagnosis in resource limited settings;

3.3 Exploring the impact of PMTCT scale-up on the acquisition of resistant viruses which could potentially affect the efficacy of first-line regimen.

4. Assess the policy implications and conduct a comprehensive assessment to inform the WHO paediatric guidelines revision process regarding the optimal first line regimen to recommend in infants and young children (less than 2 years).

3. Methods

The research work presented here was developed applying different research methodologies to acquire a more comprehensive approach to the production, evaluation and use of scientific evidence.

Different approaches were used (Figure 1) including: rigorous tools for evidence evaluation; standard methods for primary data analysis; simple modelling to assess impact of public health actions; sampling strategies to apply in population level surveys; basic element of cost-effective analysis; and international framework to translate evidence into policies.

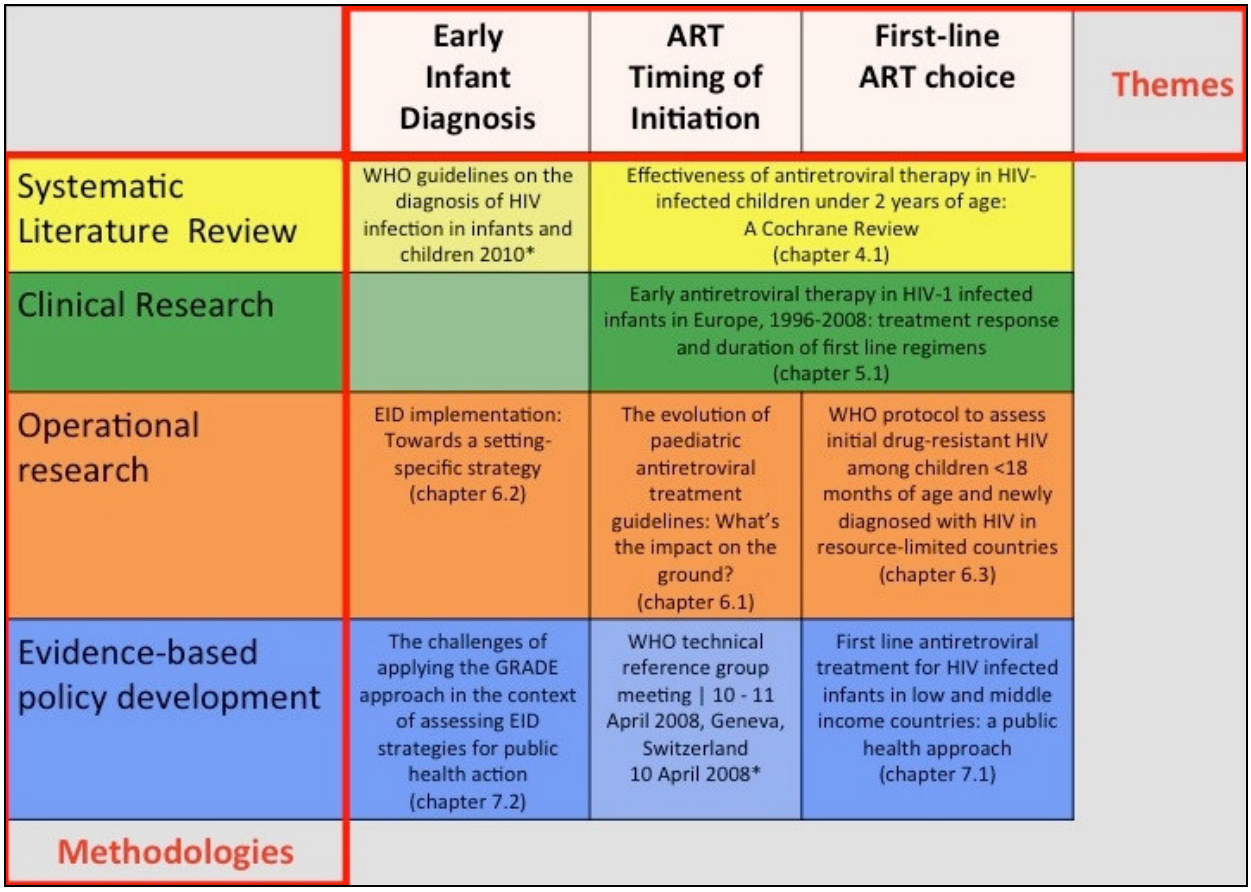


Figure 1 Conceptual Framework of the Research Project (* work produced prior the doctoral program)

The systematic literature review section was conducted by rigorously applying the Cochrane methodology for the production of meta-analysis and systematic reviews of randomized control trials. Search, studies screening, risk of bias assessment and statistical analysis were conducted in accordance with the Cochrane Handbook for Systematic Reviews [61].

In the primary data analysis section, data on HIV-infected infants, born 1996-2008 and starting ART before age 12 months, were combined from 13 European countries. Logistic and linear regressions were used to assess predictors of virological and immunological response. Competing risk methods were used to investigate switching to second-line ART and treatment interruptions under virological suppression.

For the operational research section three different approaches were used to investigate the challenges of treating HIV infected infants in resource-limited settings.

First, time-to –event methods were applied when analysing a prospective dataset of almost a thousand Ugandan children to estimate the impact of the revision of WHO treatment initiation criteria on programmes. The probability of initiating treatment at enrolment and during the subsequent follow up was estimated for different sets of WHO criteria to allow calculation of the change in treatment eligibility.

Second, core elements of cost and cost-effectiveness analysis were used to develop a concept paper and a study proposal, which could assess the cost-effectiveness of different early diagnostic strategies for HIV-infected infants in different settings.

Third, a surveillance tool was developed to investigate at a population level the acquisition of resistant virus by HIV-infected infants. Simple random sampling and adjustments to account for the impact of intra-cluster correlation were the main features of the survey methodology used.

In the section considering the policy implications of the newly acquired evidence concerning the optimal first-line regimen in infants, the GRADE framework was applied as currently endorsed by the World Health Organisation. A systematic approach was used to assess the quality of the evidence obtained from randomized control trials as well as observational studies. Risk and benefits of potential recommendations were assessed by considering toxicity, acceptability, feasibility and cost of the intervention under investigation.

Further details of methods used in each individual project are further explained within the appropriate sections of this report.

4. Research activities I. Systematic Literature Review

This section will report on the use of the Cochrane collaboration methodology as a transparent and systematic tool to review the evidence available on the effectiveness of antiretroviral therapy in HIV-infected infants and young children.

The Cochrane Collaboration is an international network, which was established in 1993 to help health care providers, policy-makers, patients, their advocates and carers, to make well-informed decisions about health care.

The Collaboration prepares, maintains and promotes systematic reviews to inform healthcare decisions. These systematic reviews seek to collate all evidence that fits pre-specified eligibility criteria in order to address a specific research question, minimising bias by using explicit and systematic methods.

The key characteristics of a systematic review are [61]:

- a clearly stated set of objectives with pre-defined eligibility criteria for studies;
- an explicit, reproducible methodology;
- a systematic search that attempts to identify all studies that would meet the eligibility criteria;
- an assessment of the validity of the findings of the included studies, for example through the assessment of risk of bias; and
- a systematic presentation, and synthesis of the characteristics and findings of the included studies.

Many systematic reviews contain meta-analyses, which apply statistical methods to summarize the results of independent studies. This approach is usually preferable as can provide more precise estimates of the effects of health care than those derived from the individual studies included within a review. Meta-analyses also facilitate the appraisal of the consistency and the investigation of differences across studies[61].

For the purpose of developing a systematic review on the effectiveness of antiretroviral therapy in HIV infected infants and young children a full protocol was developed around three

main questions: when should antiretroviral treatment be started in children?; what is the preferred regimen to start treatment with?; and are alternative strategies to long-life treatment, such us interruption or switching, safe and effective?. The protocol was submitted for peer-review and published on the Cochrane database in early 2010 [62].

The Cochrane Collaboration group provided support for the literature search phase during which we searched for published studies in the Cochrane HIV/AIDS Review Group Trials Register, The Cochrane Library, Pubmed, EMBASE and CENTRAL. We also searched for unpublished and ongoing studies by considering prospective clinical trial registries and by contacting research organizations and experts in the field.

Randomized control trials that recruited perinatally HIV-infected children under 2 years of age without restriction of setting were selected. Where the publication did not provide enough data, and this could preclude reliable estimation for treatment effect, the trial authors were contacted for further information.

Where more than one trial was identified for the questions being addressed, the treatment effect measures for each of the eligible trials were combined in meta-analysis to give a pooled measure of effect using the fixed-effect and random effect models.

I lead the work from the development of the protocol to the final writing up of the manuscript; the collaboration with one of the co-authors (AP) was particularly important during the phases of screening studies, evaluation of studies and data extraction; these activities were, however, conducted independently to minimise errors.

I also personally conducted the statistical analysis, applying statistical skills acquired during the course in advance statistical methods attended at the London School of Hygiene and Tropical Medicine as part of the Public Health in Developing Countries MSc programme.

Interpretation and discussion of the findings was later shared with the rest of the co-authors who also contributed to writing the manuscript.

The review has been submitted to the Cochrane Collaboration HIV group and is due for publication on the Cochrane Library in early 2012. The possibility of a co-publication in a peer-review journal is under consideration.

4.1 Effectiveness of antiretroviral therapy in HIV-infected children under 2 years of age: A Cochrane Review.

Martina Penazzato [1,2], Andy Prendergast [2,3], Jayne Tierney [2], Mark Cotton [4], Diana Gibb [2].

[1]Department of Paediatrics, University of Padua, Padua, Italy

[2] MRC Clinical Trials Unit, University College London, London, UK

[3]Department of Paediatrics, University of Oxford, Oxford, UK

[4] Children's Infectious Diseases Clinical Research, Tygerberg Children's Hospital, Tygerberg, South Africa

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4.1.1 Abstract

Background

In the absence of antiretroviral therapy (ART), over 50% of HIV-infected infants progress to AIDS and death by 2 years of age. However, there are challenges to initiating ART in early life, including the possibility of drug resistance in the context of prevention of mother-to-child transmission (PMTCT) programs, a paucity of drug choices, uncertain dosing for some medications and long-term toxicities. Key management decisions include when to start ART, what regimen to start, and whether and when to switch or interrupt therapy. This review aims to summarize the currently available evidence on this topic and inform the ART management in HIV-infected children less than 2 years of age.

Objectives

To evaluate 1) when to start ART in young children; 2) what ART to start with, comparing first-line non-nucleoside reverse transcriptase inhibitor (NNRTI) and PI-based regimens; and 3)

whether and when ART should be stopped or switched from a PI-based regimen to an NNRTI-based regimen.

Search methods

We searched for published studies in the Cochrane HIV/AIDS Review Group Trials Register, The Cochrane Library, Pubmed, EMBASE and CENTRAL. We screened abstracts from relevant conference proceedings and searched for unpublished and ongoing trials in clinical trial registries (http://clinicaltrials.gov -NIH and WHO International Clinical Trials Registry Platform-ICTRP).

Selection criteria

We identified RCTs that recruited perinatally HIV-infected children less than 2 years of age without restriction of setting. We rejected trials that did not include children less than 2 years of age, or did not evaluate either timing of ART initiation, choice of drug regimen or treatment switch/interruption strategy.

Data collection and analysis

Two reviewers independently applied study selection criteria, assessed study quality and extracted data. Effects were assessed using the hazard ratio (HR) for time-to-event outcomes, relative risk for dichotomous outcomes and weighted mean difference for continuous outcomes.

Results

Of 1921 records retrieved, 5 studies were eligible for inclusion in the review, addressing when to start treatment (n=2), what to start (n=2) and whether to switch regimen (n=1). Three ongoing studies that address the question of treatment interruption were also identified.

Early infant treatment was associated with a 75% reduction (HR=0.25; 95%CI 0.12-0.51; p=0.0002) in mortality or disease progression in the one trial with sufficient power to address this question. In a smaller trial, median CD4 cell count was not significantly different between early and deferred treatment groups 12 months after ART.

Regardless of previous exposure to nevirapine for PMTCT, the hazard for treatment failure was 2.01 (95%CI 1.47, 2.77) times higher in children starting ART with a NVP-based regimen compared to those starting with a LPV/r-based regimen (p<0.0001) with no clear difference in effect by age group. The hazard for virological failure was overall 2.28 (95%CI 1.55, 3.34) times higher for children starting ART with a NVP-based regimen compared to those starting with a LPV/r-based regimen (p=0.0005) with a larger difference in time to virological failure (or death) between the NVP and LPV/r-based regimens when ART was initiated in the first year of life. By contrast, increases in weight z-score (MD=0.37, 95%CI 0.08, 0.65, p=0.01) and height z-score (MD=0.23, 95%CI 0.04, 0.42, p=0.02) were larger in the NVP arm compared to the LPV/r arm.

Infants starting on a LPV/r regimen but who then switched to a NVP-based regimen after a median time of 9 months on LPV/r were less likely to develop virological failure (defined as at least one VL greater than 50 copies/mL) compared with infants who started and stayed on LPV/r (HR=0.62, 95%CI 0.41, 0.92, p=0.02). However the hazard for confirmed failure at a higher viral load (>1000 copies/mL) was higher among children who switched to NVP compared to those who remained on LPV/r (HR=10.19, 95% CI 2.36, 43.94, p=0.002).

Authors' conclusions

Immediate ART reduces morbidity and mortality among infants and may improve neurodevelopmental outcome. However, it remains unclear whether all children diagnosed with HIV infection between 1-2 years of age should start ART, as has been recommended by the World Health Organization on practical grounds.

The available evidence suggests that a LPV/r-based first-line regimen is more potent than NVP, regardless of PMTCT exposure status. However, this finding provides a dilemma to policy-makers because higher cost, poor palatability, inconvenient formulation and cold chain requirements make LPV/r a more costly and challenging first-line regimen. An alternative approach to long-term LPV/r is substitute to NVP once virological suppression is achieved. This strategy looked promising in the one trial undertaken, but may be difficult to implement in the absence of VL testing.

Ongoing trials are exploring the possibility of starting early ART and interrupting treatment beyond the critical period of rapid disease progression and neurological development. Further evidence is urgently required to better inform policy on first-line treatment recommendations in young children and more robust data addressing non-virological outcomes are also needed.

4.1.2 Plain language summary

HIV-infected children under two years of age have a high risk of dying without antiretroviral therapy, but treatment in this age group is challenging because there are few suitable drug choices. Infants are often exposed to the antiretroviral drug nevirapine around the time of birth as part of strategies to reduce mother-to-child HIV transmission, and resistance to this class of drug is rapidly acquired. Results from this systematic review show that starting ART soon after birth is preferable to delaying treatment, because infants are less likely to die or become sick. Starting a first-line combination of treatment that includes the drug lopinavir/ritonavir (a protease inhibitor) rather than nevirapine (a non-nucleoside reverse transcriptase inhibitor) seems to be preferable, because infants are less likely to discontinue treatment, whether or not they had previously been exposed to nevirapine. However, lopinavir/ritonavir is more expensive than nevirapine, tastes bitterer, and is currently only available as an inconvenient liquid, which has to be refrigerated, making it difficult to recommend lopinavir/ritonavir as first-line treatment in all parts of the world. It may be possible to switch from lopinavir/ritonavir to nevirapine once the HIV virus levels are under control, but tests to measure the amount of virus in the blood are expensive and often unavailable. Other ongoing trials are exploring other ways to give a stronger drug combination to infants and the possibility of starting ART soon after birth but then stopping medication after 1-2 years.

4.1.3 Background

The number of children under the age of 15 years living with HIV increased from 1.6 million (range, 1.4 million-2.1 million) in 2001 to 2.5 million (range 1.6 million-3.4 million] in 2009, and over 1000 newly infected infants continue to be born daily [1]. In the absence of antiretroviral therapy (ART), over 50% of HIV-infected infants progress to AIDS and death by 2 years of age [14]. The introduction of ART has dramatically changed the natural history of HIV infection in children [2, 3], and now, in well-resourced countries, over 90% HIV-infected children reach the age of 10 years [4]. However, in 2009, only 28% (range 21%-43%) of the 1270 000 (range 830 000-1700 000) children estimated to need ART in low- and middle-income countries had access to it[7].

The natural history of perinatal HIV infection differs from that of primary infection in adults. Rapid disease progression is a hallmark of HIV infection during the first 2 years of life, especially in resource-limited settings. Perinatal infection occurs either *in utero* (mostly during the third trimester), during delivery, or after birth through breastfeeding. The pattern of viraemia in vertically infected children differs from that in infected adults, with HIV RNA levels remaining high throughout infancy (first 12 months of life) and, in the absence of treatment, decreasing only slowly to adult set-point levels over the next few years in slow progressors [63]. This difference most likely reflects the immaturity of the paediatric immune system in controlling viral replication. Viral load is generally a poor predictive marker of disease progression during infancy [12].

Normal CD4 counts in young children are consistently higher than in older children or adults and slowly decline to adult levels by approximately 5 years of age [64]. Age is therefore an important consideration in assessing by CD4 count the risk of progression for HIV-infected children [12]. Percentage CD4 count tends to vary less with age and is preferred in the first 5 years of life as a marker of HIV disease progression; however, the predictive value of the CD4 cell percentage for progression to AIDS or death is lower in children under two years of age, compared with older children. Infants have a higher short-term risk of clinical progression compared to older children and are at greater risk of acquiring opportunistic infections, such as *Pneumocystis jiroveci* pneumonia (PcP) and cytomegalovirus (CMV), even at high CD4 counts [11].

The Cross Continents Collaboration for Kids (3Cs4kids) study, which combined longitudinal data from approximately 2500 untreated HIV-infected children enrolled in African studies, evaluated the prognostic value of selected laboratory and growth markers for 12-month risk of mortality [13]. There were insufficient studies focusing on sub-Saharan African infants to include children <12 months of age in this analysis. Nevertheless, prognosis for children with a given CD4 count or percentage was shown to be poorer at younger ages, and the predictive value of both markers improved with age, similar to findings for European/US children [11]. Moreover, both CD4 percentage and count were shown to be less effective in discriminating between low and high mortality risk for children in resource-limited, compared to well-resourced, settings.

Because there are no good markers to predict disease progression during infancy, criteria for initiation of ART in HIV-infected infants have varied over time. Until 2007, there was no consensus between settings: US guidelines generally recommended consideration of ART for infants more strongly than did European guidelines, which only strongly recommended treatment for those with symptomatic disease or immune-suppression, but included the option to initiate ART in all infants. World Health Organization (WHO) recommendations for resource-limited settings gave CD4 threshold criteria, but these were based on analyses of CD4 percentage and viral load from European/US cohorts [12].

Several small, observational studies in the United States and Europe have suggested benefit from early initiation of ART in infancy. Infants starting ART in the first months of life had less progression to AIDS and early-onset severe disease, compared to those starting ART later [25] [65]. Good clinical and immunological outcomes have been reported with early ART, although full virological suppression may be more difficult to achieve in infancy [24, 26, 27]. However, in most cases, HIV viral load reductions are generally poorer in children compared to adults [66, 67], which may be related to higher baseline viral load, difficulties with adherence and differences in pharmacokinetics of ART. A discordant response to ART, with an effective immunological response but sub-optimal virological response, maybe more frequently seen in HIV-infected children than in adults [68], particularly in children with advanced disease [69]. However, with availability of increasing numbers of drugs and increased clinical experience, response to ART throughout childhood has markedly improved [13, 70].

Although early initiation of ART may be beneficial for young children, lifelong treatment is problematic, given the limited availability of appropriate drugs, long-term ART toxicity, difficulties with adherence, risk of viral resistance, and cost of such a strategy. Although over

20 antiretroviral drugs are licensed worldwide for the treatment of HIV-infected adults, several are either unlicensed or do not have appropriate formulations for very young children. Treatment options are therefore limited, especially in countries where expensive drugs remain unavailable. Drug metabolism varies with age, and pharmacokinetic data are not available for many drugs in young children. Furthermore poor palatability and limited availability of liquid suspensions makes ART adherence more challenging in this age group

Efficacy of ART in young children may be also affected by maternal transmission of drug-resistant virus, arising either from multi-drug exposure in high-income countries or exposure to prevention of mother-to-child transmission (PMTCT) interventions, such as single-dose nevirapine (sd-NVP) in low- and middle-income countries. The prevalence of transmitted drug resistance in perinatal HIV infection increased by 58% between 1998 and 2002 in resource-rich countries [71, 72]. Scale-up of ART is expected to increase the prevalence of resistant transmitted virus in resource-limited settings over time. Infants who have HIV infection and are exposed to nevirapine through infant or maternal treatment or prophylaxis have demonstrable viral resistance [73-75], potentially compromising the response to nevirapine-containing first-line treatment regimens [76, 77]. The occurrence of drug resistance needs to be carefully considered in recommendations for first-line treatment regimens, especially in countries where sd-NVP remains the only affordable PMTCT intervention.

Thus, ART started in early childhood presents considerable challenges in terms of drug choice, particularly when considering the need for effective lifelong therapy with minimal toxicity. Immature renal function, altered hepatic enzyme activity and differences in drug absorption lead to variation in systemic exposure to antiretrovirals among infants. Administration of nucleoside reverse transcriptase inhibitors (NRTIs) is associated with increased rates of lactic acidosis, pancreatitis and hepatitis, due to mitochondrial toxicity [78]. Similar to HIV-infected adults, disorders of lipid metabolism and fat redistribution (the lipodystrophy syndrome) have been described in children receiving NRTIs and protease inhibitors (PIs) [79-81].

An alternative approach to the currently recommended lifelong treatment is to start early ART in infancy, followed by a period of treatment interruption. This strategy would allow the child to be protected during the period of greatest risk for HIV disease progression and mortality, but enable time off therapy beyond 1-2 years of age to reduce toxicity, cost and risk of resistance. Definitive, long-term ART would be restarted when the child meets standard age-

related treatment criteria. Ongoing trials in South Africa (CHER) and Kenya (ClinicalTrials.gov reference NCT00428116) are addressing this strategy.

This review focuses on ART for HIV-infected children under 2 years of age because of the unique issues relevant to this age group. HIV infection during the first 2 years of life is characterised by high mortality and significant morbidity during a period of rapid growth and neurodevelopment, yet prognostic markers to guide treatment decisions are poorly predictive. New treatment approaches in early life require a systematic appraisal of the available evidence in order to inform clinical decisions as well as national policies.

4.1.4 Objectives

The first objective of this review is to assess when to start ART in young children. The efficacy of early ART initiation in HIV-infected children less than 2 years of age will be compared to deferred ART, started according to standard clinical or immunological criteria. The second objective of this review is to address the question of what ART to start with, comparing first-line non-nucleoside reverse transcriptase inhibitor (NNRTI) and PI-based regimens in terms of efficacy and toxicity. If data are available, the third objective of this review is to address the issue of when to stop or switch ART.

4.1.5 Methods

Criteria for considering studies for this review

Types of studies
Randomised controlled trials.

Types of participants

Perinatally HIV-infected children under 2 years of age (since 24 months is the new WHO age limit below which universal treatment is recommended) were included from any setting.

Types of interventions
Three types of intervention were assessed:

- Timing of treatment: use of early compared to deferred ART

- Choice of treatment: use of NNRTI- versus PI-based regimens, in combination with any NRTI backbone

- Interruption or switch of treatment: continuation or switch to NNRTI-based regimens following initiation with PI-based regimens, in combination with any NRTI backbone

Types of outcome measures
The planned primary outcome measure was mortality and disease progression (defined as occurrence of new AIDS events (CDC Class C) and other serious HIV-related events (CDC Class B)). The planned secondary outcome measures were: Increase of CD4 percentage from ART initiation (as defined by each study, typically >10% from baseline); virological suppression (HIV RNA viral load below the level of assay detectability, typically 400 or 50 copies/mL plasma); virological failure (as defined by each study, typically over 1000 copies/mL plasma); change in growth from baseline values following ART initiation (absolute weight and height percentiles or z scores); neurodevelopmental outcome (Griffiths Mental Development Scales Scores: mean locomotor quotient and mean general quotient [82]; serious adverse events (SAE) and drug-related adverse events according to the NIAID SAE grade 1 to 4 rating criteria [Division of AIDS. Table for grading the severity of Adult and Paediatric Adverse Events. http://rcc.techres.com/Document/safetyandpharmacovigilance/DAIDS_AE_GradingTable_Clarification_August2009_Final.pdf]

Depending on the data available in the study report or provided by investigators the outcome definitions described above were modified for analysis and pooling. When data on only one trial were available for a question, individual trial results were described.

Search methods for identification of studies

The search was performed in consultation with the HIV/AIDS Trials Search Co-ordinator. We sought to identify all relevant studies, from 1997 to present, regardless of language or publication status, by searching the Cochrane HIV/AIDS Review Group Trials Register, The Cochrane Library, Pubmed, EMBASE and CENTRAL. In addition, the following specific search terms were used: infant, child, p(a)ediatric, highly active antiretroviral therapy, anti-retroviral agents, early antiretroviral therapy, deferred antiretroviral therapy, HIV infection, human immunodeficiency virus, acquired immunodeficiency syndrome, NNRTI, non-nucleoside reverse transcriptase inhibitors, NRTI, nucleoside reverse transcriptase inhibitors, PI, protease inhibitors, randomised controlled trial, and controlled clinical trial.

Also, abstracts from the following relevant conference proceedings were screened for potentially eligible trials: World AIDS Conference; International AIDS Society conference (IAS) and Conference on Retroviruses and Opportunistic Infections (CROI). We also searched for unpublished and ongoing studies by considering prospective clinical trial registries (http://clinicaltrials.gov -NIH and WHO International Clinical Trials Registry Platform-ICTRP) and by contacting research organizations and experts in the field.

Data collection and analysis

Selection of studies

Two of the authors (MP and AP) independently screened records identified by the search. Studies were identified if they met the eligibility criteria and were only rejected on initial screening if they did not include children less than 2 years of age or did not contain at least one comparison of ART approach in terms of initiation or drug regimen choice.

Data extraction and management

MP and AP independently extracted data from the studies on patient characteristics, interventions and outcomes onto pre-designed forms. They independently cross-checked and assessed these data, with any disagreement resolved by consensus with a third review author (JT), when necessary. When a review author had authored an eligible study, an independent party assisted in both the extraction of data and assessment of methodological quality of that study.

If insufficient data were available in the study report, further information was sought from the publication authors.

Assessment of risk of bias in included studies

Various aspects of the methodological quality of included studies were assessed independently by MP and AP using the risk of bias tool [61]. Any discrepancies were resolved by consensus with a third review author (JT).

Measures of treatment effect

For meta-analysis of time-to-event outcomes, such as death and disease progression, the most appropriate statistic is the hazard ratio (HR). Where available, the HR and associated statistics were extracted directly from the trial report. When a HR was not provided in the trial report and only Kaplan-Meier curves were available, trial investigators were contacted to obtain the relevant HRs and related statistics.

For dichotomous outcomes, such as decline by 10% in CD4%, a risk ratio (RR) of the rate of the occurrence was calculated from events and number of patients. For continuous outcomes, such as change in CD4 percentage or weight and height z score, the mean difference was calculated.

Dealing with missing data

Where the publication did not provide enough data, and this could preclude reliable estimation for treatment effect, we contacted the trial authors for further information. If insufficient data were still available for any particular outcome, this was described qualitatively rather than analysed quantitatively.

Assessment of heterogeneity

Any qualitative or quantitative heterogeneity indicated by a Chi-squared test ($p<0.2$) was investigated, where possible, by considering trial-level explanatory factors and the subgroup analyses described below.

Assessment of reporting biases

Formal methods (as described by Egger in the Cochrane Handbook, [61] were planned to investigate the presence of reporting bias, but where few trials existed, the likelihood of reporting bias was instead described.

Data synthesis

Where more than one trial was identified for the questions being addressed, the HRs or RRs for each outcome for each trial were combined in meta-analysis to give a pooled HR or RR, using the fixed-effect model (FEM). A random-effect model (REM) was also used to test the robustness of the results to the choice of model.

Subgroup analysis and investigation of heterogeneity

Pre-specified trial subgroup analyses were carried out according to age, previous antiretroviral NNRTI exposure or documented NNRTI resistance, if sufficient data were available.

Sensitivity analysis

If heterogeneity was detected and could not be explained by subgroup analyses then sensitivity analyses were conducted.

4.1.6 Results

4.1.6.1 Description of studies

Result of search

Medline/Pubmed yielded 697 records, CENTRAL 870 and EMBASE 354 to give a total of 1921 records. Of the 1308 records remaining after duplicates were removed, 4 referred to studies that were eligible for inclusion in the review. One additional study was identified in conference proceedings (Figure 2). Two of these studies were eligible for the question of when to start treatment, two for what to start and one for the strategy of switching to a NVP-based regimen. An additional study on switching to an EFV-based regimen was found but enrolment was only recently started and the study is still on going. Three studies were identified that address the question of treatment interruption, but these studies are ongoing and results are awaited.

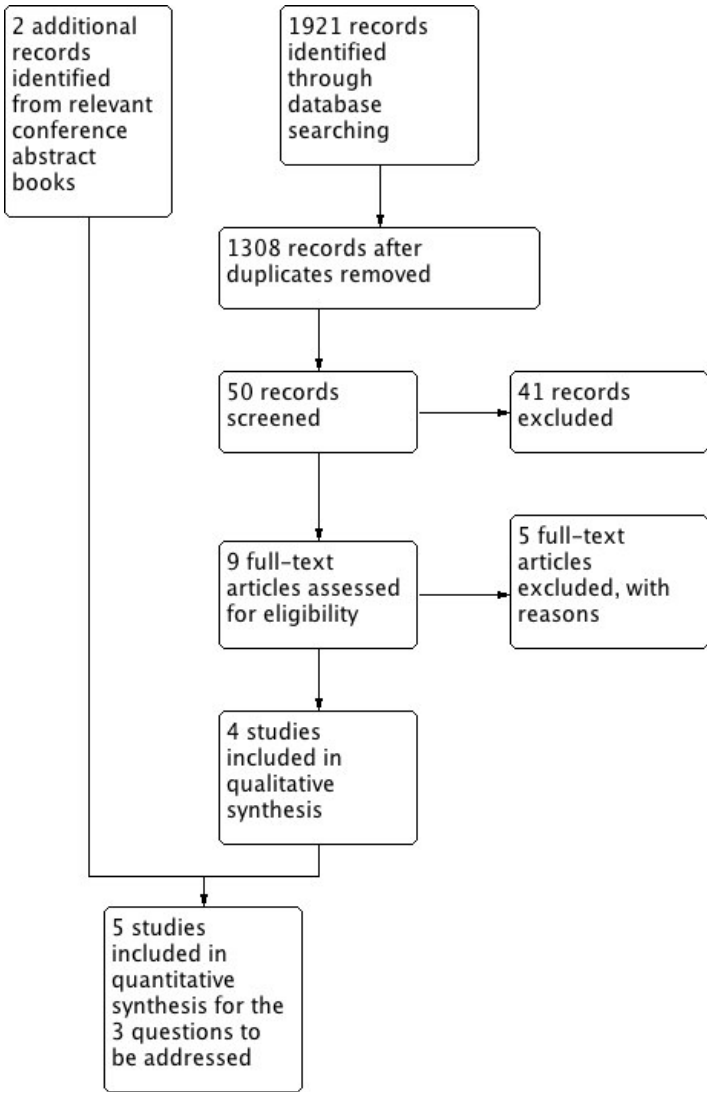


Figure 2 Study flow diagram.

Included trials

1. When to start

The CHER study [22] is a randomised controlled trial conducted in South Africa. Infants (n=377) from 6 to 12 weeks of age who had asymptomatic HIV infection and a CD4 percentage of 25% or more were randomised to immediate or deferred (according to WHO 2006 clinical or immunological criteria) antiretroviral treatment with a LPV/r-based regimen containing lamivudine and zidovudine. In two of three study arms infants were started on immediate treatment and maintained on it for one or two years before complete treatment discontinuation. The primary outcome was mortality or disease progression. Immunological response, growth and toxicity were also assessed in the three study arms. After a review by the Data and Safety Monitoring Board, the deferred-therapy group was modified, and infants in this group were all reassessed for initiation of antiretroviral therapy. The trial has continued its planned 3.5-5 year follow-up.

A second study, conducted in Durban, South Africa [83] was also included to address the timing of treatment initiation. This was a randomised controlled trial of 63 infants, designed as a feasibility pilot study to evaluate three approaches to antiretroviral treatment of HIV-infected infants. Infants were randomised at diagnosis to one of three study arms: deferred ART, started once clinical or immunological criteria were reached; immediate ART given for 1 year then stopped; or immediate ART given with up to three structured treatment interruptions to 18 months of age, and then stopped. Four-drug antiretroviral therapy (zidovudine/lamivudine/nelfinavir/nevirapine) was used as the first-line antiretroviral regimen. The primary endpoint was the proportion of infants progressing to AIDS by 3 years. However, mortality, morbidity, virological and immunological response were also measured at one year from treatment initiation and compared between study arms.

2. What to start with

P1060 was a randomised trial in six African countries of initial therapy with zidovudine and lamivudine plus either NVP or LPV/r in 164 HIV-infected children 6 to 36 months of age, who qualified for treatment according to World Health Organization (WHO) criteria . Children enrolled in cohort 1 [32] had all been exposed to single-dose nevirapine prophylaxis as part of PMTCT interventions. The primary end point was treatment failure (defined as permanent

discontinuation of the treatment regimen for any reason including death, toxic effects and virological failure) by study week 24. Secondary endpoints were virological failure (defined as a confirmed plasma HIV-1 RNA level of less than 1 log10 copies per millilitre below the study entry level at 12 to 24 weeks after the initiation of treatment or a confirmed plasma HIV-1 RNA level of more than 400 copies per millilitre at 24 weeks) or death by study week 24; time to virological failure or discontinuation over the follow-up period and time to virological failure or death over the follow-up period. Immunological response, growth and toxicity were also measured. Enrolment in this cohort was terminated early on the recommendation of the Data and Safety Monitoring Board.

In P1060 cohort 2 [34], a parallel randomised trial to P1060 cohort 1, 288 children between 2 and 36 months of age who had not been exposed to single-dose nevirapine prophylaxis (or to any antiretroviral drug taken by the mother) as part of PMTCT interventions were randomised to NVP-based or LPV/R-based first line antiretroviral therapy. The primary endpoint, similar to cohort 1, was treatment failure (defined as permanent discontinuation of the treatment regimen for any reason including death, toxic effects and virological failure) by study week 24. The secondary endpoints were: virological failure (defined as a confirmed plasma HIV-1 RNA level of less than 1 log10 copies per millilitre below the study entry level at 12 to 24 weeks after the initiation of treatment or a confirmed plasma HIV-1 RNA level of more than 400 copies per millilitre at 24 weeks) or death by study week 24; time to virological failure or discontinuation over the follow-up period and time to virological failure or death over the follow-up period. Immunological response, growth and toxicity were also measured. This study was also terminated early as the Data Safety Monitoring Board recommended un-blinding the study results in October 2010 when 24 week endpoints had been reached by all participants.

3. Switch from LPV/r to NVP

The NEVEREST study [51] was a randomised controlled trial that enrolled 323 infants under two years of age who had previously been exposed to NVP. After initiating treatment with LPV/r plus lamivudine and stavudine, 195 infants who had maintained a viral load of <400 HIV-1 RNA copies/mL for at least 3 months were randomised either to either remain on LPV/r or switch to NVP for 52 weeks. Mortality, virological suppression, immunological response, growth and toxicity were assessed in the two study groups. Follow-up data to week 156 were also collected and analysed.

Ongoing trials

The question concerning treatment interruption 1 or 2 years after initiation is currently being addressed by both CHER and the Durban study, with results expected in 2012. An additional study (NCT00428116) addressing this question, currently ongoing in Kenya, was retrieved from the clinicaltrial.gov register.

To our knowledge no other randomised control trials are underway for this specific age group to assess time to treatment initiation or safety of switching to NVP-based regimens, but and additional study (ANRS 12206- MONOD) is assessing safety and efficacy of switching from LPV/r to a once daily EFV-based regimen in children who started treatment early (less than 2 years).

4.1.6.2Risk of bias in included studies

The sequence generation was computerized and performed centrally by the trial statistician in all the studies included. Allocation was adequately concealed by using opaque envelopes or electronic interfaces, opened at the time of randomisation in all studies (Figure 3).

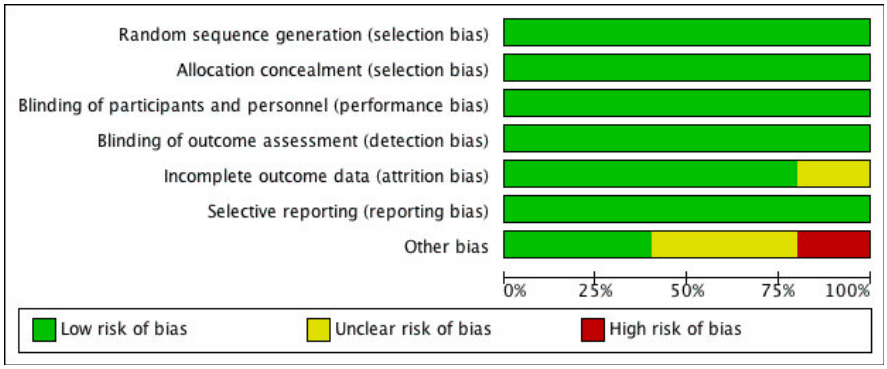


Figure 3 Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

Participants were not blinded to arm allocation, but the study endpoints were unlikely to be affected by unmasking. Blinding of outcome assessment was only reported for the CHER trial [22]; however, endpoint assessments for P1060 [32, 34] and NEVEREST [51] relied mainly on laboratory measurements, which are unlikely to be affected by unmasking.

Incomplete outcome data were reported in detail for Coovadia et al where a modified intention-to-treat analysis was conducted, but few patients were excluded. An intention to

treat analysis of all patients was performed for the CHER trial, P1060 cohort 1 and 2 such that attrition bias is not an issue. No loss to follow-up was reported for the Durban trial [83].

The primary outcome was pre-specified in the study protocols and provided in study reports or by the investigators, so selective reporting at least of this outcome is unlikely. Virological and immunological outcomes were also reported as expected given the nature of the questions under investigation.

Given the small number of studies, it was not possible to formally assess other reporting biases. However, given the extensive searches of the standard and grey literature and trial registers, and also contact with experts in the field, it is unlikely that publication bias is an issue in this review (Figure 4).

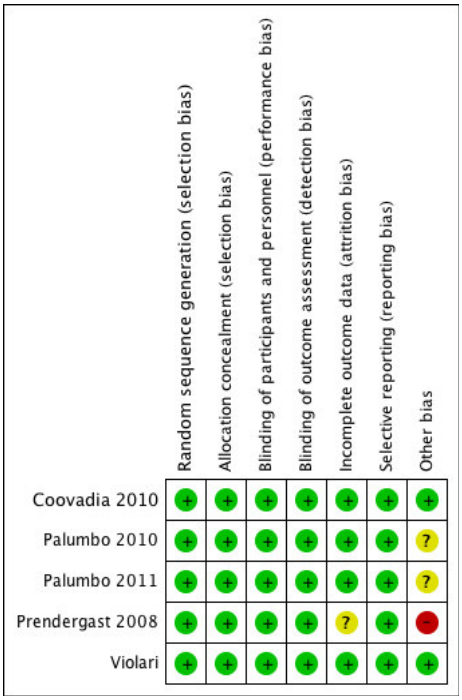


Figure 4 Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

P1060 cohort 1 and cohort 2 were terminated early as recommended by the DSMB on the basis of the pre-specified criteria, and the CHER trial was modified to recall and evaluate all deferred arm infants for immediate ART. Despite pre-specified criteria applied for termination of P1060 potential biases due to early termination of the studies should be considered. The Durban trial was originally designed as a feasibility study and was not powered to assess difference in mortality between arms. The NEVEREST trial enrolled patients quickly achieving virological suppression and it is unclear whether this may add further risk of bias and impact on generalizability.

4.1.6.3 Effects of interventions

1. When to start

Two studies assessed when to start treatment. The pooled HR for time to death for these two trials of 0.36 (95%CI 0.18-0.74) suggests a significant 64% relative reduction in mortality among infants starting ART early compared to those starting deferred ART once clinical and immunological criteria had been met (Figure 5). However, there is strong evidence (p=0.005, I^2=87%) that the effect of early ART on mortality differs between these two studies, such that the fixed effect model is not entirely appropriate. While the random-effects model suggests the effect of ART on mortality is much smaller with very large confidence intervals (HR 0.89, 95%CI 0.05-15) this gives considerable weight to the small Durban pilot study which may not be appropriate, as it was not designed or powered to address this question. Certainly, the weight of the current evidence, as provided by the CHER study, is in favour of early ART (HR=0.24, 95%CI 0.11, 0.51, p<0.001).

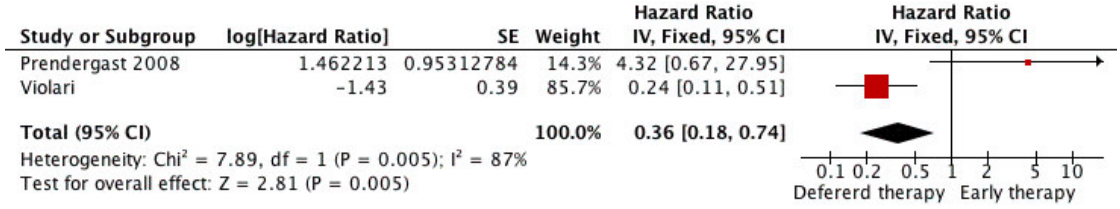


Figure 5 Forest plot of comparison: Early vs Deferred antiretroviral treatment, outcome: Mortality.

The combined outcome of mortality or disease progression could only be assessed for the CHER trial as the Durban study only reported hospitalizations. Early treatment was strongly associated with a 75% reduction (HR=0.25, 95%CI 0.12-0.51, p=0.0002,Figure 6) in time to mortality or disease progression as compared to deferred treatment.

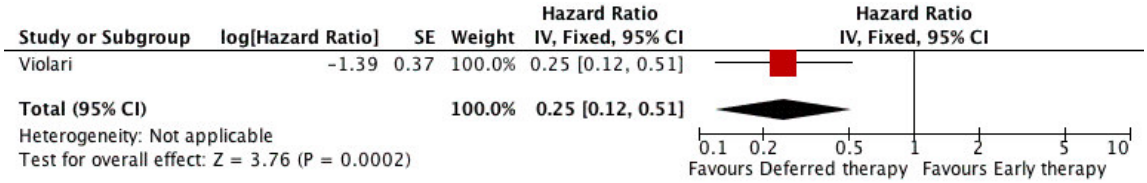


Figure 6 Forest plot of comparison: Early vs Deferred antiretroviral treatment, outcome: Mortality or disease progression.

Immunological response was not combined in meta-analysis as the two studies provided different immunological endpoints. In the Durban study median CD4 cell count was not

significantly different between groups 12 months after ART initiation (immediate group 33% vs deferred group 32%; P=0.70; Mann–Whitney test). In the CHER study the mean changes from baseline in the CD4 percentage were reported and absolute difference between the early-therapy groups and the deferred-therapy group was 12.3% (p<0.001) at 12 weeks, 11.5% (p<0.001) at 32 weeks, 9.3% (p<0.001) at 24 weeks and 6.7% by week 40.

Neurodevelopmental outcomes or growth parameters were not assessed in a randomised fashion by either study.

2. What to start

Two studies assessed what antiretroviral regimen to start in children <2 years of age. Neither study was powered to assess mortality or disease progression as independent endpoints. Overall, the hazard for treatment failure (defined as permanent discontinuation of the treatment regimen for any reason including death, toxic effects and virological failure) was 2.01 (95%CI 1.47-2.77) times higher in children starting ART with a NVP-based regimen compared to those starting with a LPV/r-based regimen (p<0.0001, Figure 7). These findings are consistent across studies (p=0.88, I^2=0%), suggesting that results are similar for NNRTI-exposed and unexposed children. There was no clear difference in effect by age group (subgroup heterogeneity: p=0.97; I^2=0%) with a similar increase in the risk of treatment failure in children starting NVP-based regimens regardless of whether they were older (HR=2.00 95%CI 1.32, 3.03, p=0.001) or younger (HR=2.03, 95%CI=1.24-3.32, p=0.005) than 12 months. The results were similar when a random-effects model was used.

The hazard for virological failure (defined as a confirmed plasma HIV-1 RNA level of less than 1 log10 copies per millilitre below the study entry level at 12 to 24 weeks after the initiation of treatment or a confirmed plasma HIV-1 RNA level of more than 400 copies per millilitre at 24 weeks) was overall 2.28 (95%CI 1.55-3.34) times higher for children starting ART with a NVP-based regimen compared to those starting with a LPV/r-based regimen (p=0.0005,Figure 8). Some modest heterogeneity was found (P=0.23, I^2=31%) across studies suggesting that a difference may exist between NNRTI-exposed and unexposed children. However, the random-effect model gives a similar result (HR=2.46 95%CI 1.48-4.08) and the evidence of association (p<0.0001) between a NVP-based regimen and a shorter time to virological failure (or death) remains very strong.

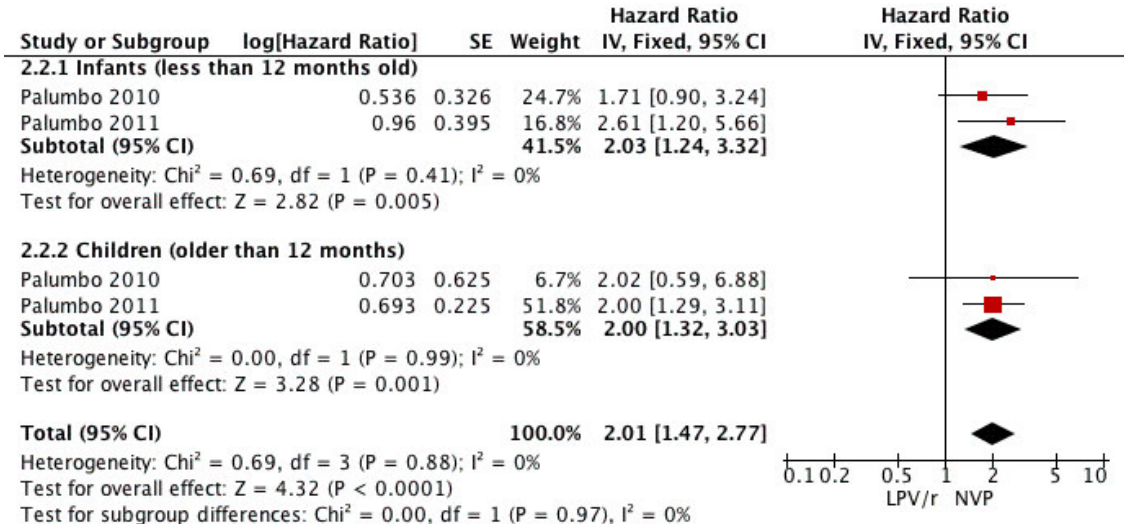


Figure 7 Forest plot of comparison: NVP-based vs LPV/r-based first line antiretroviral therapy, outcome: Treatment failure (virological failure or treatment discontinuation).

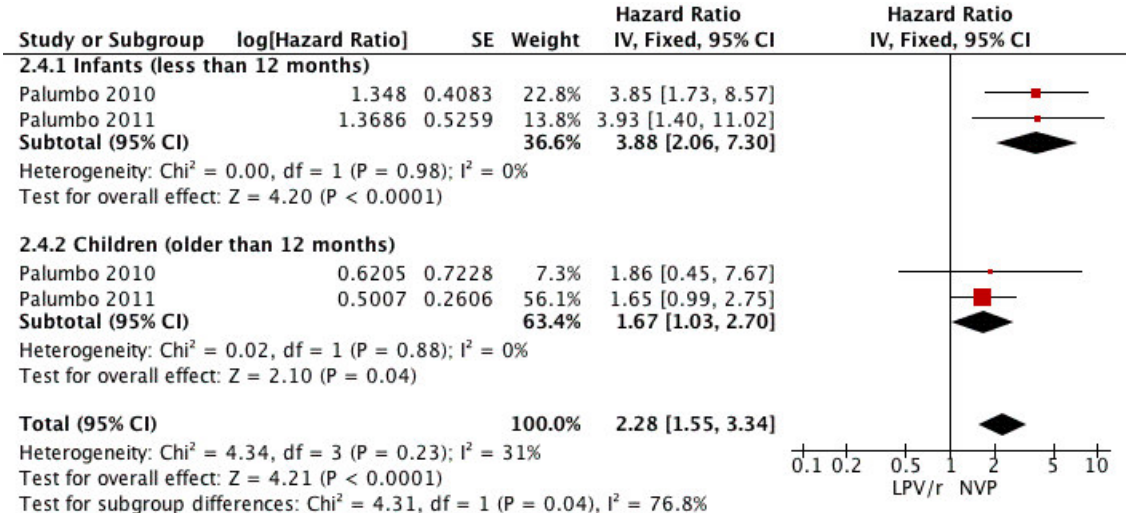


Figure 8 Forest plot of comparison: NVP-based vs LPV/r-based first line antiretroviral therapy, outcome: Virological failure or death.

The heterogeneity seems to be partly explained by a difference in effect by age group (subgroup heterogeneity: p=0.04). The HR for virological failure (or death) in the NVP-based regimen group compared to the LPV/r-based regimen group was larger in infants less than 12 months (HR 3.88, 95%CI 2.06-7.30, p<0.0001), compared to older children (HR 1.67, 95%CI 1.03-2.70, p=0.04) with no differences between trials within these age-groups. This suggests a larger difference in time to virological failure (or death) between the NVP and LPV/r-based regimens when ART is initiated in the first year of life.

Subgroup analysis accounting for the presence of documented baseline NNRTI resistance was not possible as an adequate measure of effect in the subgroups could not be obtained for both studies. However, in P1060 cohort 1 [32] it was reported that among those children with documented NNRTI resistance the difference between the proportion reaching a primary endpoint in the two study arm was much larger (83.3% vs 18.2%) than the one observed (35.8% vs 20.3%) in those without resistance (p=0.02 for interaction).

There was weak evidence of an association between treatment arm and immunological response (MD=1.56, 95%CI -0.29-3.41, p=0.10,Figure 9) and heterogeneity was limited (p=0.22; I^2 = 34%). The random-effect model gave similar results (MD=1.79, 95%CI -0.70-4.28, p=0.16). These results provide some indication that the increase in CD4% maybe greater in the NVP arm as compared to the LPV/r arm.

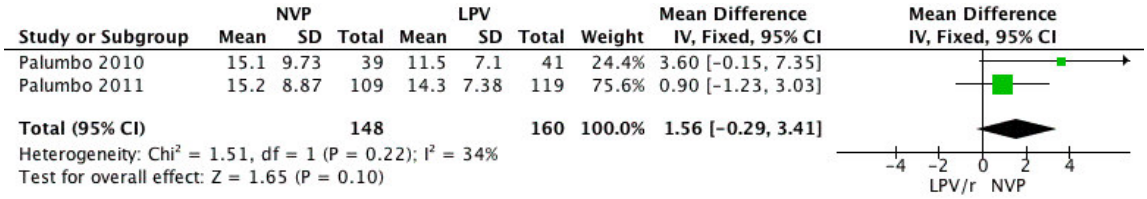


Figure 9 Forest plot of comparison: VP-based vs LPV/r-based first line antiretroviral therapy, outcome: Change in CD4% from baseline.

In contrast to the findings for primary endpoints, the increase in weight z-score was greater in the NVP arm compared to the LPV/r arm (MD=0.37, 95%CI 0.08-0.65, p=0.01,Figure 10). These findings are consistent across studies (p= 0.63; I^2 = 0%), suggesting that results are similar for NNRTI-exposed and unexposed children. The robustness of these findings was further confirmed with the random-effects model (MD=0.37, 95%CI 0.080, 0.65).

Figure 10 Forest plot of comparison: NVP-based vs LPV/r-based first line antiretroviral therapy, outcome: Change in weight zscore from baseline.

Similarly, a weak association was found between treatment arm and change in mean height z-score whether the fixed (MD=0.23, 95%CI 0.04-0.42, p=0.02,Figure 11), or random-effects

model (0.26, 95%CI -0.01-0.53, p=0.06) was used, with the change being higher in the NVP group compared to the LPV/r group. Heterogeneity detected across studies was modest (p=0.23, I^2=29%).

Figure 11 Forest plot of comparison: NVP-based vs LPV/r-based first line antiretroviral therapy, outcome: Change in height zscore from baseline.

Adverse events associated with treatment were not significantly more frequent in the NVP arm with either the fixed (RR=1.34, 95%CI 0.81-2.23, p=0.26,Figure 12) or random effects model (RR=1.44, 95%CI 0.60-3.46, p=0.42). Some difference in effect across studies was found (heterogeneity: p=0.12; I^2=58%).

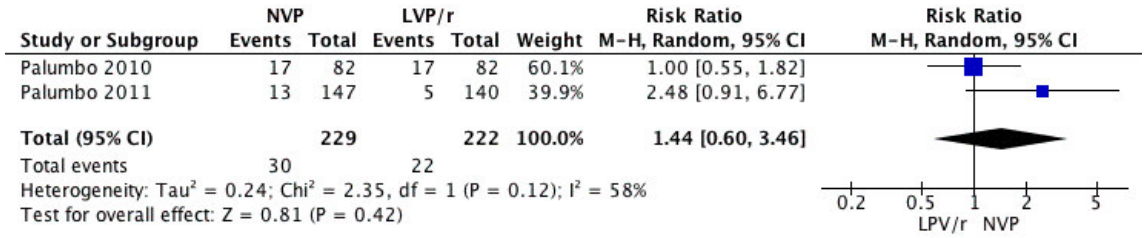


Figure 12 Forest plot of comparison: NVP-based vs LPV/r-based first line antiretroviral therapy, outcome: Adverse events

3. Switch from LPV/r to NVP

Only one trial addressing the question of treatment switching was included. In the NEVEREST trial [51], investigating infants and young children who had previously been exposed to NVP, the risk of having at least one VL greater than 50 copies/mL was lower in children switching to a NVP-based regimen after a median of 9 months on LPV/r-based regimen (having achieved virological suppression) compared to those remaining on a LVP/r-based regimen (HR=0.62, 95%CI 0.41-0.92, p=0.02,Figure 13).

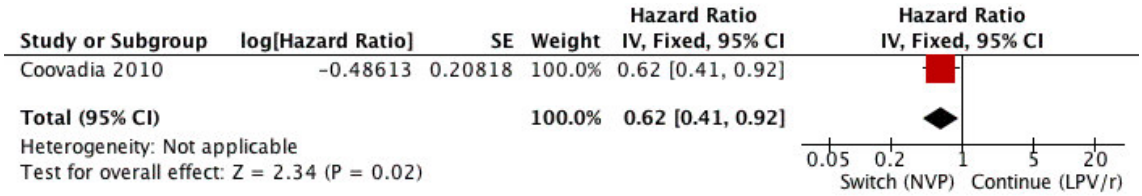


Figure 13 Forest plot of comparison: Switch to NVP vs continue on LPV/r, outcome: Virological failure (any VL>50 copies/mL).

However, the hazard for confirmed virological failure (>1000 copies/mL), however, was higher among children switching to NVP as compared to those remaining on LPV/r (HR=10.19, 95% CI 2.36, 43.94, p=0.002,Figure 14). CD4% increase was lower in the control (LPV/r) group compared to the switch (NVP) group (RR=0.22, 95% CI 0.07, 0.74, p=0.01,Figure 15). Weight-for-age Z scores were similar on average, but fewer children in the switch group experienced a decline in weight-for-age (RR=0.32, 95% CI 0.11-0.94, p=0.04,Figure 16).

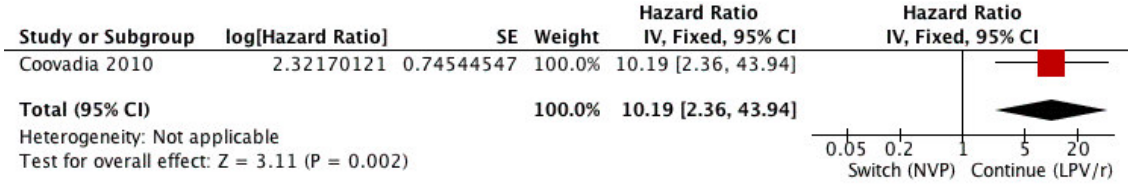


Figure 14 Forest plot of comparison: Switch to NVP vs continue on LPV/r, outcome: Virological failure (confirmed VL>1000 copies/mL).

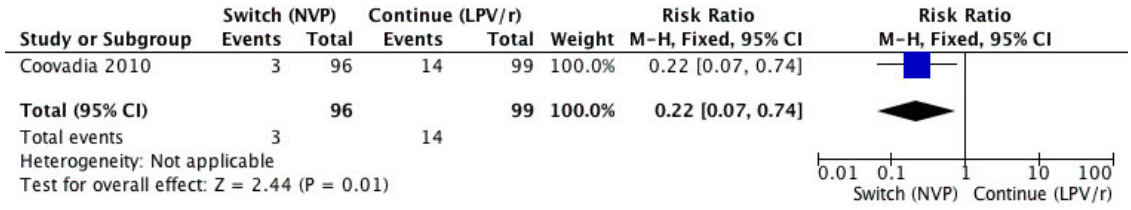


Figure 15 Forest plot of comparison: Switch to NVP vs continue on LPV/r, outcome: Decline by 10% in CD4% at week 52.

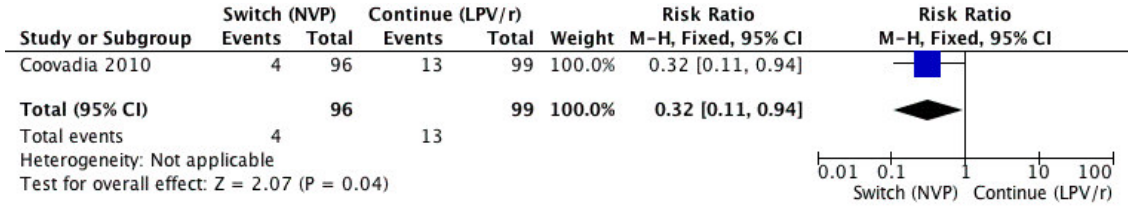


Figure 16 Forest plot of comparison: Switch to NVP vs continue on LPV/r, outcome: Decline by 1 z-score in weight-for-age at week 52.

4.1.7 Discussion

Paediatric HIV infection remains an important public health problem, with an estimated 1000 infants newly infected each day [1]. Treating young children is challenging because of lack of appropriate antiretroviral formulations, adherence difficulties, concerns regarding pharmacokinetics and reliance on caregivers. Furthermore, there are concerns regarding long-term drug toxicity, development of resistance and the cost of lifelong treatment when starting young children on ART. However, it has long been recognised that disease progression in infancy is rapid, and that the markers used to guide treatment decisions in older children and adults do not reliably identify those infants at highest risk of morbidity and mortality [11]. Mindful of these competing factors, and in the absence of randomised evidence, clinicians have historically varied in their practice regarding when to start ART in young children. Furthermore, decisions around which ART regimen to start are complicated by the possibility of drug resistance in the context of nevirapine exposure, the paucity of drug choices for infants, uncertain dosing for some medications and long-term toxicities. However, as reviewed here, there are now data from randomised trials to address these issues and guide clinicians in treatment choices for young children.

The question of when to start ART has been clarified by results from the CHER trial. In this trial, which recruited South African infants between 6-12 weeks of age, the data were so clear at interim DSMB review that results were released early and infants randomised to deferred ART were recalled to assess the need to start immediate treatment. There was a significant difference in both morbidity and mortality between infants randomised to immediate versus deferred ART. Deaths were often sudden and were not classically AIDS-defining; gastroenteritis and pneumonia predominated and mortality occurred even among infants with high CD4 counts. This highlights the difficulty of following up untreated infants, even in the setting of a trial, because of the rapidity of disease progression. Data from another trial included in this analysis, conducted in Durban, South Africa, showed a reduction in morbidity, but no significant difference in mortality between infants randomised to immediate versus deferred ART. Although there appears to be heterogeneity in these results, the Durban trial was a much smaller (n=63) feasibility study of different approaches to antiretroviral management in infancy; this trial was neither designed nor powered to address the question of 'when to start' ART. The results of the CHER trial were felt by policy makers to be generalisable

to all settings, as reflected in recent changes in US [9], European [84] and WHO guidelines [85] which all now recommend immediate initiation of ART in infancy. It should be noted, however, that most infants in CHER had been exposed to pMTCT (as sdNVP) and thus were infected despite prophylaxis, suggested in-utero acquisition to HIV infection, which maybe associated with faster disease progression compared with those infected in the perinatal period or post-natally through breast milk [86, 87]. Infants in CHER [22] were also identified early in life, as would be anticipated from a well functioning pMTCT programme. However, in many settings early infant diagnosis (EID) is delayed or even unavailable, and loss-to-follow-up from EID programmes is high. Infants recruited to CHER had relatively well preserved CD4 counts (CD4$\geq$25% was an entry criterion), but given the speed of disease progression after birth, many infants will have progressed to a CD4 count below this threshold by 6-12 weeks. It may therefore be anticipated in ART programmes that mortality will be higher than that reported for immediate therapy arms in CHER. This highlights the critical nature of an integrated and effective PMTCT/EID programme so that infants can benefit from early diagnosis and immediate initiation of ART.

The trials analysed here do not address the question of when ART should be initiated in young children beyond infancy. Whilst the majority of perinatally infected infants progress rapidly, a minority will be long-term non-progressors. An asymptomatic, HIV-infected child who is diagnosed in late infancy or beyond the first year of life may not require immediate initiation of ART. WHO guidelines [21], in the absence of trial data, and recognising that disease progression remains high in children from 1-2 years of age, made a pragmatic recommendation for universal ART below 2 years. However, this has not been adopted by all countries and the option exists to watch and wait for immunological or clinical disease progression beyond infancy.

Immediate ART, at least in young infants, reduces morbidity and mortality and may improve neurodevelopmental outcome. Data from the trials presented here show that good virological and immunological outcomes are achievable in early life. However, there are concerns regarding cost, long-term toxicity and viral resistance, in addition to the feasibility of early ART in terms of availability, palatability and adherence. The choice of first line therapy is therefore critical, particularly in the context of lifelong treatment.

The P1060 trial [32, 34] addressed the question of what ART to start, comparing a NVP-based regimen with a LPV/r-based regimen in young children below 3 years. Two parallel trials

recruited both sdNVP-exposed children (cohort 1) and sdNVP-unexposed children (cohort 2). It is well recognised that a substantial proportion of infants and young children exposed to sd-NVP at birth develop NNRTI resistance [73], and early data suggested that this may compromise the efficacy of NVP-based regimens [76]. This concern was further consolidated by the P1060 cohort 1 data, which showed a significantly higher discontinuation rate among sdNVP-exposed children randomised to an NNRTI, compared to LPV/r, regimen. Unexpectedly, sdNVP-unexposed children in cohort 2 also showed a higher treatment failure rate if randomised to a NVP, compared to LPV/r, regimen. This trial was not powered to evaluate mortality or disease progression as independent endpoints. The primary outcome, treatment failure, was a composite endpoint defined as permanent discontinuation of the regimen for any reason, including toxicity, virological failure and death. Methodologically, the P1060 trial was robust and investigators were as rigorous as possible in ensuring correct allocation of NVP exposure status, although it is difficult to completely rule out the possibility that some infants in cohort 2 were NVP exposed. Children in cohort 2 were older than children in cohort 1. After stratifying by age, there was no difference between infants and children older than 12 months for the primary endpoint of treatment failure (composite of death, toxicity and virological failure); however, a considerable difference was detected for the secondary endpoint comprising virological failure or death. This inconsistency in the subgroup analyses could be explained by a higher number of interruptions due to toxicity in children older than 12 months in the NVP arm. The strength of association between starting ART with NVP and having a higher risk of virological failure in infants (less than 12 months) was much larger than the risk observed in older children, suggesting that NNRTI resistance may have an attenuated effect in older children, who benefit from a "wash out" period; these findings would be consistent with data obtained from the NEVEREST cohort where detection of NNRTI-resistant strains was inversely correlated with increasing age at treatment initiation [88].

Overall, comparison of the data presented here shows that results from the two cohorts were consistent and that, in meta-analysis, no clear differences in effect were found across the two studies. It remains unclear why LPV/r should be superior to NVP even in children not previously exposed to sdNVP for PMTCT, as this has not been observed in adults. There are several theoretical explanations. First, infants may be disadvantaged by a NVP-based regimen because of the low genetic barrier to resistance in the context of high viral loads during early life. Of note the dose was increased during the course of the P1060 trial following release of new

WHO guidelines (from 4 mg/kg for 14 days and 7 mg/kg twice a day to 160-200 mg/m2/dose once daily for 14 days then 160-200 mg/m/dose). However, the authors did not find evidence for a dose effect to explain the observed failure rate among NVP-treated children. Second, it has been proposed that the use of lead-in dosing, whereby NVP is given only once-daily for the first 2 weeks, may lead to under-dosing and thereby facilitate NNRTI resistance and virological failure. However, in the CHAPAS 1 trial where children were randomised to initiate ART with full dose versus 'lead-in' for 2 weeks with half dose NVP, the viral load responses at 48 and 96 weeks were the same in the two groups [36]. Further investigation of early pharmacokinetics and viral load with 'lead-in NVP dosing is planned in the IMPAACT P1103 trial. Third, it is possible that infants without documented NNRTI exposure were, in fact, exposed to NVP, although the Authors made every effort to ensure that exposure history was accurate for each infant. Studies in South Africa have indicated that levels of transmitted resistance remain low[89] despite treatment scale-up, so acquisition of NNRTI resistance mutations as a consequence of multiresistant strains circulating at a population level seems unlikely. Although population sequencing was conducted in a subset of the P1060 study population, more sensitive allele-specific resistance assays of baseline samples from the 1060 Cohort 2 trial are ongoing to rule out the possibility of undocumented NVP exposure.

Although from the P1060 trial suggest that LPV/r-based regimens are preferable for all infants, regardless of PMTCT exposure status, these data are in contrast to those from the PENPACT-1 study [33], a multinational trial of first-line NNRTI versus PI therapy in children. Over 4 years of follow up, PENPACT-1 found no significant difference between children starting NNRTI- or PI-based regimens in primary (change in log10 HIV-1 RNA VL between baseline) or secondary endpoints (regimen switch, change in CD4% from baseline, VL <400c/ml at week 24 on first-line ART, VL <400c/ml at 4 years, continued VL suppression on first-line ART, failure of second-line ART, grade 3/4 adverse events, new CDC stage C events and resistance). PENPACT-1 was not included in the current systematic review because subgroup data were not available for children <2 years of age. Children in this trial were older (median age 6.5 (IQR: 2.8-12.9) years), and principally living in Europe and USA. Furthermore first-line ART in the PI arm included un-boosted PIs (Nelfinavir and Ritonavir), which are known to be sub-optimal, compared to ritonavir-boosted PIs. Nonetheless, a non-randomised analysis by individual drug in children aged <3 years (n=68) showed no significant difference in virological outcome between children receiving NVP or LPV/r (personal communication).

To our knowledge, no other trial is addressing the question of PI versus NNRTI 3-drug ART regimens started in early life, as the one trial of sdNVP-exposed infants underway in Kenya (ClinicalTrials.gov reference NCT00427297 trial) was terminated early following the results of the P1060 trial. A large observational study of ~400 infants followed in national cohorts for ~5 years in Europe [90] did not find evidence to support the P1060 trial findings. In this study, no difference was observed in viral load suppression and Cd4 response between infants initiating ART with PI versus NNRTI; however, children starting with 4-drug, 2 class (NNRTI+3NRTI) regimen did have superior virological and immunological responses after controlling for other factors in multivariate analysis. This approach is also being evaluated in the ongoing ARROW trial (ISRCTN24791884), which has currently enrolled 370 children less than 2 years and is due to complete in 2012.

Data presented here provide a dilemma to policy-makers: high-quality data from two randomised controlled trials suggest a benefit to LPV/r; however, the reasons for this finding (in non-NVP-exposed children) are not completely understood, and there are currently disadvantages to LPV/r over NVP in infancy, including cost, palatability, cold chain requirements and formulation. An alternative approach to long-term LPV/r, which was tested in the NEVEREST trial, is to start ART with a LPV/r-based regimen and switch to NVP once virological suppression is achieved. Interpretation of the NEVEREST results for the two virological endpoints would suggest that, at least for selected patients (without pre-treatment NVP resistance), switching to NVP could be a successful strategy. Children who switched to NVP were more likely to maintain viraemia below 50 copies/ml compared to those continuing LPV/r. By contrast, virological failure (>1000 copies/mL) was more common in those who switched to NVP and was strongly related to presence of pre-treatment NNRTI mutations. The authors suggest that the apparent inconsistency between the two virological endpoints may be explained by the sub-optimal adherence profile of infants continuing LPV/r, leading to occasional blips of HIV viraemia, whereas virological failure (VL>1000 copies/mL) occurred more commonly in those switching to NVP, because of the low genetic barrier to resistance.

Although the NEVEREST strategy appears promising and results are now confirmed by the week 156 follow up[91], the findings may not be easily generalisable since enrolled children had all achieved and sustained viral load <400 copies/mL for at least 3 months within the first 12 months of treatment. This selected population is likely to have better adherence, fewer problems with ART tolerability and an improved prognosis, compared to an unselected

population. Furthermore, this strategy would ideally require virological monitoring, both to identify eligible children and to detect early virological failure on NVP, together with resistance testing to identify those who would not be suitable for switching. Additional data to further address similar PI-sparing strategies will be investigated in the under two years old with the MONOD trial (ANRS 12206- MONOD) and in children between 3 and 5 years of age with the NEVEREST-3 trial (NCT01146873).

An alternative approach to the currently recommended lifelong treatment is to start early ART in infancy, followed by a period of treatment interruption. This strategy would allow the child to be protected during the period of greatest risk for HIV disease progression and mortality, but enable time off therapy beyond 1-2 years of age to reduce toxicity, cost and risk of resistance. Definitive, long-term ART would be restarted when the child meets standard age-related treatment criteria. Long-term data from trials in South Africa (CHER and Durban) and Kenya (ClinicalTrials.gov reference NCT00428116) will inform this potential strategy.

4.1.8 Authors' conclusions

4.1.8.1 Implications for practice

Recommendations for treatment initiation in children <2 years of age have already been updated in all settings, because of the unequivocal benefit of early treatment initiation in infancy. Policymakers are currently deliberating over the findings of the P1060 trial because of the implications of recommending PI-based therapy for all infants. Despite recommendations for LPV/r in NVP-exposed HIV-infected infants, only a few countries are currently using LPV/r as first-line treatment; the overwhelming majority of infants on this regimen are from South Africa. LPV/r is much more expensive than NVP and is currently only available for young infants as an unpalatable liquid formulation. However, a multi-unit particle preparation of LPV/r, stored in a capsule designed to be opened and sprinkled, is currently under evaluation in the CHAPAS 2 trial in Uganda and Zambia (ISRCTN01946535). Given these feasibility issues, it currently remains uncertain whether international guidelines will be updated in the light of the P1060 trial results. If results of the ARROW trial (ISRCTN24791884) which are due in 2012, confirm superiority of an induction-maintenance approach to first line ART using NVP with 3

NRTIs (ZDV, 3TC, ABC), as observed in the non-randomised EPPICC cohort [90], this could provide an alternative to LPV/r.

4.1.8.2 Implications for research

It remains unclear whether all children diagnosed with HIV infection between 1-2 years of age should start ART, as has been recommended on practical grounds by WHO. Although the risk of disease progression remains high at this age, a substantial proportion of children surviving beyond the first year of life will be slower progressors, who may not fulfil clinical or immunological criteria to start ART until late childhood or adolescence. However, there may still be a benefit to starting early ART, in terms of neurodevelopmental outcome and amelioration of the inflammatory consequences of HIV (so-called 'non-AIDS' morbidity). Future trials may provide clarity on this issue. Data are awaited from trials that are exploring the possibility of starting early ART and interrupting treatment beyond the critical period of rapid disease progression and neurological development. However, even if this strategy proves promising, it will be necessary to undertake an adequately powered trial that compares important endpoints (mortality and disease progression) between infants randomised to this treatment interruption strategy and infants taking continuous ART from early infancy, which is now the standard of care in this age group.

Further evidence is urgently required to better inform policy on first-line treatment recommendations in young children. Data from non-randomised, observational studies might usefully be combined to inform an individual patient meta-analysis to further address the question of what regimen to start in children under 2 years of age. More robust data addressing non-virological outcomes are also needed.

More definite answers on the effectiveness of switching to nevirapine after virological suppression with LPV/r-based regimens are needed, particularly regarding how best to identify those children who may benefit from such a strategy and the mid- and long-term impact on treatment sequencing and virological control.

4.1.9 Appendices

4.1.9.1 Acknowledgements

We are particularly grateful to Jane Lindsey, Paul Palumbo, Avy Violari, Elaine Abrams and the P1060 trial team investigators on the who provided supplementary data for the analyses; Louise Kuhn and the NEVEREST team; Steve Welch; Tim Clayton; Katja Doerholt.

4.1.9.2 Contributions of authors

MP and AP were the lead authors. MP and AP scrutinised identified studies for eligibility, extracted data and assessed the methodological quality of included studies. MP and JT performed the analysis. All authors critically reviewed the manuscript before submission.

4.1.9.3 Declarations of interest

Mark Cotton and Di Gibb are co-investigators on the CHER trial. Andrew Prendergast is a co-investigator on the Durban trial.

4.1.9.4 Characteristics of studies

a) Characteristics of included studies

Coovadia 2010 [51]

Methods	Randomized open label trial
Participants	323 children less than 2 years old, median age 20 months (IQR 10-36) in the control and 19 (IQR 9-43) months in the switch group. Inclusion criteria: met clinical and immunological criteria for treatment initiation (WHO 2006), exposed to NVP and started on PI-based regimen; achieved and sustained less than 400 copies/ml for at least 3 months within the first 12 months of treatment Exclusion criteria: children receiving tuberculosis treatment, having abnormalities in ALT greater than grade 2 (grading from Division of AIDS guidelines) and needing acute treatment for opportunistic infections (except TB) or tumours
Interventions	Control: continuous PI based cART Intervention: switch to NVP-based cART
Outcomes	Mortality; Any viral load >50 copies/ml; Confirmed viral load >1000 copies/ml; Change in CD4%; Change in weight and height Z scores; Adverse events

Risk of bias table

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	quote:"Randomization was done in cohort blocks of variable size between 8 and 12. Allocations were generated by the study statistician" .
Allocation concealment (selection bias)	Low risk	quote:"allocations were concealed in opaque envelopes opened on site at the time of randomisation"
Blinding of participants and personnel (performance bias)	Low risk	None, but outcomes are unlikely to be biased by unmasking
Blinding of outcome assessment (detection bias)	Low risk	None, but outcome measurements are unlikely to be biased by unmasking
Incomplete outcome data (attrition bias)	Low risk	quote:"Modified intent-to-treat analyses were conducted excluding those children (n = 3) missing virologic outcome data" Missing data were reported in details
Selective reporting (reporting bias)	Low risk	Study protocol available in the public domain, risk of selective reporting is very unlikely
Other bias	Low risk	

Palumbo 2010 [32]

Methods	Phase II randomised clinical trial
Participants	123 children less than 36 months old Age:> 6 months to < 36 months (up to but not including the 3rd birthday) Inclusion criteria: If either the mother or the child had received NVP but subsequently amended to require specifically that the child had received nevirapine Had to have diagnosis or AIDS by 60 days from birth and be formula fed from birth. Treatment eligible as defined by the WHO paediatric algorithm. HIV-1 RNA >5,000 copies/mL within 60 days prior to study entry/randomisation Exclusion criteria: TB treatment or not met inclusion criteria
Interventions	NVP-based vs LPV/r-based first line antiretroviral therapy
Outcomes	Treatment failure (virological failure or discontinuation for any cause included death); virological failure; change in CD4%; change in weight and height z scores; adverse events.
Notes	Enrolment was terminated early on the recommendation of the data and safety monitoring board.

Risk of bias table

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	quote from the protocol:"dynamic permuted block system [stratified (<12months>)] "
Allocation concealment (selection bias)	Low risk	quote from correspondence: " The Subject Registration and Randomization System provides a web-based interface that leads site personnel through the checklist. If the subject satisfies the entry criteria, the system stores the subject's information in the central database and assigns a treatment based on a permuted block algorithm"
Blinding of participants and personnel (performance bias)	Low risk	None (from correspondence), but outcomes are unlikely to be biased by unmasking
Blinding of outcome assessment (detection bias)	Low risk	None (from correspondence), but outcome measurements are unlikely to be biased by unmasking
Incomplete outcome data (attrition bias)	Low risk	ITT was performed and attrition bias is unlikely
Selective reporting (reporting bias)	Low risk	Protocol available, selective outcome reporting is unlikely
Other bias	Unclear risk	Early termination of the study: "Enrollment in this cohort was terminated early on the recommendation of the data and safety monitoring board."

Palumbo 2011 [34]

Methods	Phase II randomised clinical trial
Participants	288 Age: > 2 months to < 36 months (up to but not including the 3rd birthday) Inclusion criteria: HIV-1 RNA >5,000 copies/mL within 60 days prior to study entry/randomisation. This can also be considered as the confirmatory HIV test in patients in whom only one positive HIV test is available at the time of screening, at sites where only one HIV PCR is normally performed for diagnosis of HIV infection. ARV naïve except for ART used in attempts to prevent intrapartum MTCT. (Infant ART use for < 1 week postpartum for prevention of MTCT is allowed.) Treatment eligible as defined by the WHO paediatric algorithm. Parent or legal guardian able and willing to provide signed informed consent and to have the subject followed at the clinical site. Maternal use of ARVs during pregnancy and/or during labor is permitted with the exception of NNRTIs. Documentation of lack of NVP exposure. Exclusion criteria: Grade >2 AST or ALT at screening. Any Grade >3 laboratory toxicity at screening. Receipt of ART other than for prevention of intrapartum MTCT. Infants who received ART past the first week of life (e.g. for prevention of breast milk transmission) were excluded from study entry. Acute, serious infections requiring active treatment (prophylaxis allowed [e.g. PCP, cryptococcal meningitis]). Subjects could be receiving treatment for active TB if this did not include rifamycin drugs, and with approval by the study chairs. Report of any maternal NVP or other NNRTI exposure prior to or during the pregnancy and during breastfeeding with this child, including single dose NVP, documented by either verbal report or through the clinic or hospital record (use of ART from the NRTI or PI classes was allowed). Report of infant NVP exposure at any time, including during the first week of life, documented by either verbal report or through the clinic or hospital record.
Interventions	NVP-based vs LPV/r-based first line antiretroviral therapy
Outcomes	Treatment failure (virological failure or discontinuation for any cause included death) at 24 weeks; virological failure or death at 24 weeks; time to treatment failure (virological failure or discontinuation for any cause included death); time to virological failure or death; change in CD4%; change in weight and height z scores; adverse events
Notes	In October 2010, the DSMB recommended un-blinding results.

Risk of bias table

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	quote from the protocol:"dynamic permuted block system [stratified (<12months>)] "
Allocation concealment (selection bias)	Low risk	quote from correspondence: " The Subject Registration and Randomization System provides a web-based interface that leads site personnel through the checklist. If the subject satisfies the entry criteria, the system stores the subject's information in the central database and assigns a treatment based on a permuted block algorithm"
Blinding of participants and personnel (performance bias)	Low risk	None (from correspondence), but outcome measurements are unlikely to be biased by unmasking
Blinding of outcome assessment (detection bias)	Low risk	None (from correspondence), but outcome measurements are unlikely to be biased by unmasking
Incomplete outcome data (attrition bias)	Low risk	ITT was performed and attrition bias is unlikely
Selective reporting (reporting bias)	Low risk	Protocol available, selective outcome reporting is unlikely
Other bias	Unclear risk	Early termination of the study:"In October 2010, the Data Safety Monitoring Board recommended un-blinding the study results. "

Prendergast [83]

Methods	Pilot randomised control trial
Participants	Age: 63 HIV infected infants from birth Inclusion criteria: born in one of the study hospitals and had confirmed intrauterine/intrapartum HIV infection and caregivers gave consent for enrolment. Exclusion criteria: gestational age less than 37 weeks, birth weight less than 2 kg, evidence of other congenital infections or severe abnormalities.
Interventions	Arm A: deferred ART Arm B: immediate ART given for 1 year, then stopped Arm C: immediate ART given with up to three structured treatment interruptions to 18 months of age, then stopped.
Outcomes	Mortality; morbidity; virological suppression; change in CD4%
Notes	Data regarding stopping or interrupting strategy are currently being analysed

Risk of bias table

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer generated sequence by trial statistician
Allocation concealment (selection bias)	Low risk	quote from correspondence: " Opaque envelope at study sites were used for concealment allocation"
Blinding of participants and personnel (performance bias)	Low risk	None, but outcome are unlikely to be biased by unmasking
Blinding of outcome assessment (detection bias)	Low risk	None, but outcome measurements are unlikely to be biased by unmasking
Incomplete outcome data (attrition bias)	Unclear risk	No loss to follow up was reported.
Selective reporting (reporting bias)	Low risk	Major outcomes to address efficacy and safety of antiretroviral therapy were all reported, therefore risk of bias is unlikely.
Other bias	High risk	The trial was a feasibility study and was not designed or powered to assess difference in mortality between the two arms.

Violari [22]

Methods	Phase 3 open-label randomised control trial
Participants	377 infants 6 to 12 weeks of age who had HIV infection (DNA PCR positive; RNA above 1000copies/ml) Inclusion criteria: CD4 percentage of 25% or more. Exclusion criteria: Presence of a severe CDC Stage B or Stage C disease, Grade 3 or 4 laboratory values for ALT and AST, absolute neutrophil count, haemoglobin, electrolytes, creatinine or clinical toxicity at screening, as defined by age appropriate toxicity tables; presence of any major congenital abnormalities that were life-threatening and any acute and clinically significant medical event at randomisation; inability to tolerate oral medication; 4) birth weight <2 kilograms; use of investigational drugs or any medications that are disallowed with protease inhibitors or non-nucleoside reverse transcriptase inhibitors and; inability of parent or legal guardian to attend regularly scheduled study visits.
Interventions	Infants were randomly assigned to receive one of three treatments: early limited antiretroviral therapy for 96 weeks, early limited antiretroviral therapy for 40 weeks, or deferred therapy
Outcomes	By week 24: Time to death or failure of the first-line antiretroviral therapy; HIV disease progression; change in CD4% during follow up; grade 3 and 4 drug-related events.
Notes	After a review by the data and safety monitoring board, the deferred-therapy group was modified, and infants in this group were all reassessed for initiation of antiretroviral therapy.

Risk of bias table

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	quote: "The randomisation schedule was prepared centrally by the trial statistician and faxed to the study sites."
Allocation concealment (selection bias)	Low risk	quote from correspondence: " prepared by the trial statistician and communicated by fax to the sites"
Blinding of participants and personnel (performance bias)	Low risk	None, but outcomes are unlikely to be biased by unmasking
Blinding of outcome assessment (detection bias)	Low risk	quote: "An independent end-point review committee reviewed all deaths and CDC stage C and severe stage B events without knowledge of CD4 values, status of antiretroviral therapy, or randomised treatment assignments.
Incomplete outcome data (attrition bias)	Low risk	Loss to follow up was 6% in the early treatment arm and 3% in deferred treatment arm. ITT analysis was performed by censoring LTFU.
Selective reporting (reporting bias)	Low risk	Protocol available, selective outcome reporting is unlikely.
Other bias	Low risk	Early stop: "After a review by the data and safety monitoring board, the deferred-therapy group was modified, and infants in this group were all reassessed for initiation of antiretroviral therapy". The guiding statistical criterion for "proof beyond reasonable doubt" was based on a difference of at least 3 SD in the log relative hazard (or nominal P<0.001) in any interim analysis (according to the Haybittle–Peto rule).

b) Characteristics of excluded studies

Ananworanich 2008	
Reason for exclusion	Children younger than 1 year were excluded from the study.
King 2005	
Reason for exclusion	Children included in the study were not treatment naive.
Krogstad 2002	
Reason for exclusion	Children included in the study were not treatment naive.
Lockman 2007	
Reason for exclusion	No comparison between NNRTI-based and PI-based was provided.
Luzuriaga 2004	
Reason for exclusion	This trial was not randomised.
NCT00427297	
Reason for exclusion	This trial was interrupted for lack of equipoise.
PENPACT-1 2011	
Reason for exclusion	Children less than 2 years old were poorly represented and no stratification by age was provided for the measure of effect.
Wiznia 2000	
Reason for exclusion	Children included in the study was not treatment naive.
Wongsawat 2010	
Reason for exclusion	Addressing a different comparison: CD4 count versus CD4% as criteria to start treatment

c) Characteristics of ongoing studies

ANRS 12206- MONOD

Study name	ANRS 12206 – MONOD: International phase 2b-3 randomized clinical trial to assess two once-daily simplified antiretroviral triple therapies among HIV-infected children early treated by a 12-month twice daily triple therapy between 6 weeks and 24 months of age and in virological success in Africa: the MONOD Project (Burkina Faso, Ivory Coast, Rwanda)
Methods	Open label randomized clincial trial
Participants	Inclusion Criteria for antiretroviral treatment initiation: - infant follow-up in one of the trial site - HIV-1 infection diagnose by RT PCR after 6 weeks of life - age between 3 and 12 month at the antiretroviral treatment initiation - naive of antiretrovirals except if received for the prevention of mother to child HIV transmission - HB>=7 g/dl, neutrophiles>750/mm3, creatinin<3xULN, TGO and TGP<3xULN - signed informed consent Exclusion Criteria for antiretroviral treatment initiation: - HIV-2 infection or HIV-1/HIV-2 co-infection - Known intolerance to one of the trial treatment - HB<7 g/dl, neutrophiles<750/mm3, creatinine>3xULN, TGO or TGP>3xULN Inclusion Criteria for randomisation at 12 months in the simplification phase: - age 24 months at most - virological success define as 2 consecutive indetectable HIV RNA measured by RT PCR at least 3 months apart. Exclusion Criteria for randomisation at 12 months in the simplification phase: - virological failure after the first 12 months of antiretroviral treatment
Interventions	Drug: AZT-3TC-LPV/r twice a day Drug: ABC-3TC-EFV once a day Drug: ABC-3TC-LPV/r once a day
Outcomes	Primary Outcome Measures: Virological success at 25 months (HIV RNA < 50 copies / mL) Secondary Outcome Measures: Virological success at 12 months (HIV RNA < 400 copies / mL) Immunological response at 12 and 25 months (CD4+ lymphocyte absolute count and percentage) Antiretroviral and cotrimoxazole pharmacokinetic parameters at 6, 19 and 25 months. Tolerance at 12 and 25 month (occurrence of grade 3 and 4 adverse events related to the trial treatment, particularly occurrence of immune reconstitution inflammatory syndrome) Adherence at 12 and 25 months Resistance to antiretroviral at 12 and 25 months
Starting date	June 2011
Contact information	Dr. Valeriane Leroy Valeriane.leroy@isped.u-bordeaux2.fr

NCT00428116

Study name	Optimizing Pediatric HIV-1 Treatment, Nairobi, Kenya (NCT00428116)
Methods	Open label randomised clinical trial
Participants	One hundred and fifty infants who initiated HAART at <13 months of age Inclusion Criteria: A. Infants newly initiating HAART Less than 13 months of age HIV-1 DNA detection with confirmation (positive on two HIV-1 DNA filter paper tests) Caregiver of infant plans to reside in Nairobi for at least 3 years (reported by caregiver) Caregiver is able to provide sufficient location information B. Infants already receiving ART Initiated HAART at <13 months of Records confirming HIV positive status Documentation of CD4% and weight prior to HAART initiation Must be on 1st line drug regimen Eligibility for randomisation: Completed 24 months of treatment with HAART Normalized growth: weight for height z-score > -0.5; Child's weight must be above the 5th weight-for-age percentile and the weight curve must not be flat or falling (i.e. cross 2 major percentile lines or more over the past 3 months) CD4% >= 25 Children who recently initiated or who require anti-tuberculosis treatment at the time of randomisation will be ineligible for randomisation.
Interventions	Infants will be treated with HAART regimen for 24 months after which those who have immune -reconstitution and adequate growth (~100) will be randomised to continue versus deferred therapy. Clinical outcomes will be compared in these children to determine if interruption is a safe and beneficial strategy.
Outcomes	Primary outcome: growth Secondary outcome: Incidence of morbidities, specifically pneumonia, diarrhoea, and hospitalisation.
Starting date	September 2007 (Estimated Study Completion Date: April 2013)
Contact information	Grace C John-Stewart, MD, PhD gjohn@u.washington.edu
Notes	Follow-up in this studies will be closely monitored by an external Data Safety and Monitoring Board (DSMB).

4.1.9.5 Data Analyses

a) Early versus deferred treatment

Outcome or Subgroup	Studies	Participants	Statistical Method	Effect Estimate
1.1 Mortality	2		Hazard Ratio(IV, Fixed, 95% CI)	0.36[0.18, 0.74]
1.2 Mortality or disease progression	1		Hazard Ratio(IV, Fixed, 95% CI)	0.25[0.12, 0.51]

b) NVP-based versus LPV/r- based first line antiretroviral therapy

Outcome or Subgroup	Studies	Participants	Statistical Method	Effect Estimate
2.1 Treatment failure (virological failure or treatment discontinuation)	2		Hazard Ratio(IV, Random, 95% CI)	2.02[1.47, 2.77]
2.2 Treatment failure (virological failure or treatment discontinuation)	2		Hazard Ratio(IV, Fixed, 95% CI)	2.01[1.47, 2.77]
2.2.1 Infants (less than 12 months old)	2		Hazard Ratio(IV, Fixed, 95% CI)	2.03[1.24, 3.32]
2.2.2 Children (older than 12 months)	2		Hazard Ratio(IV, Fixed, 95% CI)	2.00[1.32, 3.03]
2.3 Virological failure or death	2		Hazard Ratio(IV, Fixed, 95% CI)	2.24[1.51, 3.32]
2.4 Virological failure or death	2		Hazard Ratio(IV, Fixed, 95% CI)	2.28[1.55, 3.34]
2.4.1 Infants (less than 12 months)	2		Hazard Ratio(IV, Fixed, 95% CI)	3.88[2.06, 7.30]
2.4.2 Children (older than 12 months)	2		Hazard Ratio(IV, Fixed, 95% CI)	1.67[1.03, 2.70]
2.5 Change in CD4% from baseline	2	308	Mean Difference(IV, Fixed, 95% CI)	1.56[-0.29, 3.41]
2.6 Change in weight z-score from baseline	2	309	Mean Difference(IV, Fixed, 95% CI)	0.37[0.08, 0.65]
2.7 Change in height z-score from baseline	2	323	Mean Difference(IV, Fixed, 95% CI)	0.23[0.04, 0.42]

c) Switch to NVP versus continue on LPV/r

Outcome or Subgroup	Studies	Participants	Statistical Method	Effect Estimate
3.2 Virological failure (any VL>50 copie/ml)	1		Hazard Ratio(IV, Fixed, 95% CI)	0.62[0.41, 0.92]
3.3 Virological failure (confirmed VL>1000 copies/ml)	1		Hazard Ratio(IV, Fixed, 95% CI)	10.19[2.36, 43.94]
3.4 Decline by 10% in CD4% at week 52	1	195	Risk Ratio(M-H, Fixed, 95% CI)	0.22[0.07, 0.74]
3.5 Decline by 1 z-score in weight-for-age at week 52	1	195	Risk Ratio(M-H, Fixed, 95% CI)	0.32[0.11, 0.94]

5. Research activities II. Clinical Research

Randomized control trials are considered to provide the highest quality of evidence [92] and are worldwide the gold standard in clinical research to inform guidelines development on new drugs and on treatment or prevention strategies. However the setting up of clinical trials requires substantial resources and, even where resources are available, clinical trials are not always a feasible option.

Prospective cohort studies are therefore a good opportunity to consolidate or complete the evidence from RCT by gathering good quality data and producing descriptive and analytical studies. In particular, cohort collaborations allow answering specific questions, which otherwise single cohort may not be able to answer by making available a considerable amount of data.

The European Pregnancy and Paediatric Collaboration (EPPICC) was set up to conduct epidemiological research on the prognosis and outcome of HIV-infected pregnant women, children and children exposed to HIV in utero across Europe. The EPPICC research activity aims to focus on scientific questions requiring a large sample size of patients which the contributing cohorts cannot answer individually and/or which do not overlap with existing collaborations between participating EPPICC.

Currently about 17 cohorts representing 12 countries are part of the collaboration and, in 2009, 9 observational cohorts joined the first EPPICC project which was focused on the management of antiretroviral treatment in HIV-infected infants. Each cohort submitted data using a standardised template (the HIV Collaboration Data Exchange Protocol (HICDEP) to the coordinating centre at the MRC Clinical Trial Unit and data collected were subject to rigorous checks and quality assurance.

Standard practice regarding cART management in HIV infected infants varies across Europe and has changed over time. As mentioned in previous sections of this report, two recent trials have investigated the effectiveness of NNRTI- vs. PI-based first-line ART regimens in children obtained contradictory results [33, 34]. In studies including children who have received ART for prevention of mother-to-child transmission (pMTCT), exposure to single-dose nevirapine reduced subsequent response to NNRTI-based ART [32, 77, 93]. An alternative “induction-

maintenance" approach of starting with a 4-drug NNRTI-based regimen and reducing to 3-drug ART later [50] has been reported to be promising in the UK and Irish CHIPS cohort [70] but tis was based on non comparative cohort data; this approach is now under long-term evaluation in PMTCT exposed and unexposed children in the ongoing Ugandan/Zimbabwe ARROW trial (www.arrowtrial.org, completion in 2012).

This specific study was developed within the EPPICC collaboration to report on the use of cART for treatment of HIV in infants in Europe over time (1996-2008), to investigate factors associated with virological and immunological response to cART at 12 months after treatment initiation and to describe duration of first line therapy and predictors of stopping and switching.

This cohort analysis adds to the current debate about which is the optimal regimen for infants starting treatment early. These findings suggest that outside trial settings, an effective treatment response can now be achieved in infants who start ART early in life, with 65% of infants remaining on first-line ART without treatment interruption after 5 years. Results are in line with evidence in the PENPACT-1 trial, suggesting similar responses to initial 3-drug NNRTI-based and PI-based regimens, but in addition, suggest that a 4-drug NNRTI-based initial regimen maybe superior. However, this approach needs further evidence from on-going randomised trials, as do strategies of 4- to 3-drug NNRTI induction-maintenance and treatment interruption following early ART in infancy.

I carried out this work in close collaboration with senior staff (Ali Judd and Trinh Duong who conducted the statistical analysis) at the of the Medical Research Council clinical trial unit in London. Contribution was made on the study concept and design, the interpretation of results and the writing up of the manuscript. The findings were presented with a poster at the XVIII World AIDS Conference in Vienna and I subsequently presented the full set of results in an oral communication session at the 3rd HIV Paediatric Workshop in Rome. A paper entitled "Early antiretroviral therapy in HIV-1 infected infants in Europe, 1996-2008: treatment response and duration of first line regimens" has recently been published by AIDS (October 2011).

5.1 Early antiretroviral therapy in HIV-1 infected infants in Europe, 1996-2008: treatment response and duration of first line regimens

The European Pregnancy and Paediatric HIV Cohort Collaboration (EPPICC) study group in EuroCoord

5.1.1 Abstract

Objective: To investigate virological and immunological response to ART, and predictors of switching and interrupting treatment among infants starting ART across Europe.

Design: Cohort study.

Methods: 9 cohorts from 13 European countries contributed data on HIV-infected infants born 1996-2008 and starting ART before age 12 months. Logistic and linear regression, and competing risks methods were used to assess predictors of virological (viral load <400c/mL) and immunological (change in CD4 Z-score) response, switching to second-line ART and treatment interruptions with viral load <400c/mL.

Results: 437 infants were followed for median 5.9 (interquartile range 2.3-7.6) years after starting ART; 30% had an AIDS diagnosis prior to ART initiation. 53% had suppressed viral load <400c/mL at 12 months in 1996-1999, increasing to 77% in 2004-2008. Virological and immunological responses at 12 months varied by initial ART type ($p<0.001$ and $p=0.03$ respectively), with 4-drug NNRTI-based regimens being superior (virological response <400c/mL adjusted OR=3.00, 95%CI 1.24-7.23; mean increase in CD4 Z-score coefficient=0.64, 95%CI 0.10-1.17) to both 3-drug NNRTI-based (reference) and boosted PI regimens which were similar. Rates of switching to second-line ART were lower among children starting 4-drug NNRTI-based and boosted PI-based regimens compared to 3-drug NNRTI regimens ($p=0.03$). 65% of infants remained on first-line ART without treatment interruption after five years.

Conclusions: Effective and prolonged responses to first-line ART can now be achieved in infants starting early ART outside trial settings. Superior responses to 4-drug NNRTI compared with boosted PI or 3-drug NNRTI regimens need further evaluation, as does treatment interruption following early ART.

5.1.2 Introduction

Over 1000 HIV-infected infants are born each day worldwide.[7] Several studies [22, 23, 25, 83, 94] have shown that early initiation of combination antiretroviral therapy (ART) in HIV-infected infants, irrespective of clinical, immunological or virological condition, increases survival and reduces disease progression, and international guidelines have been changed accordingly [21, 84]. Although high levels of viral replication occur in vertically HIV-infected infants, early initiation of ART can result in sustained viral suppression and maintain CD4 values at protective levels [25-28]. However, some studies have reported that rates of virological failure are higher in infants starting therapy than in older children and adults [4, 24, 30, 31]. The efficacy, safety and tolerability of first-line ART regimens is therefore critical for HIV-infected infants who are likely to need life-long treatment.

Two recent trials investigated the effectiveness of different first-line ART regimens in children, with contradictory results. In the PENPACT-1 trial, 266 children from Europe, the USA and South America, aged one month to 18 years (26% ≤3 years) were randomised to start protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART [33]. At four years, >80% of children in both arms had viral load <400c/mL with no differences in CD4 responses; after 5 years 71% were still taking their first-line regimen. There was no evidence (but low power) to suggest that this result was any different in children initiating ART at <3 years. Conversely in the IMPAACT 1060 trial, conducted mainly in Africa, 288 children aged 2-36 months (median 20 months) and not exposed to nevirapine-based ART for the prevention of mother-to-child transmission (PMTCT) showed a significantly higher rate of treatment failure by 24 weeks in those starting nevirapine-based compared with lopinavir/ritonavir-based ART (40% v 19% respectively) [34].

In studies including children who have received ART for prevention of mother-to-child transmission (pMTCT), exposure to single-dose nevirapine reduced subsequent response to NNRTI-based ART, unless a PI-based ART regimen precedes simplification to an NNRTI-based regimen, as reported in the NEVEREST trial [95]. An alternative “induction-maintenance” approach of starting with a 4-drug NNRTI-based regimen and reducing to 3-drug ART later [50] has been reported to be promising in the UK and Irish CHIPS cohort [70, 96] and is under

evaluation for long-term efficacy in PMTCT exposed and unexposed children in the Ugandan/Zimbabwe ARROW trial (www.arrowtrial.org).

Standard practice regarding ART management in HIV-infected infants has varied across Europe and over time. Using data from the European Pregnancy and Paediatric HIV Cohort Collaboration (EPPICC, 1996-2008), we investigated factors associated with 12-month virological and immunological response to first-line ART and predictors of switching and interrupting therapy up to 5 years from treatment initiation.

5.1.3 Methods

Data from 9 observational cohort studies (5 national or multi-country cohorts and four city-based cohorts; 5 birth cohorts and 4 prevalent cohorts) in 13 European countries were merged using a standardised format [97]. Six cohorts with <25 infants each were combined into an "other" category. HIV-infected infants born between 1996 and 2008 and who started combination ART naïve were included.

Definitions and statistical methods

ART during infancy was defined as the first time ≥3 antiretroviral drugs were started within two weeks of each other and before 12 months of age, excluding ART for neonatal prophylaxis. Timing of ART initiation was categorised *a priori* as <3, 3-6, or 6-12 months of age [23, 98]. Baseline CD4 and HIV-1 RNA viral load values were defined as the latest pre-treatment measurements within three months before ART initiation. Virological and immunological responses were defined as viral load <400c/mL and mean change in CD4 Z-score at 12 months (±3 months) after ART initiation respectively [4]. CD4 Z-scores were used because of normal age-related changes in CD4 counts (and to a lesser extent CD4 percentages) during infancy [64].

Switching to second-line ART was defined as changing ≥3 drugs simultaneously irrespective of reasons, or changing two drugs with documented treatment failure (virological, immunological and/or clinical) [99]. Drug substitutions with undetectable viral load were likely related to toxicity or simplification, and were not included. Treatment interruption was defined as discontinuation of all medication for ≥14 days; our analyses focussed on interruptions with viral load <400 c/mL because they are most relevant as potential future treatment strategies. Viral loads and CD4 values at switch and treatment interruption were the latest measurements within 3 months before the event. We used virological and immunological measurements closest to 12 months after switching (±3 months).

The effects of potential predictors of virological and immunological responses to ART were analysed using logistic and linear regression respectively. Competing risk methods separately estimated the cumulative incidence of switching and of treatment interruption with viral load <400c/mL, and assessed potential predictors. Loss to follow-up, death and treatment

interruption with detectable viral load >400c/mL (in analysis of treatment interruption with viral load <400 c/mL only) were considered competing events [100].

A priori confounders in analyses of treatment response included in multivariate models were age and calendar year at ART initiation, type of initial ART regimen, and baseline CD4 z-score (for CD4 response only). Other potential predictors considered were: country, sex, ethnic group, baseline viral load, pre-treatment AIDS diagnosis, maternal ART during pregnancy, neonatal prophylaxis, and breastfeeding status; these factors remained in multivariable models if the corresponding p-values in univariable and multivariable models were <0.10.

A priori confounders in analyses of switching and of treatment interruption with viral load <400 c/mL were type of initial ART regimen, age at ART initiation, and country. Other potential predictors were sex, ethnic group, baseline viral load and CD4 z-score, pre-treatment AIDS diagnosis, and most recent CD4 Z-score. Finally, calendar period of follow-up, having a viral load <400c/mL, and confirmed rebound of viral load (defined as 2 consecutive viral loads >400c/mL within 12 months after having suppressed <400c/mL) were also considered, all fitted as time-dependent covariates. Children enrolled in planned treatment interruption trials (namely PENTA 11, n=8) were excluded in analyses of treatment interruption [52].

Missing data for covariates at ART initiation and for viral load at treatment interruption were imputed using chained equation methods with 20 imputations for regression analyses assessing potential predictors and for estimating cumulative incidence of treatment interruption with viral load <400 c/ml [101]. Statistical analyses were performed using Stata version 11 (Stata Corporation, College Station, Texas, USA).

5.1.4 Results

A total of 437 infants born between 1996 and 2008 started ART before 12 months of age at a median of 3.7 (IQR 2.1-5.8) months. Approximately 40% were from the UK/Ireland, 20% from Italy, and 20% from France (Table 1).

Table 1: Characteristics of infants at the time of ART initiation

	n (%) or median [IQR]	
Country of cohort		
UK or Ireland	169	(39%)
Italy	100	(23%)
France	82	(19%)
Other*	86	(20%)
Female sex	235	(54%)
Ethnic group		
White	145	(33%)
Black	216	(49%)
Other**	43	(10%)
Not known	33	(8%)
Maternal ART in pregnancy	150	(34%)
Infant neonatal prophylaxis* **	122	(28%)
Breastfed		
No	244	(56%)
Yes	144	(33%)
Not known	49	(11%)
AIDS diagnosis before ART initation	136	(31%)
Age at ART initiation		
<3 months	166	(38%)
3-5 months	165	(38%)
6-12 months	106	(24%)
Calendar year of ART initiation		
1996-1999	121	(28%)
2000-2003	180	(41%)
2004-2008	136	(31%)
Type of initial ART		
3-drug NNRTI-based	107	(24%)
4-drug NNRTI-based	61	(14%)
Boosted PI + 2 NRTIs****	67	(15%)
Unboosted PI + 2/3 NRTIs	166	(38%)
PI + NNRTI +/- NRTI or 3NRTIs	36	(8%)
CD4 count at ART initiation (cells/mm^3) (n=274)	1291	[460-2073]
CD4% at ART initiation (n=257)	29%	[17%-39%]
CD4 Z-score at ART initiation (n=274)	-1.7	[-3.1- -0.7]
Viral load at ART initiation (log_{10}c/mL) (n=330)	5.7	[4.9-5.9]

ART, antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

***** Includes infants from Belgium (n=17), Germany (n=5), the Netherlands (n=1), Poland (n=2), Romania (n=13), Spain (2 cohorts, n=29 total), Sweden (n=1), Switzerland (n=16) and Ukraine (n=2).

**Other includes: 7 hispanic; 3 Asian; 2 mixed; 31 'other' (of whom 30 were European nationals and one was from sub-Saharan Africa).

******* Neonatal prophylaxis was defined as ART initiated within three days of birth and stopped within three months (90 days).

******** Includes 6 children taking boosted PI + 3 NRTI regimens

Half were female; half were black ethnic origin; 34% had been exposed to maternal ART in pregnancy, of whom 29 (19%) were exposed to nevirapine (3 as single-dose). Additionally, 28% received neonatal prophylaxis. One third, in whom HIV was undiagnosed antenally, was breastfed. Thirty per cent had an AIDS diagnosis, a median age of 3.6 (IQR 2.7-5.3) months prior to ART initiation. 26 infants developed AIDS at median 40 days after ART initiation (IQR 14-189). The most common AIDS events were *Pneumocystis jiroveci* pneumonia (n=81 before and n=7 following ART initiation), cytomegalovirus infection (n=52 and n=7 respectively), and HIV encephalopathy (n=33 and n=10 respectively). Median duration of follow-up after starting ART was 5.9 (IQR 2.3-7.6) years, during which time 20 patients died and 32 were lost to follow-up (16 and 13 by 12 months respectively). Median CD4% and viral load at ART initiation were 29% and 5.7 $\log_{10}$c/mL respectively (Table 1).

Seventy-six per cent (331/437) infants started ART before six months of age (Table 1). Twenty-four per cent (107/437) of ART regimens contained an NNRTI (mostly nevirapine) with 2 NRTIs, most commonly didanosine with stavudine (36%, 8/22) in 1996-1999, and zidovudine with lamivudine (55%, 47/85) from 2000 onwards. Four-drug nevirapine-based regimens were more common in later years (3% (4/121) of regimens in 1996-1999, and 18% (57/316) from 2000 onwards), almost all with 3 NRTIs (zidovudine, lamivudine and abacavir (98%, 60/61)); most (58/61) were from UK/Ireland. Boosted PI regimens were used only from 2001, increasing from 11% (21/180) of all regimens in 2000-2003 to 34% (46/136) in 2004-2008; the most common NRTI backbones being zidovudine with lamivudine (48%, 32/67) and lamivudine with abacavir (27%, 18/67). Use of unboosted PI-based regimens, mainly nelfinavir (86% (143/166) of all unboosted regimens), declined from 68% (82/121) in 1996-1999 to 17% (23/136) in 2004-2008.

Virological and immunological response to ART

Table 2 shows factors associated with virological and immunological response 12 months after ART initiation. Viral load at 12 months was missing for 26% of children; these patients were more likely to be born in 2004-2008 (38%, versus 29% for those with an available viral load, p=0.022) and reported from the Italian cohort (36%, versus 18%, p=0.002), and less likely to have been on a 4-drug NNRTI regimen (9%, versus 16%, p=0.011, as expected, given most missing data were from Italy). However, there were no differences by all other factors included in multivariable models.

Table 2: Factors associated with virological and immunological response 12 months after ART initiation

Factor*	Virological response (<400c/mL)** (*n*=322)				Change in CD4 Z-score (*n*=203)			
	n	%	Adjusted OR (95% CI)	p value	n	Median	Coefficient (95% CI)	p value
Age at ART initiation								
<3 months	127	66%	1.00		69	0.19	0.00	
3-5 months	117	53%	0.97 (0.53-1.76)		82	1.38	-0.12 (-0.55-0.31)	
6-12 months	78	68%	1.98 (0.92-4.25)	0.059	52	1.62	0.05 (-0.44-0.53)	0.70
Calendar year of ART initiation								
1996-1999	85	53%	1.00		53	0.98	0.00	
2000-2003	145	57%	0.65 (0.36-1.16)		91	0.90	-0.41 (-0.82-0.00)	
2004-2008	92	77%	1.39 (0.71-2.69)	0.09	59	0.92	-0.24 (-0.74-0.27)	0.14
Type of initial ART regimen								
3-drug NNRTI-based	81	64%	1.00		40	0.65	0.00	
4-drug NNRTI-based	51	84%	3.00 (1.24-7.23)		39	2.29	0.64 (0.10-1.17)	
Boosted PI + 2 NRTIs	42	71%	1.39 (0.62-3.13)		32	0.91	0.16 (-0.42-0.73)	
Unboosted PI + 2/3 NRTIs	116	51%	0.58 (0.32-1.03)		69	0.61	-0.16 (-0.64-0.31)	
PI + NNRTI + NRTI / 3 NRTIs	32	47%	0.49 (0.21-1.13)	<0.001	23	1.27	0.18 (-0.44-0.80)	0.035
CD4 Z-score at ART initiation								
per unit increase	-	-	-	-	203		-0.76 (-0.88- -0.63)	<0.001
Viral load at ART initiation (log 10c/mL)								
per log10 increase	322	-	0.67 (0.50-0.89)	0.006	-	-	-	-
Maternal ART in pregnancy								
No	-	-	-	-	124	1.69	0.00	
Yes	-	-	-	-	79	0.27	-0.46 (-0.83- -0.08)	0.016

CI, confidence interval; ART, antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; OR, odds ratio

*Both models were adjusted for *a priori* confounders of treatment response (age and calendar year at ART initiation, type of initial ART regimen, and baseline CD4 count (for CD4 response only); other potential predictors were included if their corresponding p value was <0.10.

** Five viral loads measured at 12 months after ART initiation were reported as <500c/mL and have been assumed to also be <400c/mL in this analysis

Overall, 62% (199/322) of infants achieved virological suppression <400c/mL by 12 months after ART initiation. There was a trend towards improved viral suppression with calendar time, from 53% for those initiating ART in 1996-1999, to 57% in 2000-2003, and 77% in 2004-2008 (adjusted p=0.09, Table 2). Age at ART initiation was weakly associated with 12month virological response, 6-12 month-old infants being more likely to suppress virus than <3 month-olds (adjusted odds ratio (AOR) 1.98, 95%CI 0.92-4.25; p=0.06). Infants on 4-drug NNRTI regimens had significantly better viral load suppression (AOR 3.00, 95%CI 1.24-7.23) compared to 3-drug NNRTI regimens, whilst boosted PI regimens (AOR 1.39, 95%CI 0.62-3.13) were not statistically different from 3-drug NNRTI regimens. In addition, the likelihood of achieving virological suppression declined with increasing baseline viral load (AOR 0.67 per $\log_{10}$c/mL, 95%CI 0.50-0.89; p=0.01).

Half (47%; 203/437) all infants had baseline and 12-month CD4 values available. As with viral load, infants with missing CD4 values were also more likely to be reported from the Italian cohort (32% versus 12%, p<0.001), and less likely to be on 4-drug NNRTI regimens (9% versus 19%, p=0.002). In addition, they were more likely to be white (44% versus 21%, p<0.001), and less likely to have had infant prophylaxis (21%, versus 36%, p<0.001). However, there was no association between missing CD4 count and all other factors.

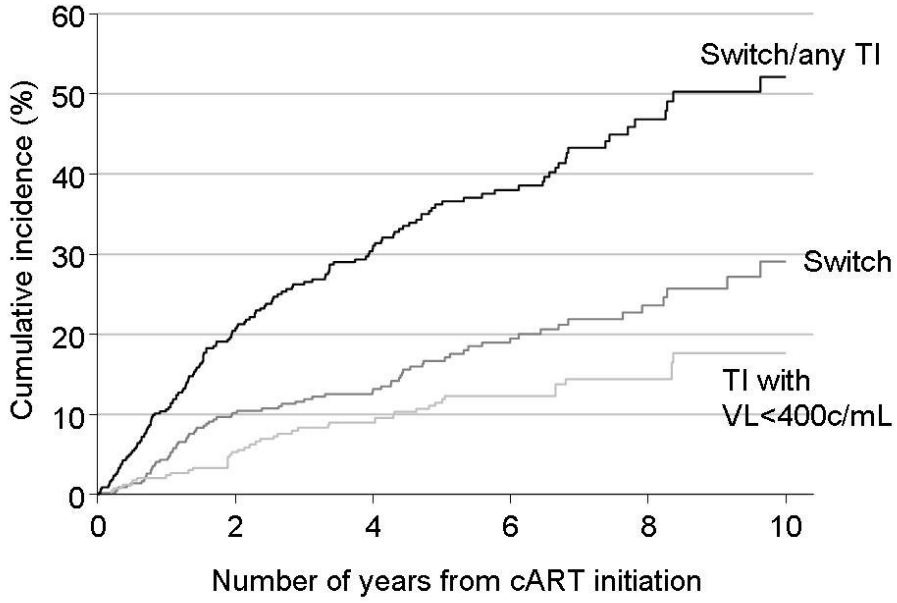
For infants with baseline and 12-month CD4 values, median (IQR) changes in CD4 count, CD4% and CD4 Z-score were 520 cells/mm^3 (271-1340), 6% (-6- 16%) and 0.92 (-0.14- 2.34), respectively. Median CD4 Z-score increase was 2.29 in infants receiving 4-drug NNRTI regimens compared with 0.65 in those receiving 3-drug NNRTI regimens and 0.91 for boosted PI regimens (overall adjusted p=0.04) (Table 2). In addition infants with lower baseline CD4 Z-scores had larger increases in CD4 at 12 months than those with higher baseline values (p<0.001), and infants whose mothers received ART in pregnancy had a smaller increase in CD4 Z-score at 12 months than those whose mothers did not receive ART (median Z-score increase 0.27 v 1.69, p=0.02).

Switching to second-line ART and treatment interruption

Eighteen per cent (77/437) infants switched to second-line therapy. The cumulative incidence fsof switching by two and five years from ART initiation was 10.2% (95% CI 7.5-13.4%) and 16.7% (13.0-20.7%) respectively (Figure 1). As expected, the main reported reason for

switching was treatment failure (84%, 41/49 with information available). Three-fifths (61%, 43/70) of these infants never achieved virological suppression (<400c/mL) by the time of switch, and 31% (22/70) had a confirmed virological rebound before subsequently switching after a median interval of 8.9 (IQR 1.9-27.6) months from initial rebound. In the remaining five who had achieved virological suppression, treatment was switched without a confirmed virological rebound.

Figure 1: Cumulative incidence of switch to second-line therapy or treatment interruption from initiation of ART



Note: Cumulative incidence conditional on a child being alive and still in study follow-up, and cumulative incidence for treatment interruption with viral load <400 c/mL further conditional on having not had a treatment interruption with viral load >400 c/mL

Those starting with either 4-drug NNRTI or boosted PI regimens were slower to switch (adjusted HR 0.41, 95% CI 0.15-1.14, and adjusted HR 0.26, 95% CI 0.06-1.19, respectively, p=0.03) compared to other regimens (Table 3), though data were sparse. Risk of switching decreased considerably once a child had a viral load <400c/mL (HR 0.23, 95% CI 0.15-0.37; p<0.001), and increased substantially once a child with viral load suppression had a confirmed virological rebound (HR 22.8, 95% CI 5.47-95.14; p<0.001). However, among all children with a

confirmed rebound while on first-line ART, only an estimated 10.7% (95% CI 5.8-17.2%) switched within 12 months of initial rebound.

Over half (56%, 13/23) of children switching from an NNRTI-based first-line regimen went on to a boosted PI as second-line ART, and 6 to an unboosted PI regimen. Two-fifths (42%, 19/45) of children switching from an unboosted PI-based first-line regimen went on to a NNRTI-based second-line regimen, and 11 to a boosted PI regimen with another PI drug. Overall, only 2 of the 67 children initiating ART with a boosted PI switched to second-line ART; one to an NNRTI-based and the other to a dual PI second-line regimen. Half (53%, 31/58) of those switching to second-line ART achieved a viral load <400c/mL within 12 months of switching.

Twenty-eight percent (121/429) of children had at least one treatment interruption lasting >14 days; 21 (17%) had 2 and 4 (3%) had 3 interruptions. Of those with a viral load available, 38% (36/94) had an interruption while viral load was suppressed, after a median 29 (IQR 16-54) months on ART; most (92%, 33/36) were on first-line therapy. The cumulative incidence of interruption with viral load <400 c/mL by 2 and 5 years was 5.3% (95%CI 3.2-8.0%) and 11.5% (8.2-15.3%), respectively (Figure 1) and no factors predicted interruption, though data were sparse. Fifty-eight per cent (21/36) restarted ART following their first interruption after an estimated median duration off therapy of 21.4 (IQR 3.7-68.6) months.

Children remaining on first-line without treatment interruption

Two-thirds (65%, 278/429) of children had neither switched to second-line ART nor experienced any treatment interruption by last follow-up, and of these, 36% (100/278) had been treated for at least 5 years. At last follow-up, 81% (213/262 with measurement available) had viral load <400c/mL and median CD4% was 36% (IQR 30-42%). The estimated probability of remaining on first-line ART without interruption was 79.3% (95%CI 75.1-83.1%) and 63.8% (58.7-68.9%) by 2 and 5 years from ART initiation, respectively

Table 3: Factors associated with a) switching to second-line therapy (n=437) and b) first treatment interruption with viral load <400 c/mL (n=429*)

Factor**	a) Switch to second-line			b) Treatment interruption with viral load <400 c/mL		
	Rate per 100 pyar (events/pyar)***	Adjusted hazard ratio (95% CI)	p value	Rate per 100 pyar (events/pyar)***	Adjusted hazard ratio (95% CI)	p value
Type of initial ART						
3-drug NNRTI-based	3.9 (18/464)	1.00		3.6 (15/419)	1.00	
4-drug NNRTI-based	2.1 (5/236)	0.41 (0.15-1.14)		0.5 (1/217)	0.18 (0.02-1.50)	
Boosted PI + 2 NRTIs	1.3 (2/154)	0.26 (0.06-1.19)		2.9 (4/137)	0.67 (0.19-2.39)	
Unboosted PI + 2/3 NRTIs	5.4 (45/837)	1.41 (0.78-2.53)		1.4 (11/765)	0.53 (0.22-1.25)	
PI + NNRTI + NRTI / 3 NRTIs	3.7 (7/187)	0.99 (0.40-2.45)	0.033	2.1 (36/1697)	0.73 (0.24-2.23)	0.40
Viral suppression						
Before viral load <400c/mL	1.9 (27/1398)	1.00		****	****	
After initial viral load <400c/mL	10.4 (50/481)	0.23 (0.15-0.37)	<0.001			
Viral rebound***						
Before confirmed rebound	5.7 (22/386)	1.00		2.2 (6/267)	1.00	
After initial confirmed rebound	0.4 (4/1012)	22.80 (5.47-95.14)	<0.001	3.0 (29/978)	0.48 (0.19-1.19)	0.11

CI, confidence interval; ART, antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; pyar, person-years

* Analyses exclude 8 children enrolled in the PENTA 11 treatment interruption trial.

** Results are only presented for factors with p value <0.1 associated separately with either switching and/or treatment interruption.

*** Rates are conditional on a child being alive and still in study follow-up, and the rate of treatment interruption with viral load <400 c/mL further conditional on having not had a treatment interruption with detectable viral load

**** Since the outcome is treatment interruption with viral load <400 c/mL, the effect of viral load <400 c/mL cannot be assessed.

***** Based on 319 children for analysis of switching to second-line therapy and 329 children for analysis of treatment interruption who had a viral load <400 c/mL before experiencing the outcome of interest. Follow-up time was considered from the date of initial viral load <400 c/mL.

5.1.5 Discussion

In our study, virological response in infants starting ART before 12 months of age showed improvement with calendar year of ART initiation, and virological and immunological responses were better in those starting with 4-drug NNRTI-based regimens compared with 3-drug NNRTI-based and boosted PI regimens, which were similar. The rate of switching to second-line ART was low, and almost 65% of children remained on first-line ART without treatment interruption after five years.

Our study has several limitations. We were unable to assess the influence of unmeasured confounders, and there is a risk of attrition and selection bias, particularly for data acquired from non-birth cohorts. Clinicians' preference in first-line treatment choice, influenced by patient presentation and adherence patterns, cannot be ruled out. However, there was no evidence of differences across countries (data not shown), nor by pre-treatment AIDS diagnosis. Missing data for viral load and CD4 were higher for the Italian cohort, indicating that selection bias may be present, although multiple imputation techniques were employed to address this bias [102]. Data on HIV resistance mutations were not available for most children, and thus the impact of resistance could not be assessed.

Our findings demonstrate that across Europe, virological responses have improved over calendar time in infants starting ART early in life. Possible explanations include better regimen efficacy, better dosing and improved management resulting in better caregiver adherence. Of note, in the CHER trial, the proportion of children on lopinavir/ritonavir with viral load <400c/mL at 12 months was similar to that observed here for 2004-2008 (77%).[22, 103] Similarly in the NEVEREST trial of young children <2 years of age starting on a PI-based regimen, the proportion with viral load <400c/mL at 9 months was 84% [104].

Firm evidence of better virological response to boosted PI-based versus NNRTI-based regimens is lacking in our study, after controlling for potential confounders. This is in agreement with the PENPACT-1 trial [33], but in contrast to the short-term IMPAACT 1060 trial findings [34]. However, power to detect small differences was low in our

study as relatively few infants started boosted PI-based ART. African children in IMPAACT 1060 started ART according to clinical and immunological criteria and were more severely immune-suppressed (median CD4% 15% overall, versus 29% in our study), and were assessed for a different study endpoint after only 24 weeks. PENPACT-1 included few infants and, like our study, included some started on early ART when asymptomatic with high CD4 values; duration of follow-up in both PENPACT-1 and our study was considerably longer than in IMPAACT 1060.

Of interest, use of 4-drug NNRTI-based regimens resulted in improved virological and immunological responses compared with other regimens. Given high viral loads in infancy and potential advantages of a PI-sparing regimen in terms of tolerability, adherence, lack of interaction with other drugs, preservation of effective second-line options, and cost, an NNRTI-based 4- to 3-drug induction-maintenance strategy could be valuable in infants not exposed to single-dose nevirapine for pMTCT. However, as infants initiating 4-drug regimens in our study were mainly from UK/Ireland, potential biases in indication for treatment cannot be excluded; the results of the ARROW trial, which is evaluating this strategy, are awaited in 2012.

Immunological recovery was better in those initiating therapy with a lower CD4 Z-score at baseline, confirming good thymic activity in young children [2, 96] and a possible "ceiling effect" of CD4 response in infants, as noted elsewhere [4]. In addition, the negative association with exposure to maternal ART *prima facie* suggests the possibility, supported by previous findings, that infants acquiring HIV despite maternal ART in pregnancy may have a worse prognosis and potentially suboptimal immunological response to treatment [86, 87, 105]. However, exposure to maternal ART was varied in our study, with infants being exposed to many different regimens, and we had insufficient data to fully evaluate this association.

Five years after starting ART, two-thirds of infants in our study were still on their first-line regimen without interruption, and a fifth had switched to second-line. Similarly in the UK and Ireland paediatric cohort (median age 5 years), 22% switched to second-line after a median of seven years [99]. In our study children starting ART on 4-drug NNRTI-based or boosted PI regimens switched to second-line ART more

slowly, in line with evidence that these regimens maybe more durable, and result in a more sustained virological response and/or a higher genetic barrier to drug resistance[48, 49].

Despite treatment interruption not being currently recommended in international paediatric guidelines, a quarter of infants interrupted treatment during follow-up. Sixty-two per cent of interruptions occurred with detectable viral load, likely reflecting challenges encountered with tolerability, acceptability and adherence, which may be exacerbated in young children, with unclear impact on subsequent treatment response. Five-year results of the CHER trial comparing outcomes in infants randomised to planned interruption at 12 or 24 months of age after early ART versus deferred ART are awaited later this year. In our study, children remained on first-line therapy longer than adults [106], even following the occurrence of viral load rebound. This is likely due to a more conservative approach to the clinical management of young children, especially in earlier years, and limited treatment options.

In conclusion, our findings suggest that outside trial settings, an effective treatment response can now be achieved in infants who start ART early in life, with the majority remaining on first-line ART after five years. However issues around choice of first-line ART including potential treatment sequencing, feasibility and cost, require careful consideration. Our findings are in line with evidence in the PENPACT-1 trial, suggesting similar responses to initial 3-drug NNRTI-based and PI-based regimens, but in addition, suggest that a 4-drug NNRTI-based initial regimen maybe superior. However, this approach needs further evidence from on-going randomised trials, as do strategies of 4- to 3-drug NNRTI induction-maintenance and treatment interruption following early ART in infancy.

5.1.6 Acknowledgements

Writing Committee (ordered by project team first and in last place, followed by working group, and finally all others alphabetically by name):

Ali Judd (Medical Research Council Clinical Trials Unit (MRC CTU), London, UK), Martina Penazzato (MRC CTU, London, UK and University of Padova, Italy), Claire Townsend (MRC CTU, London, UK)*, Trinh Duong (MRC CTU, London, UK)*, Hannah Castro (MRC CTU, London, UK); Tessa Goetghebuer (Hospital St Pierre, Brussels, Belgium), Josiane Warszawski (INSERM, Paris, France), Luisa Galli (University of Florence, Italy), Elena Chiappini (University of Florence, Italy); Maurizio de Martino (University of Florence, Italy), Luminita Ene (Victor Babes Hospital, Bucharest, Romania), Carlo Giaquinto (University of Padova, Italy), Christoph Königs (University of Frankfurt, Germany), Jerome LeChenadec (INSERM, Paris, France), Hermione Lyall (Imperial College Healthcare NHS Trust, London, UK), Antoni Noguera Julian (Hospital Sant Joan de Déu, Barcelona, Spain), Jose T. Ramos (Hospital Universitario de Getafe, Madrid, Spain), Pablo Rojo-Conejo (Hospital 12 de Octubre, Madrid, Spain), Christoph Rudin (Universität Basel, Switzerland), Claire Thorne (UCL Institute of Child Health, University College London (UCL), UK), Pat Tookey (UCL Institute of Child Health, UCL, UK), Gareth Tudor-Williams (Imperial College Healthcare NHS Trust, London, UK); Di M. Gibb (MRC CTU, London, UK).

Contributions: Ali Judd, Martina Penazzato, Hannah Castro and Di Gibb were responsible for the study concept and design. Claire Townsend, Trinh Duong and Hannah Castro were responsible for undertaking the analyses; Trinh Duong acts as guarantor for the analyses and has full access to the dataset. Ali Judd, Martina Penazzato, Claire Townsend, Trinh Duong and Di Gibb wrote the manuscript. Ali Judd, Di Gibb, Elena Chiappini, Maurizio de Martino, Luminita Ene, Luisa Galli, Tessa Goetghebuer, Jerome LeChenadec, Hermione Lyall, Antoni Noguera Julian, Jose T. Ramos, Pablo Rojo-Conejo, Christoph Rudin, Claire Thorne, Pat Tookey, Gareth Tudor-Williams and Josiane Warszawski provided data for the study. All members of the Writing Committee participated in discussions about the design of the study, the choice of statistical analyses, interpretation of the findings, and critically reviewed the manuscript.

Contributing cohorts (listed alphabetically by cohort name): ANRS CO1 EPF/ ANRS CO11 OBSERVATOIRE EPF, France (Josiane Warszawski, Jerome LeChenadec); Collaborative HIV Paediatric Study (CHIPS), UK & Ireland (Ali Judd, Di Gibb); CoRISPE-cat, Spain (Antoni Noguera-Julian); European Collaborative Study (ECS) (Claire Thorne); Italian Register (Luisa Galli, Elena Chiappini, Maurizio de Martino); Madrid Cohort, Spain (Jose T. Ramos, Pablo Rojo-Conejo); MoCHiV, Switzerland (Christoph Rudin); National Study of HIV in Pregnancy and Childhood (NSHPC), UK & Ireland (Pat Tookey); St Pierre Paediatric Cohort, Belgium (Tessa Goetghebuer); Victor Babes Cohort, Romania (Luminita Ene).

6. Research activities III. Operational Research

While interventions can be proven effective in clinical research, testing them in specific populations is essential, as is researching their usefulness, appropriateness and applicability given the health care resources, processes and structures in a given country [107].

In addition, research in developing countries can provide the contextual knowledge essential for research to have an impact on improvement of care settings, by illustrating how the results of previous research can be applied to a specific population or country and what the potential challenges are. Operational research is therefore critical for findings of basic research to have an impact.

Early treatment for HIV-infected infants and young children less than 2 years has been recommended by the WHO since July 2010 [21] on the basis of robust evidence obtained by a randomized control trial conducted in South Africa [22] and the need of pragmatic recommendations to reduce the high mortality observed in the first two years of life. However, the impact of these new treatment initiation criteria at programmatic level is still unclear and so is the extra proportion of children which is now in need of treatment.

The implementation of early treatment recommendations requires that infants are diagnosed early in life by using virological testing. In 2010 a new testing algorithm has been released by the WHO but its validity depends very much on the HIV prevalence in the countries and on the points of entry to EID services. Virological testing is still very expensive. Significant lost to follow-up before children can be started on ART is considerable. The cost-effectiveness of the testing algorithm is therefore still unknown and information about it is needed to help countries to allocate their budget and tailor their EID strategy.

When prompt diagnosis is achieved and early treatment possible, the choice or first-line regimen presents a further challenge. Exposure to different PMTCT regimens has

been shown to lead to the acquisition of HIV resistant strains which can affect the efficacy of early treatment in infants and young children [32, 77]. However the evidence so far available has been produced within research settings and population level data on the magnitude and type of acquired resistance is still largely lacking.

This section of the report will focus on the above issues and will explore ways in which operational research could inform the implementation of diagnosis, care and treatment of HIV-infected infant's in middle and low-income countries.

The following three projects will be described in detail:

A. Impact of the revised WHO ART initiation criteria in a resource limited setting: by using observational data obtained through an HIV treatment program in Uganda (Tukula Fenna), the change in treatment eligibility due to the last WHO recommendations revision will be assessed.

B. EID implementation in resource limited settings: a study proposal was developed to investigate the cost-implications and the cost-effectiveness of different EID algorithms with different HIV prevalence in a variety of countries.

C. Transmitted drug-resistance in HIV-infected infants: a population survey has been developed by using DBS left over form EID services to investigate frequency and predictors of the acquisition of HIV resistant strains to inform national guidelines.

6.1. Impact of the revised WHO ART initiation criteria in resource limited setting

Despite significant progress in diagnosis and treatment of paediatric HIV infection, access to treatment and care is still limited in most countries and only 23% of children who need treatment were accessing it by end 2010 [108]. Limited health care systems are currently struggling to meet the demands of national paediatric diagnosis, treatment and care.

WHO guidelines for paediatric HIV, which were first issued as stand-alone guidelines in 2006, are based on a public health approach with the aim of simplifying and facilitate treatment scale-up. Treatment initiation criteria were revised and expanded in 2008 and 2010 following the CHER trial results [22] with the additional aim of further simplify treatment strategies at the programmatic level. However the real impact of this change remains unknown and estimation of the increase in number of patients becoming eligible for treatment is critical to understand the burden on programmes and the additional resources needed.

In this project data from a Ugandan cohort of HIV-infected infants accessing care in an urban district of Kampala was used to assess the potential impact of the changing WHO paediatric guidelines on the number of children in need of treatment. Adoption of 2010 WHO paediatric guidelines for starting ART would significantly increase the number of children eligible for treatment at enrolment into the programme, but this effect would lessen over the subsequent 5 years.

I led this work in collaboration with other senior international colleagues. Direct involvement in the data collection occurred in the previous 5 years as part of the collaboration with the Tukula Fenna project in Kampala (Uganda). Major contribution was given to the design, analysis and manuscript preparation. Results were shared through oral presentations at the 2nd HIV Paediatrics Workshop and the XVIII World AIDS Conference in Vienna in 2011. A concise communication is under submission to AIDS.

The evolution of paediatric antiretroviral treatment guidelines: What's the impact on the ground?

Penazzato M[1,2], Crowley S[3], Mofenson L[4], Lodi S[5], Franceschetto G[1], Morelli E[1], Nabachwa S[6], Nannyonga M[6], Mazza A[7], Giaquinto C[1].

[1] *Department of Paediatrics University of Padova, Italy*
[2] *MRC Clinical Trial Unit, London, UK*
[3] *UNICEF South Africa*
[4] *Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA*
[5] *Instituto de Salud Carlos III, Madrid, Spain*
[6] *St. Raphael of St. Francis Nsambya Hospital, Kampala, Uganda*
[7] *Trento Santa Chiara Hospital, Trento, Italy*

6.1.1 Abstract

Antiretroviral treatment guidelines for children evolve quickly as new evidence emerges. The treatment initiation criteria have been revised in 2010 but the likely impact of these changes is still unknown.

Methods: Using a cohort of 985 children (median age 70 months) followed since 2003 by the Tukula Fenna project (Kampala-Uganda). WHO ART initiation criteria in 2006, 2008 and 2010 guidelines were applied separately and the proportion of children eligible for ART identified. The probability of initiating ART over time was also assessed. Children initiated on ART were considered and least used criteria for those not starting treatment despite being eligible identified.

Results: By applying 2006, 2008 and 2010 criteria respectively 40%, 57% and 66% of the children of the cohort would have been eligible for ART at enrolment and 76%, 84% and 88% by 2 years later. The proportion of eligible children who started ART within 6m and 12m according to criteria being applied were respectively 39% and 50% until 2008 and 50% and 52% afterwards. The criterion least applied was clinical stage (OR=2.0, I.C.95%=1.2-3.2) until 2008 and being an infant (OR=10.5, I.C.95%=3.8-31.1) for thereafter.

Conclusions: Adopting 2010 WHO paediatric guidelines for starting ART does increase the number of children eligible for treatment, even though not dramatically. A different impact may be expected in paediatric programmes with strong links with PMTCT services. Additional efforts will be needed to ensure infants and young children are initiated on treatment in a timely manner. In this context, Early Infant Diagnosis (EID) and new guidelines dissemination are critical.

6.1.2 Introduction

HIV infection in infants is rapidly progressive and often fatal disease. Infected infants frequently present with clinical symptoms in the first year of life; in resource-limited settings, without effective therapy, by age 1 year an estimated one-third of infected infants will have died and by age 2 years over half will have died [14]. In resource-rich countries, early initiation of effective antiretroviral therapy has transformed HIV infection into a chronic disease, with survival of HIV-infected infants and children into adolescence and adulthood [109]. In the U.S., well over 90% of HIV-infected children are receiving antiretroviral therapy [110].

In resource-limited countries, there has been significant progress in diagnosis and treatment of paediatric HIV infection. The World Health Organization publishes guidelines for treatment of paediatric HIV infection in resource-limited countries based on a public health approach, using standardized and simplified antiretroviral regimens based on the best available scientific evidence that offer both a durable response and preserve future treatment options. Guidelines were first published in 2004 combined with guidelines for adults; in 2006, stand-alone guidelines for children were developed and published. Given rapidly evolving evidence of the optimal time to start therapy in children in resource-limited settings, criteria for initiation of treatment were revised and expanded in 2008 and 2010.

However, only 28% of children who need treatment are accessing it [108]. Significant obstacles to scaling up paediatric care remain, including limited screening for HIV, lack of early diagnostic capabilities in young infants, lack of human capacity,

and lack of affordable and manageable paediatric antiretroviral drug formulations. As a result health care systems in resource-limited countries are struggling to meet the demands of national paediatric treatment.

Guidelines for treatment initiation are based on age, WHO clinical stage, and immune status. In 2006, treatment guidelines were more complex: treatment was recommended for all children with WHO stage 4 disease regardless of CD4 count; all infants <12 months with WHO stage 3 disease regardless of CD4 count and all older children with the exception of those who met WHO stage 3 disease based on tuberculosis, oral hairly leukoplakia, lymphoid interstitial pneumonia or thrombocytopenia, where CD4 was recommended for decision making if available; treatment of WHO stage 1 or 2 disease was recommended only if CD4 count was available; 4 age-related CD4 treatment thresholds were used (<11 months; 12-36 months; 36-59 months; >5 years). In 2008, based on clinical trial data demonstrating that initiation of treatment at age <12 weeks significantly improved survival [22], guidelines were modified to recommend treatment for all infants <12 months regardless of WHO clinical stage or CD4 count. In 2010, these criteria were further broadened to recommend therapy for all children age <24 months; all children with WHO clinical stage 3 or 4 disease, regardless of CD4 count; for children>24 months with WHO stage 1 or 2 disease, CD4 criteria were simplified into 2 age-related categories (24-60 months and >5 years) and the CD4 threshold for initiation of treatment was increased.

We used data from a Ugandan cohort of HIV-infected infants accessing care in an urban district of Kampala to assess the potential impact of the changing WHO paediatric guidelines on the number of children in need of treatment and to estimate the degree of implementation of the guidelines achieved within the program.

6.1.3 Methods

Data from a cohort of HIV infected children followed since January 2003 by Tukula Fenna project (St Francis of St Rafael Hospital in Kampala – Uganda [13] were analysed. Children are enrolled in the program from a variety of points of entry, such as programs to prevent mother to child transmission and outpatient and inpatient services. HIV is diagnosed by HIV DNA PCR in infants <18 months or by HIV antibody testing in those 18 months or older. Patients are followed with monthly clinical examinations and laboratory monitoring (CD4 count/per cent, full blood count, and liver function tests, amylase and creatinine) at baseline and every 6 months. Cotrimoxazole prophylaxis, psychosocial and nutritional supports are also provided.

In order to evaluate the impact of serial WHO guidelines revision on the proportion of children eligible for treatment, treatment initiation criteria recommended by WHO 2006[111], 2008 [112] and 2010[21] paediatric treatment guidelines were independently applied to the same cohort over time. Children were followed from enrolment and censored at the time they first met WHO treatment criteria or if lost to follow-up (defined as a child not seen for more than 6 months since last visit). The cumulative proportion of patients becoming eligible for treatment was calculated and Kaplan Meier method was used to estimate the probability of becoming treatment eligible over time after enrolment in the program.

WHO criteria became more comprehensive over time allowing for an earlier initiation of treatment, thus a child who was eligible according 2006 criteria would also be eligible based on the 2008 and 2010 criteria. The overall proportion of patients becoming eligible for treatment during follow-up was considered for each WHO set of criteria and reasons for becoming ART eligible (e.g., age, WHO clinical stage, or CD4 count/percentage) were separately examined.

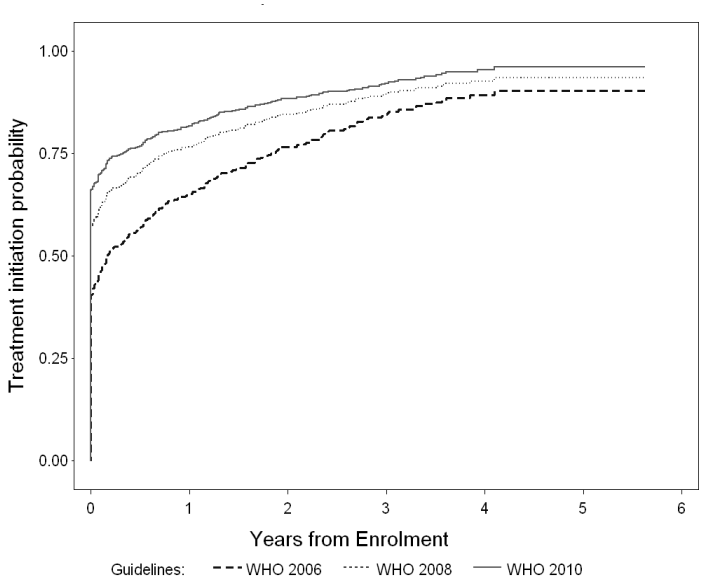
To estimate the actual implementation of the guidelines in the programme we then assessed the proportion of children starting treatment as recommended. Two periods of time were defined on the basis of the treatment initiation criteria being

used: the first from January 2006 when antiretroviral therapy became widely available in Uganda until July 2008 when universal treatment for infants under 12 months was recommended by WHO, and the second from July 2008 until of July 2009 (data freeze).

6.1.4 Results

The cohort included 985 children and a total of 1899.55 person/year of follow up; 483 were male and 492 female, with a median age at enrollment of 5.8 years (IQR 1.7 - 10.1). The median baseline CD4% was 13.7% (IQR 7.1-21.8%) and 63.6 % of patients presented with symptomatic disease (45.9% WHO stage 2, 13.9 % WHO stage 3 and 3.8 % WHO stage 4).

Fig 1. Probability to become eligible for treatment over time from enrolment

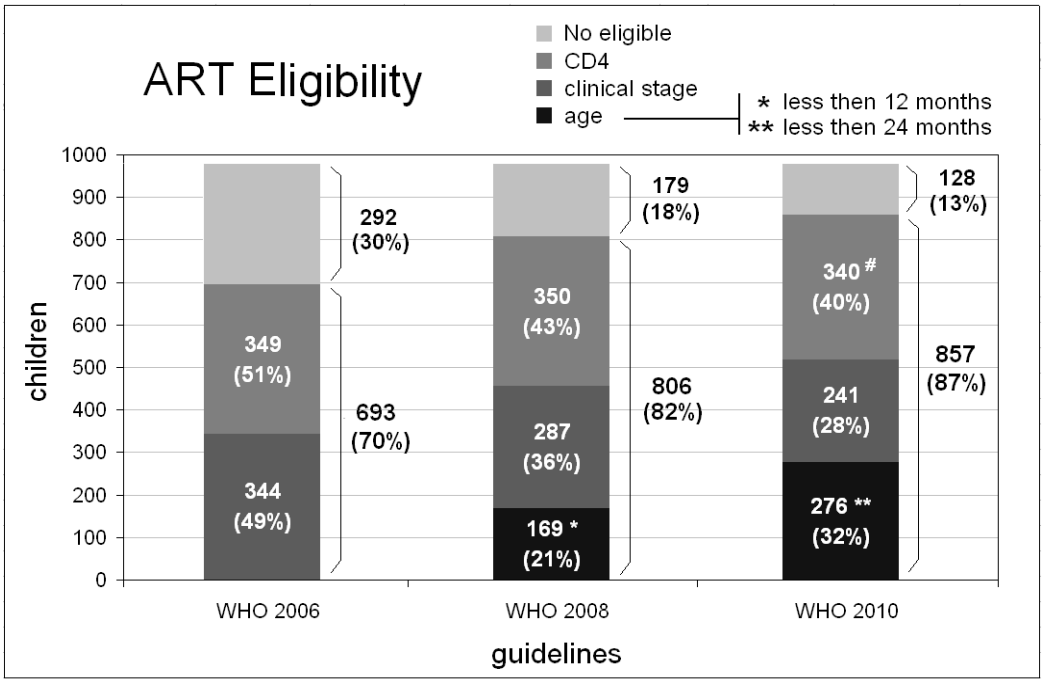


According to the 2006, 2008 and 2010 WHO criteria respectively 40%, 57% and 66% of the children were already treatment-eligible at enrolment and by 2 years from enrolment the probability of being eligible for treatment increased to 76%, 84% and 88% respectively (Fig.1).

By applying 2006, 2008 and 2010 WHO guidelines during the 6 years of observation, the number of children in need of treatment increased from 693 (70%) to 806 (82%)

up to 857 (87%) of children in the cohort, respectively (Fig.2). Overall, a 17 % increase in the total number of patients requiring initiation of treatment was observed when applying 2010 guidelines as compared to the 2006 criteria, and 5% more comparing 2010 to 2008 guidelines (Fig.2).

Fig 2. Proportion of children in need of treatment and reasons for eligibility (2003-2009).



CD4 criteria was simplified as follows: 36-59 months CD4 < or cell/ml, over 59 months CD4<350 cell/ml

Of those eligible for treatment, age (<12 months) accounted for 21% of eligibility in 2008, when universal treatment for infants was recommended. This proportion increased to 32% in 2010 when the age threshold for universal therapy was raised to 24 months.

Using the 2006 treatment guidelines, WHO clinical staging accounted for 49% of children being eligible for treatment, whereas, according 2008 and 2010 guidelines, WHO clinical stage would be responsible for treatment initiation in the 36% and 28% of children, respectively. A CD4 criterion for immune suppression was responsible for treatment eligibility in 51%, 43% and 40% of children in 2006, 2008 and 2010.

Twelve per cent of infants and 26% of the young children (less than 2 years) who would have been eligible for treatment based on 2008 and 2010 guidelines based on their age also had clinical or immunological criteria that met the 2006 treatment thresholds at the enrolment. Out of the remaining 148 (88%) and 205 (74%) children eligible for treatment solely due to their age in 2008 and 2010, 25% and 30% showed subsequent disease progression and met clinical or age-specific immunological criteria for treatment by the end of observation time.

The proportion of potentially treatment-eligible children who actually started treatment within 6 and 12 months from becoming eligible for treatment based on the existing treatment guidelines were 39% and 50%, respectively, until 2008 and 50% and 52% afterwards. In the period prior to 2008, 20% of children did not start treatment despite meeting treatment eligibility criteria; in the period after 2008, 25% of children did not start treatment despite being eligible. Prior to 2008, the most common eligibility criteria met in children who did not start treatment was WHO clinical stage criteria (OR=2.0, I.C.95%=1.2-3.2); after 2008, the most common eligibility criteria met in children who did not start treatment was age <12 months (OR=10.5, I.C.95%=3.8-31.1).

6.1.5 Discussion

The programmatic impact of adopting 2010 WHO paediatric guidelines for starting treatment is still unknown and there are concerns they will significantly increase the workload in already

overburdened programmes. We described the potential scenario that programmes may encounter after implementation of the new recommendations. Comparing 2006 guidelines with 2010, an overall increase of 17% of children in need for treatment was observed in our cohort. However this rise was primarily driven by the introduction of universal treatment for infants <12 months already recommended in 2008.

In cohorts with similar characteristics, universal treatment for children under 2 years is expected to increase by 65% (from 40% to 66%) the number of patients already treatment-eligible at enrolment as compared to using only clinical and immunological criteria (fig.1). According our estimates only few children would remain treatment-free at 2 years from enrolment. However, the age at enrolment in our cohort (5.8 years) is relatively old, and the impact of the change in guidelines would be expected to differ in cohorts with lower age at enrolment into care and hence have more children diagnosed by age 2 years. However, these paediatric programmes have usually a strong link with prevention of mother to child transmission programs and therefore are very likely to diagnose HIV and start treatment in infancy, thereby being significantly affected by the 2008 universal treatment recommendations but not as much affected by the 2010 extension of treatment eligibility to under 2 years old.

Because patients were more likely to present with advanced disease in the earliest years of the Tukula Fenna programme, differences in baseline characteristics and calendar year of enrolment should be taken into consideration and caution should be used when applying our estimates to other settings.

In this cohort only half of treatment-eligible children were started on antiretroviral therapy within 12 months of the time they met eligibility criteria. A significant delay in treatment initiation was observed, highlighting the need of a prompt clinical and immunological assessment, faster CD4 results turnaround time, as well as a streamlining pre-treatment counselling process.

In conclusion, we believe that in order to achieve a successful implementation of new standard for treatment initiation in children, additional efforts will be needed to ensure infants and young children are initiated on treatment in a timely manner. In this context, strengthening of early infant diagnosis services and the dissemination of the new guidelines are critical.

6.2. EID implementation in resource limited settings

Early treatment in HIV infected children requires access to early infant diagnosis (EID). New 2010 WHO guidelines [17] were developed to improve the uptake of EID in resource-limited settings and they recommend that every child being seen in a healthcare setting should have their HIV exposure status ascertained and that a confirmatory virological test should be performed after a first positive result to improve sensitivity and maximize the PPV.

Although there is clear evidence of the clinical benefits of identifying and treating infected infants early, it is important to consider whether diagnosis by virological testing can be considered cost-effective. Cost and performance of virological tests are influenced by multiple factors. HIV prevalence has a major impact on positive and negative predictive value of virological testing, which in turn will impact the number tests undertaken.

The proposal here presented aims to explore the economic implications of different diagnostic strategies accounting for diverse entry paths. The differences between all the possible entry paths, such as HIV prevalence in the target population, need for service decentralization, context-specific barriers and different cost implications may support the recommendation of alternative EID strategies. This could provide key information and generic models, which may support countries in their mid- and long-term planning of EID country strategies as their HIV epidemics and PMTCT services mature.

This section of the work was developed in close collaboration with an experienced health economist (Paul Revil, York University) and other senior colleagues at the MRC clinical trial unit. I particularly contributed by revising barriers and challenges to EID implementation and by further investigating the effect of HIV-prevalence on NPV and PPV of the virological test algorithm at the different service entry points. A study proposal was finalised and a position paper to highlight the need of such studies in the field of EID is currently under preparation.

EID implementation: Towards a setting-specific strategy

Penazzato M [1,2], Revill P[3], Prendergast A[2,4], Gibb DM[2]

[1] *Department of Pediatrics, University of Padova, Italy*

[2] *Clinical Trial Unit, Medical Research Council, London, UK*

[3] *Health Economics Unit, York University, York, UK*

[4] *Centre for Paediatrics, Blizard Institute, Queen Mary, University of London, UK*

6.2.1 Abstract

Almost 1000 HIV-infected infants continue to be born daily globally. WHO guidelines were revised in 2008 [113] to recommend early ART initiation by the first year of life, to reduce mortality and disease progression when they occur most rapidly [114]. However, implementation of early treatment requires access to early infant diagnosis (EID).

Virological assays, are the gold standard for HIV diagnosis of infants and children younger than 18 months [17]. Despite becoming progressively more available, the technology for EID remains expensive and complex, requiring specialized equipment and trained technicians. The best approach to virological testing for EID in different settings remains uncertain and the cost per infant diagnostic test continues to be more than 10 times that for older children and adults.

The huge financial challenges that country are currently facing, requires optimisation of resources and appropriate use of the existing budget available. Two main questions which concern the financing of EID in low-income countries have been identified: How should given budgets for EID be utilized to greatest effect? What level of resources from the overall healthcare budget should be devoted to EID?. This protocol aims to outline challenges and suggests an approach to assess the cost implications of alternative diagnostic strategies could be investigated, with the objective of developing future research able to inform decision makers and facilitate a better allocation of the available resources.

6.2.2 Introduction

By current estimates, almost 1000 HIV-infected infants continue to be born daily globally. Mortality amongst HIV-infected children in sub-Saharan Africa exceeds 50% by 2 years of age because of rapid disease progression during early life [14]. The South African CHER study [22] demonstrated that mortality was reduced 4-fold (from 16% to 4% [HR 0.24 (CI 0.11-0.51)]) by initiation of antiretroviral therapy (ART) before 12 weeks of age compared to waiting for immunological or clinical disease progression in infants infected with HIV. As a result, WHO guidelines were revised in 2008 [113] to recommend that ART is started as soon as possible after HIV infection is diagnosed during the first year of life; this recommendation was subsequently extended to the less than 2 years old to facilitate treatment management at programmatic level [85]

However, implementation of early treatment requires access to early infant diagnosis (EID). Standard HIV antibody testing cannot identify infected infants in the first year of life due to the presence of maternal anti-HIV antibody. Virological assays, especially HIV-1 nucleic acid amplification tests (NAATs), are the gold standard for HIV diagnosis of infants and children younger than 18 months [17]. With such testing, the diagnosis in non-breastfed infants can be established within the first few weeks of life. Important factors when considering choice and timing of HIV-1 diagnostic assays for children include the age of the child, the potential timing of infection (perinatal or through breastfeeding), whether the infection status of the child's mother is known or unknown, the antiretroviral exposure history of the mother and the child, and characteristics of the virus.

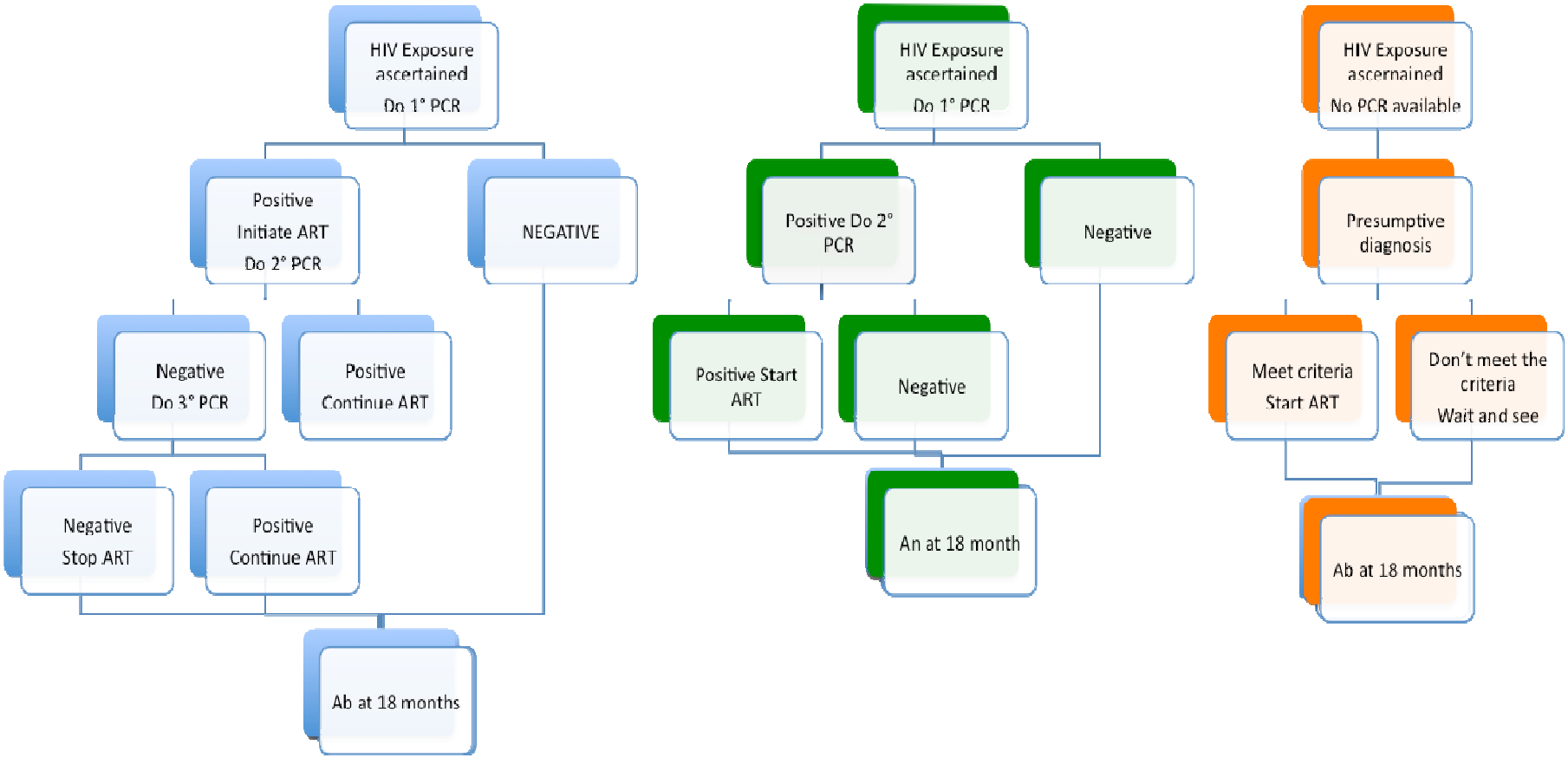
Despite becoming progressively more available, the technology for EID remains complex, requiring specialized equipment and trained technicians, and tests are expensive. Considering the large number of children who require testing, the implementation of EID is challenging for resource-limited countries. A study examining the predictive value of using the standard serological testing with Abbott Determine (DET) rapid test versus DNA PCR (PCR) for early diagnosis of HIV in infants in Uganda estimated the costs at $1.20 per DET and $15 per PCR,

including kit price, labour, logistics and repeat runs inherent to each test [115]. The cost per infant diagnostic test is still more than 10 times that for older children and adults.

New 2010 WHO recommendations[17], were developed to improve uptake of EID in resource-limited settings, and they state that wherever possible, all infants should have their exposure status ascertained and that any virological test (NAAT or Agp24 on liquid or dried blood spot (DBS) sample) can be used to undertake EID in those born to HIV-infected mothers. In order to improve sensitivity and maximize the positive predictive value, a confirmatory test is now recommended for those with initial positive results and a further virological test should also be considered in the case of discordant results. WHO guidelines had previously recommended that confirmation of an initial positive test result should be done by repeating the test on a separate specimen, where possible. However at that time it was recognized that in severely resource-constrained settings, repeat virological testing may not be either feasible or affordable. In these situations the reliability of the laboratory (determined by standard quality assessment) was fundamental to ensure reliable test results (see Fig. 1).

Confirmatory testing, as discussed in WHO 2010 guidelines, can increase pressure on constrained health systems (cost and capacity), but using two different virological tests, each with at least 98% sensitivity and 98% specificity to minimize false positive and false negative test results, is recommended. While a high value was placed on not treating infants unnecessarily (cost of treatment and treatment-related adverse events), the consequence of missing HIV infection could be death given the very high mortality observed in HIV infected infants.

Fig.1 Diagnostic algorithm (a. WHO 2010; b. WHO 2006; c. presumptive diagnosis)



Confirmatory testing is particularly important in settings where the prevalence of HIV in the population being tested is less than 5%. At an HIV prevalence of 2% in the infant population being tested (i.e. a well-performing PMTCT programme providing combination antiretroviral therapy), only 294 positive infants will be identified for every 10 000 virological tests performed among babies born to HIV infected mothers who have accessed pMTCT during pregnancy, of whom only 196 will be HIV infected, i.e. two out of three infants who initially test positive are actually infected. One out of three infants starting ART after the first test is therefore not infected, and the confirmatory test will be negative. This infant will need to have a negative HIV DNA test result before ART is discontinued. Where the prevalence of HIV in infants is higher (assuming a prevalence of 30% in the population undergoing testing, such as within inpatient provider-initiated testing and counselling [PITC] settings), of 3010 positive tests results identified for every 10 000 assays performed, 2940 infants will be truly infected and 70 infants who are uninfected would start ART, 60 infants who are infected would be missed due to false negative test results. 3010 repeat tests would be required, to find these 70 uninfected infants (see Tab.1 and 2) [17].

Table 1. Likely performance of HIV assays (HIV serological or HIV virological tests) meeting or exceeding thresholds for sensitivity of 95% and specificity of 98% at various HIV prevalence levels [WHO 2010]

Prevalence in population being tested (%)	**1**	**2**	**5**	**10**	**20**	**30**	**50**
No. of positive results per 10 000 tests (TP+FP)	293	386	665	1130	2060	2990	4850
No. truly infected in 10 000	100	200	500	1000	2000	3000	5000
No. uninfected in 10 000	9900	9800	9500	9000	8000	7000	5000
No. uninfected testing positive/10 000 tested (FP)	198	196	190	180	160	140	100
Infected testing negative/10 000 tested (FN)	5	10	25	50	100	150	250
No. uninfected testing negative (TN)	9702	9604	9310	8820	7840	6860	4900
No. infected testing positive (TP)	95	190	475	950	1900	2850	4750
PPV (%)	32.4	49.2	71.4	84.1	92.2	95.3	97.9
NPV (%)	99.9	99.9	99.7	99.4	98.7	97.9	95.1

Table 2. Serial testing using tests with a sensitivity of 95% and specificity of 98% [WHO 2010]

Prevalence in the population initially being tested (%)	1-test algorithm PPV (%)	1-test algorithm NPV (%)	2-test algorithm PPV (%)	2-test algorithm NPV (%)
1	32.4	99.9	95.8	97.6
2	49.2	99.9	97.9	95.3
5	71.4	99.7	99.2	88.7
10	84.1	99.4	99.6	78.8
20	92.2	98.7	99.8	62.3
30	95.3	97.9	99.9	49.1
50	97.9	95.1	100.0	29.2

Source: Calculations courtesy of and checked by J. Schüpbach and M. Penazzato (WHO 2010)[17].

Although there is clear evidence of the clinical benefits of identifying and being able to treat HIV-infected infants early, a key component for scale-up is consideration of the cost-effectiveness of EID. It is noteworthy that in the CHER trial 5985 infants born to mothers in PMTCT programmes were screened in order to identify 405 HIV-infected infants (6.8% of those screened). This was at a time when most infants received only single dose neviripine for pMTCT; much lower transmission rates will be observed with WHO 2010 recommendations for pMTCT, particularly if mothers are taking HAART during pregnancy (1-2% - i.e. for every 100 infants screened, only 1-2 might be HIV-infected). However, identification of HIV-exposed infants who are uninfected facilitates follow-up care and may help to ensure they remain uninfected [116].

Expanding access to HIV counselling and testing is increasingly recognized as the building block to the scale-up of HIV prevention, treatment and care [108]. However, EID using virological testing is currently unavailable, unaffordable or unfeasible in most settings. Adoption of universal treatment guidelines requires expansion of EID, but the feasibility and the cost-effectiveness of this expansion in different settings and among infants presenting in different ways needs to be assessed.

This concept proposal aims to explore the cost implications of different diagnostic strategies, accounting for diverse entry paths, and therefore is meant to inform decision makers and facilitate a better allocation of the available resources.

6.1.3 Potential Strategies for Roll-Out

The best approach to virological testing for EID in different settings remains uncertain. Cost and performance of virological tests will be influenced by multiple factors. HIV prevalence has a major impact on positive and negative predictive value of virological testing, which in turn will impact the number and the cost-effectiveness of tests undertaken. In addition to direct health service costs of diagnosis and treatment, the non-health service costs to families need to be assessed.

Antenatal care provides an opportunity for testing and counselling of pregnant women. For HIV-negative women, it is an opportunity to gain information and the skills needed to remain HIV-negative. For women living with HIV, it is an opportunity to be informed about the importance of measures to reduce transmission to the infant and an entry point to assessing their own need for antiretroviral therapy or prophylaxis. PMTCT sites are therefore the setting where mothers are selected according to their HIV status and their babies can be ideally followed up. If effective PMTCT interventions are in place, 2-10% (when HAART or sdNVP are used, respectively) of babies will be infected by birth and EID services linked to PMTCT sites could effectively identify them. However, even though significant progress has been made during the past few years, it is currently estimated that only 45% of HIV-infected pregnant women in low and middle-income countries received at least some antiretroviral drugs to prevent HIV transmission to their child[108]

In addition, the vast majority of infants who have HIV are currently not identified through PMTCT services, but instead when they present for inpatient and outpatient health services because of signs and symptoms of HIV infection (see Fig.2).

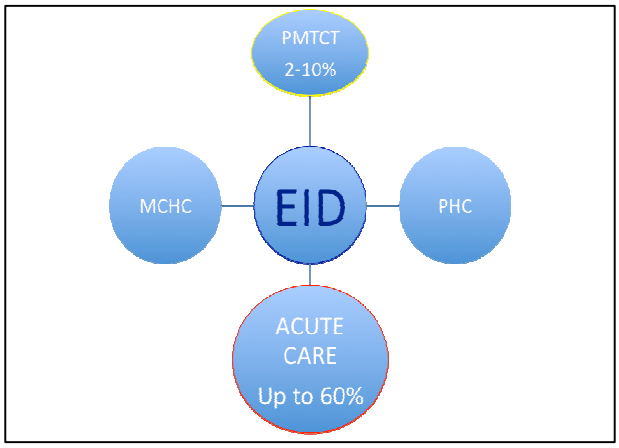


Fig. 2 Potential entry points for EID services and related HIV prevalence estimates reported.

Significant numbers of children who have HIV have been identified in many countries through PITC (provider initiated testing and counselling) in acute care settings, including Cameroon, Uganda[117], Zambia[118] and others. A particularly striking example comes from a study of referral patterns for HIV care and treatment for children in Malawi, which found that only 1% of the children referred for antiretroviral therapy, came from PMTCT services; the vast majority, almost 80%, came from children's wards or nutritional rehabilitation units.

EID is therefore primarily required to diagnose HIV infection in infants presenting with symptomatic disease. Acute paediatric units, nutrition units, outpatient departments and community clinics will all have a substantial number of infants presenting with signs and symptoms suggestive of HIV infection. In many African countries, policies of routine HIV antibody testing of children presenting to care are increasingly being adopted.

Mother and child health clinics (MCHC) and primary health care clinics (PHC) offer additional entry paths, particularly relevant in rural settings where HIV testing can be offered along with other basic interventions such as immunizations, vitamin A supplementation or insecticide-treated bed net distribution. HIV prevalence in this population is very different from country to country and the use of DBS or filter paper-based HIV DNA or HIV RNA testing and p24 antigen assays offers significant logistical and financial advantages for resource-limited settings and clearly offers a potential for EID service decentralization. The Fifth Stocktaking Report undertaken by UNICEF in partnership with WHO

reports that while the availability of early infant diagnosis services has increased dramatically in many countries, coverage in low- and middle-income countries still remains low. In 2007, 57 of 109 (52%) reporting countries had the capacity to provide virological testing to infants within two months of birth, in 2008 the number of further increased to 83 of 123 (67%) and availability of such testing is progressively expanding [18]. However, in 2010 only 6 percent of children born to HIV positive mothers received virological testing by 2 months of age and only 15% receive it at any time before 18 months of age [7].

A study of 11 sites in Cameroon by the Department of Disease Control, Ministry of Health, in conjunction with the Clinton Foundation HIV/AIDS Initiative found that only 32% of infants with a positive PCR test result were alive and undergoing treatment almost 18 months after data collection began on the provision of HIV early diagnostic services. The greatest loss to follow-up of infants in care (45%) occurred even before the mother received her child's positive test result[119]. Similar results were reported from Swaziland [120]. A meta-analysis of data from eight countries carried out by the Clinton Foundation estimated that 53% children were lost to follow-up after testing positive for HIV by DNA PCR[119, 120].'

A comparative analysis was recently published; the study was undertaken by UNICEF in four national programmes showed a remarkable increase in EID testing volumes between 2006 and 2009; data were considered representative of high (Namibia, Uganda) and lower (Cambodia, Senegal) HIV prevalence with diverse health system capacities. However, it was observed that despite the rapid expansion of the number of tests, the public health impact was still poor since in 3 of the 4 countries reviewed infants being diagnosed with HIV by virological testing were only in 22% to 38% of the cases, initiated on ART [121]. In this analysis it was also found that decentralization alone is not sufficient to greatly increase utilization of services at lower-level sites.

The benefits of decentralization to people receiving services must be balanced with the ability of the health care system to provide high-quality services. Whilst decentralization of the service, both geographically and through various levels of the health system, is highly desirable in high-burden countries, in low-burden countries, decentralizing treatment programmes for children to a more

peripheral level may not be practical, as the number of HIV-infected children managed by each health provider will drop below the level necessary to maintain clinical skills. The provision of cotrimoxazole preventive treatment or referral of symptomatic children, however, can usually be decentralized to the lowest levels of the health system[122].

By contrast, a successful implementation has been achieved by ANRS in the PEDIACAM study [123] where overall, the EID process was complete for 83.9% (95%CI: 82.0–85.6) of infants before the age of 7 months. This rate is similar to that reported in one clinical setting in Botswana (81%)[124], but remarkably higher than those in other surveys such as those conducted in Tanzania(55%)[125] and in South Africa (65%) [126]. However, should be noted that the PEDIACAM study, which involved a large prospective cohort designed to evaluate the feasibility of early HAART for HIV-infected infants, was conducted at three reference hospitals in the two main cities in Cameroon, strongly linked to PMTCT services and an intensive active follow up was performed; reasons which would potentially explain the significant better outcome being observed.

The differences between all the possible entry paths, such as HIV prevalence in the target population, need for service decentralization (diagnosis, general care and ART provision), context-specific issues and the cost implications may support the identification of alternative EID strategies. For instance, targeted strategies where repeat virological testing is implemented primarily where most needed and most cost-effective could allow countries to better allocate limited available resources. Information regarding the cost implications of similar approaches may provide a dynamic model which could also support countries in their mid- and long-term planning of EID country strategy as the HIV epidemic and the PMTCT service mature.

6.2.4 Additional Policy Background on Roll-Out of EID

The Inter-Agency Task Team on PMTCT (IATT) lab working group was formed in 2008 in recognition of the urgent need to address laboratory obstacles to the diagnosis of HIV infection in infants. Attention to infant diagnostics arose in part due to the need to promote access to immediate ART based on 2008

WHO recommendations. The initial focus of the working group was on addressing laboratory elements of diagnostic testing for infants.

In February 2010 the Laboratory Working Group, the group discussed the significant scale up of National early infant diagnosis services worldwide, as were the challenges that continue to face service delivery even as coverage increases. Across both high and low prevalence countries with rapidly expanding EID services, challenges were seen with regards to late identification and testing of HIV exposed infants, verticalization of the EID service such that exposed infants were receiving EID without other essential services such as cotrimoxazole prophylaxis, sub-optimal result return ratios, attrition of infants testing positive and in some cases substandard or delayed clinical care of infants enrolment in HIV care.

Program reviews of the national EID service were considered from 6 countries[1] . The Ministry of Public Health, Cameroon, conducted a review of both of their EID testing laboratories as well as 16 EID collection sites across 9 of 10 provinces from October 2008 to June 2009. In late 2009, the Ministries of Health of Senegal, Cambodia, Namibia and Uganda led EID reviews to examine 16-25 EID collection sites per country and to understand past and current practice after 1.5-3 years of service implementation [121]. CDC Nigeria undertook an EID service review of a representative sample of 37 EID collection sites and all 8 EID testing laboratories to identify bottlenecks and understand program trends. All reviews examined infant identification and sample collection through to central lab processing, result return to patient, and infant follow-up, as well as national courier and central laboratory practices. CDC also surveyed all CDC laboratories providing EID services in early 2010 and shared supplemental data from these surveys. These reviews, spanning a variety of national programs, have informed the highlighted potential gaps and barriers so far encountered.

At the end of the February 2010 meeting, plans for deliverables of the IATT Laboratory Working Group were defined and committed to by various member organizations. All plans and deliverables related to the strengthening of EID services within PMTCT programs.

The next phase is meant to keep supporting the current scale up of EID in different countries and a cost-effectiveness analysis was recognized as a priority to be developed and mutually undertaken in

order to support decision-making on EID service design and on cost-effective investments in laboratory infrastructure [WHO/UNICEF, KEMRI, CHAI, CDC].

6.2.5 Economic Considerations for Roll-Out

Economic analysis can help to inform the appropriate use of budgets within and across healthcare interventions. The tools of economic evaluation therefore enable the comparison of alternative courses of action in terms of both their *costs* and *effects*.

Two questions are pertinent for the financing of EID in low-income countries:

How should given budgets for EID be utilized to greatest effect?

What level of resources from the overall healthcare budget should be devoted to EID?

The methods of economic evaluation can help to inform both of these questions.

The first question can be addressed if an effect of interest common across EID strategies can be established, but which is achieved to different degrees. The effect should capture the immediate outcomes of interest from EID. Assuming this is a positive effect, it should then be maximized from available budgets. Outcomes are often expressed in a '*Cost/Effect*' ratio. Strategies that achieve the desired effect at lowest cost (i.e. have the lowest cost-effectiveness ratio) are chosen first, followed by the next most cost-effective strategies, until the available budget is exhausted. The appropriate method of analysis is often termed *Cost-Effectiveness Analysis[58]*.

To undertake a full Cost-Utility Analysis of alternative EID strategies it is necessary to understand how a positive test result would impact the treatment choice, the long-term health outcomes and the long-term healthcare costs associated with infants compared to the no testing alternative.

6.2.6 Methods for Establishing the Costs and Consequences of Alternative Strategies for EID

Countries are currently rolling out EID but may lack the information base to make rational choices over the alternative strategies for testing. This proposal will establish the costs and consequences of implementing two alternative testing algorithms at different entry points, in each of the countries in which the study will be undertaken.

Resource Use

Resource use data to be collected at each of the sites includes use of testing equipment, consumables, outpatient visits or additional length of inpatient stays for testing, costs of reorganizing clinics, and human resource requirements to deliver testing, laboratory costs, and transport costs to deliver and return samples to labs.

A *'time and motion'* study of human resource commitments may be undertaken in selected sites. Any additional resource use should be informed by practitioners at the sites.

Unit Costs

The costing analysis should follow the ingredients approach, whereby all resource items are assigned unit costs and are aggregated to determine costs per case.

Costs of testing equipment, consumables and any additional medical items required should be obtained from national purchase lists and international market rates. Costs of inpatient stays and outpatient visits should be obtained from nationally available unit cost estimates. Human resource costs should be obtained based on wage rates. Transport, reorganization and other costs should be informed by national practitioners and local market rates.

A societal perspective for costs should be used in the base case, but narrower perspectives need to be investigated in sensitivity analysis, in particular a health sector perspective. Societal costs falling on families of infants include travel costs to attend clinics and lost productivity through time off work. These should be determined through a survey of parents and caregivers at selected sites. Productivity costs can be informed by local wages.

Outcomes

Outcomes from testing have to be established by determining the probability of a tested case being true positive (TP), false positive (FP), true negative (TN) and false negative (FN), for the two testing algorithms, at each of the alternative entry points. This should be based on prevalence levels and the sensitivity/specificity of the tests (Tab. 1 and 2).

6.2.7 Identifying HIV-Infected Infants within Existing Budget Constraints for EID

Low-income countries currently have budgets that are earmarked for EID. Although these are being implemented through national systems they are largely funded by development partners and philanthropic organizations – for instance, PEPFAR and the Gates Foundation. It is therefore possible to provide guidance on strategies to maximize identification of true positives from EID budgets that for the purposes of the study can be taken to be exogenously determined and from within which decisions are then made.

The immediate objective of EID is the diagnosis of HIV-infected infants and it is therefore useful to represent the results of alternatives for testing in terms of an estimated 'cost per HIV-infected infant identified'. However, it is important to recognize that in the countries investigated a positive test result does not automatically lead to treatment. Previous work has shown that loss-to-follow-up is highly variably across different entry points, due to factors such as the distance from patients' homes and their likelihood to return to appointments [121]. An outcome of particular interest in this study is therefore *'Cost per HIV-infected infant accessing treatment as a result of EID'.*

The different entry points will therefore be assessed, for each of the testing algorithms, according to this measure. This will indicate how existing EID budgets can be utilized to maximize the number of true positives identified and ultimately accessing treatment for each of the testing algorithms.
Two provisos should, however, be noted:
The entry points may differ in access to diagnostic testing for HIV infected infants. The path of those starting with a condition to eventually enjoying improved health outcomes after treatment can be represented by a "staircase" type model, covering: access to an entry point, diagnosis, treatment, adherence and ultimately outcomes; in which the numbers moving to each step gradually reduce. Although pre-diagnosis access is undoubtedly important, coverage of testing and treatment is still far from universal. Unless there is interest in the equity implications of infants of different socioeconomic status having differential access depending upon entry point, the priority of decision-makers is likely to be getting increased numbers of infants diagnosed and onto treatment within given budgets.

However, reducing barriers to access should, over the longer term, be a key objective, and this would be a valuable area for future research.

The above analysis assumes the decision-maker is only interested in maximizing the number of TPs from available budgets, not in considering the costs and health consequences associated with FPs, TNs, and FNs. It is reasonable to assume that the role of EID is primarily to identify TPs, but equally the outcomes associated with the other consequences of testing are likely to have relevance.

The analyses should be repeated for the two- and three- test strategies. The major advantage of the three- compared to the two-test option is the cost saving of withdrawing treatment from a FP case after the 2nd test that would subsequently be correctly identified as HIV-uninfected with the 3rd test. The analyses should illustrate the treatment cost savings that may be achieved from the 3rd test and these can be compared to additional testing costs to provide some indication of the value of the 3rd test.

6.2.8 Budget Impact Analysis (BIA)

Based on the costing models and the above analyses, budget impact analyses should be undertaken to estimate the costs of fully implementing identified favoured strategies at the national levels in the study countries. This will indicate the appropriate path for scale-up of EID, and the associated financial commitments required by national authorities and/or development partners

The data requirements for the BIAs should include the number of entry points of each type across the countries, their size and associated utilization, some estimation of mean travel times for users, and distances of entry points from testing laboratories.

6.2.9 Draft Plan of Work

The first phase should be focused on the collection of the information regarding all the potential variables to be included in the model. We therefore envisage undertaking fieldwork in each country to be assessed. This will allow us to:

- Perform a comprehensive retrospective mapping of 2011 (first or second half) EID services in the country (by liaising with the Ministry of Health) – to include lab, PCR performed, DBS collection, uptake of the service etc.

- Develop a very basic preliminary costing model to identify data gaps
- Collect data on the virological testing algorithm implemented in the country
- Explore the sample volume received by referral labs from different entry points
- Sample specific sites representative of the diverse areas in the country (potentially between 18-25)
- Review the turnaround time of the results for the different sites
- Follow up the diagnostic cascade reviewing the number of children being diagnosed and initiated on treatment at the different sites
- Collect resource use and unit cost data for the different entry points

This work should thereafter comprise the following activities to be undertaken remotely:

- Estimation of the (societal and health sector) per unit costs of implementing the two EID algorithms at the different entry points;

- Estimation of the likelihood of TP, FP, TN and FN results, based on associated prevalence rates and the sensitivity and specificity of tests;

- Elimination of dominated strategies based on the above;

These above results should be used in a future CUA of full diagnosis-treatment pathways, utilizing also the findings of the CHER cost-effectiveness analysis. The following will also be undertaken to guide EID given existing information:

- Estimation of cost per positive case 'identified' and 'accessing treatment', for each of the testing algorithms across the different entry points (subject to the stated provisos)

- Budget impact analyses of implementing the favoured strategies.

The study should be undertaken across a range of countries representative of different types of health systems and HIV/AIDS epidemics. Some countries, such as those in sub-Saharan Africa, have generalized epidemics whereas others, such as Cambodia and India, have more concentrated epidemics.

6.2.10 Additional Work

Cost-Utility Analysis of EID

A comprehensive CUA of EID strategies is expected be undertaken using the results of this study and the results of the CHER economic evaluation, based on sourcing research funding. This could then

inform policy-makers in their response to infant HIV/AIDS epidemics through the whole pathway of identification, diagnosis and treatment.

Barriers to Access

Even if strategies are shown to be cost-effective, there are likely to be *barriers to access* that prevent the delivery of the interventions to infants in need of testing, and later treatment. Barriers can be identified on the "supply-side" - such as inadequate coverage of human resources, breakdown in supply chains and absence of transport between facilities and laboratories. Alternatively they can be on the "demand-side" – such as costs to families to present at clinics, fear of stigma, or lack of awareness. They may be identified at the health system, community or at household levels.

Further research would identify and determine the significance of these barriers through examination of the national and international literature, focus group discussions and surveys of potential users. If measures to overcome the barriers can be costed and their effectiveness determined an empirical case can be established to address these barriers.

It may also be informative to examine whether addressing particular barriers would increase access for vulnerable socio-demographic groups. Cost-equity analyses can be used to determine whether interventions may be favoured based on equity as well as efficiency criteria[59].

6.3 Transmitted drug-resistance in HIV-infected infants

Over the past five years antiretroviral treatment for HIV-infected individuals has been rapidly scaled up in resource-limited countries. Despite the initial fears of the widespread of HIV drug resistance (HIVDR) reports from resource-limited countries suggest that HIV drug resistance has been limited by an effective management of treatment programmes [127].

The acquisition of drug-resistant virus, the lack of availability of appropriate second-line drugs for children and the additional accumulation of resistance mutations in the absence of viral load testing to determine failure remain a concern.

Moreover, studies have evaluated HIVDR among children associated with various PMTCT regimens administered to mothers and children. Scale-up of efficacious PMTCT regimens should result in a decrease in pediatric HIV incidence, however, the widespread use of NNRTIs for PMTCT (or as part of an ART regimen for pregnant or breastfeeding women) are likely to result in a substantial proportion of children which may acquire NNRTI-resistant HIV, compromising their response to NNRTI-based ART. Despite the revised 2010 WHO recommendations advocating LPV/r-based ART as the regimen for HIV-infected children with prior NNRTI exposure, in many countries all children are started on NNRTI-based regimens, because of cost and feasibility. Even where an LPV/r based regimen is available, children initiating ART with previous NNRTI exposure may go unrecognized because of poor documentation.

The WHO's Global Network HIVResNet, which benefits from technical support of HIVDR experts worldwide, was formed to address concerns regarding HIVDR development by providing standardized tools, training, technical assistance, laboratory quality assurance and recommendations for guidelines and public health action [127].

Surveillance to assess HIVDR prevalence among young HIV-infected children eligible for ART initiation is relevant for improving health outcomes among HIV infected children and minimizing subsequent accumulation of drug resistance mutations.

Several studies have evaluated the prevalence of HIVDR among infants associated with various PMTCT regimens administered to mothers and infants. However, no widespread surveillance systems have

been implemented to evaluate the association of PMTCT and HIVDR among infants in the field, because of expense, difficulties with specimen collection, and the difficulty of comparing infants in different programs and receiving different regimens. With new virological diagnostic methods involving dried blood spots (DBS) being implemented in a large number of resource-limited countries, [68] remnant DBS are likely to be available for drug resistance surveys. This represents an important opportunity to evaluate resistance routinely among infants newly diagnosed with HIV, providing critical information to support optimal regimen selection.

The WHO HIVDR Prevention and Assessment Strategy

WHO recommends that countries adopt a seven-element HIVDR Prevention and Assessment strategy [67]. The assessment portion of the strategy includes already two paediatric components:

1. HIV Drug Resistance Early Warning Indicators (EWI) at sites providing ART to paediatric populations. The HIVDR EWI do not involve genotyping, but monitor factors associated with the emergence or prevention of HIVDR in a representative sample of children starting ART at representative ART sites in each country. Factors monitored include: ART prescribing practices, Losses to follow-up at 12 months, the proportion of children still on appropriate first-line ART at 12 months, the proportions of children whose ARV drugs are picked up and who attend appointments on-time, and ARV drug stock-outs.

2. Surveys to Monitor HIV Drug Resistance Prevention and Associated Factors in Sentinel Paediatric Antiretroviral Treatment Sites. WHO has developed a protocol to evaluate the effect of maternal and infant ARV history, baseline drug resistance, and other factors on ART outcomes in children. The protocol specifies obtaining a baseline genotype in a cohort of approximately 130 children before ART is started and following them for 12 months of ART to evaluate the factors associated with viral suppression and the development of drug resistance, including the child's and the mother's ARV history intrapartum, at delivery, and postpartum. A viral load and a drug resistance sequence are obtained after twelve months of treatment. As ART is expanded to more infants, this protocol could be used in a cohort of children $\leq$ two years of age. The survey has been piloted in a cohort of older children in one country; data are being analyzed.

WHO and CDC have developed a surveillance approach to estimate the prevalence of NNRTI and NRTI resistance in children newly diagnosed with HIV who are <18 months of age. This project will be included in the current WHO HIVDR prevention and assessment strategy activities, which are ongoing in several countries (see box above). This survey will be conducted with the aim of informing and supporting national, regional and global decision-making on optimal selection of children's first-line ART.

I was directly involved in the development of the protocol in close collaboration with senior colleagues who are part of the HIVResNet group. The contribution of the candidate has been particularly relevant in acquiring background data to inform the implementation of the survey; in order to identify countries to be potentially targeted for the pilot phase a questionnaire has been developed and sent to WHO country offices in the African region. Representatives of Ministry of Health and National HIV programmes were contacted and a questionnaire was compiled to obtain information on the current state of EID implementation in the country. Information were collected through 12 questions concerning: laboratories, sample volume being processed in the last year, positive PCR results, sample collection and conservation, logistic of sample transport across sites, availability of remnant DBS samples and specification of data collected by the EID lab form in use. This data were subsequently analyzed to assess the feasibility of the project at country level.

The candidate has also given a major contribution to the writing up of the manuscript, which is currently in press by the Clinical Infectious Diseases Journal.

WHO protocol to assess initial drug-resistant HIV among children <18 months of age and newly diagnosed with HIV in resource-limited countries

Silvia Bertagnolio[1]; Martina Penazzato[2]; Michael R. Jordan[1,3]; Deborah Persaud[4]; Lynne M. Mofenson[5]; Diane Bennett[6]

Author affiliations

[1] World Health Organization, Geneva, Switzerland

[2] Department of Pediatrics, University of Padova, Italy

[3] Tufts University School of Medicine, Boston, MA, USA

[4] Johns Hopkins University School of Medicine, Baltimore, MD, USA

[5] National Institutes of Health, Rockville, MD, USA

[6] United States Centers for Disease Control and Prevention, Global Aids Program, Atlanta, GA, USA

Keywords: HIV drug resistance, surveillance, children, pediatric, resource-limited setting

Running title: HIVDR in children <18 months newly diagnosed with HIV

6.3.1 Abstract

Studies have evaluated HIV drug resistance (HIVDR) prevalence among children associated with various prevention of mother-to-child HIV transmission (PMTCT) regimens administered to mothers and children. In 2009, an estimated 53% of pregnant women living with HIV and 35% of children born to them received PMTCT regimens[7]. Scale-up of efficacious PMTCT regimens should result in a decrease in pediatric HIV incidence. The widespread use of non-nucleoside reverse transcriptase inhibitors (NNRTI) for PMTCT or as part of an antiretroviral treatment (ART) regimen for pregnant and or breastfeeding women will result in a substantial proportion of children infected despite PMTCT acquiring NNRTI-resistant HIV, which will compromise their response to NNRTI-based ART. Despite the revised 2010 WHO recommendations advocating lopinavir/ritonavir (LPV/r)-based ART as the regimen for HIV-infected children with prior NNRTI exposure, in many countries children are started on NNRTI-based regimens despite previous NNRTI exposure, because of cost and feasibility. Even where an LPV/r based regimen is available, children initiating ART with previous NNRTI exposure may go unrecognized because of poor documentation.

Surveillance to assess HIVDR prevalence among young HIV-infected children eligible for ART initiation is crucial for improving health outcomes among HIV infected children and minimizing subsequent accumulation of drug resistance mutations; surveillance has not been implemented because of cost and logistics. WHO and CDC have developed a surveillance approach to estimate the prevalence of NNRTI and NRTI resistance in children newly diagnosed with HIV who are <18 months of age.

Results will support national, regional and global decision-making on optimal selection of children's first-line ART.

6.3.2 Introduction

As of December 2009, more than 2 million children were estimated to be living with HIV [108] . In 2009, an estimated 370,000 [230 000-510 000] children under 15 years were newly infected with HIV and 260,000 (150,000-360,000) died because of AIDS related diseases.

The World Health Organization (WHO) and the President's Emergency Plan for AIDS Relief (PEPFAR) support countries to scale-up services for the prevention of mother-to-child transmission (PMTCT) of HIV. In 2009, 53% [37–57%] of pregnant women infected with HIV living in low- and middle-income countries received antiretroviral drugs to prevent HIV transmission to their infants, compared to 45% in 2008 [108]. Current WHO PMTCT guidelines [128] recommend that, in addition to women requiring antiviral therapy (ART) for their own health, pregnant women who do not require ART should be initiated on a prophylactic regimen as early as 14 weeks gestation. Infants born to women receiving ART either for their own health or as prophylaxis should also receive standard prophylaxis with daily zidovudine (ZDV) or nevirapine (NVP) started at birth and continued to four-six weeks regardless of breastfeeding. For breastfed infants of mothers not receiving ART, daily NVP prophylaxis should be started at birth and continued until one week after stopping breastfeeding.

As recommended by WHO, infants known to be exposed to HIV should receive a diagnostic HIV Polymerase Chain Reaction (PCR) test at four to six weeks of age using dried blood spots (DBS), or at the earliest opportunity thereafter. For breast-fed children, repeat HIV testing six weeks after cessation of breastfeeding (when the child is <18 months old) is recommended [85]. Additionally, WHO recommends that all HIV-infected infants and children $\leq$24 months start ART at time of diagnosis. It is recommended that all children not known to have previously received the NNRTI, NVP for PMTCT start standard NVP-containing ART. Children with previous exposure to an NNRTI, either because their mothers received an NNRTI-based regimen during pregnancy, labor and delivery or breastfeeding, or because they received an NNRTI directly, should initiate an ART regimen that includes the protease inhibitor LPV/r [21].

The implementation of early ART with boosted-PI regimens in infants and children in limited-resource settings represents a challenge. The public health approach to global ART scale-up is based on use of two nucleoside reverse transcriptase inhibitors (NRTIs) in combination with an NNRTI. This antiretroviral drug (ARV) combination is more widely available, relatively inexpensive, and is available in generic formulations and co-formulated tablets for pediatric populations. Pediatric boosted- PI regimens, which are commonly reserved for second-line ART have limited availability, relatively high cost and present a number of challenges which limit ART success. Challenges include: poor palatability, need for cold chain supply, interaction with rifampicin used for treatment of tuberculosis co-infections, and association with long-term metabolic complications. While NNRTI-based PMTCT regimens reduce HIV infection risk in infants/children, the increased risk of acquiring drug-resistant HIV infection in children who become infected despite PMTCT, particularly with extended NVP prophylaxis poses a challenge. In addition, initiating ART with two NRTIs and LPV/r may further limit second line treatment options if thymidine-analogue mutations (TAMs) and/or K65R have been selected when the child's ART fails.

In children, NNRTI-resistant HIV can be selected by exposure to NNRTIs used for maternal ART or child prophylaxis in the antenatal, intra-partum, and postpartum periods (including during breastfeeding). Furthermore, primary infection with NNRTI-resistant virus through mother-to-child transmission via *in utero*, peri-partum and breastfeeding is documented. A meta-analysis of seven studies of HIV-exposed infants who became infected despite NVP PMTCT showed an overall prevalence of NNRTI HIVDR in 52.6% (n=201; 95% confidence intervals [CI] 37.7-67.0) at four-eight weeks following NVP exposure using standard HIV genotyping assays. The risk of acquiring NNRTI-resistant virus is increased further when NVP is given daily for prophylaxis against breast-milk transmission.

Infants who received six-week extended-dose NVP during breastfeeding were significantly more likely to carry NNRTI resistance mutations detected by standard genotyping than those who received sd-NVP . NNRTI resistance was reduced to 16.5% when NVP prophylaxis was combined with zidovudine (ZDV) or ZDV+ lamivudine (3TC). The addition of extended ZDV to extended NVP prophylaxis also reduces the risk of NVP resistance for infants infected *in utero* . A negative correlation between the level and detected NVP resistance and infant age is reported ($p<0.001$), which may have implications for timing of re-use of NVP for ART in infants with prior NNRTI exposure .

Concerns have been raised for infants exposed to NRTIs prophylaxis or whose mothers are receiving NRTIs for ART and are breastfeeding. In the Pediatric AIDS Clinical Trials Group Protocol (PACTG) 076 study, in which NRTIs were used for PMTCT, no NRTI resistance was detected in infected infants. However, the PACTG185 study showed high ZDV resistance prevalence among infected infants whose mothers had ZDV PMTCT. In the Stopping Infection from Mother-to- Child via Breastfeeding in Africa (SIMBA) study infant prophylaxis with lamivudine (3TC) for prevention of breast-milk transmission resulted in acquisition of the M184V/I mutation (69%) of infants, which was no longer detectable five months after discontinuation of 3TC . In the Kisumu Breastfeeding Study (KiBS), the use of a nelfinavir (NFV)-based triple ARV regimen in breastfeeding women was associated with higher prevalence of infant NRTI resistance (mainly to 3TC) compared to infants infected with maternal NVP-based triple ARV prophylaxis (100% vs. 67%). No major protease inhibitor (PI) mutations were detected. Multi-class resistance mutations to both NNRTI and NRTI were detected in 11 of 37 breastfeeding infants whose mothers initiated NNRTI-based ART for their own health postpartum. The infants had received either single-dose NVP (sdNVP) or extended NVP prophylaxis. Initiation of maternal ART within 14 weeks of delivery was associated with detection of multi-class HIVDR in breastfeeding infants.

Selection of HIVDR may be due to low levels of ARV exposure to the infant/child through breastfeeding, though studies are limited. NVP levels in breast-milk were found to be measurable for up to 16 days after maternal sdNVP. While NNRTI exposure in breast-milk may prevent infection, it may also select for drug-resistant virus, depending on the level to which the child is exposed. Sub-optimal levels of NNRTIs have been documented in breast-milk but in KiBS, NVP and 3TC were detected in breastfeeding infants at levels sufficient to suppress virus replication. Drug-resistant HIV may also be transmitted through breast-milk; NNRTI resistant virus has been detected in breast-milk of women who received intra-partum sd-NVP. The risk of NNRTI resistance transmission is reduced when sd-NVP prophylaxis in women is combined with a seven-day course of ZDV/3TC (“tail”).

Selection of drug- resistant HIV during PMTCT has important implications for ART in children in whom prophylaxis fails. In an initial study in a small number of infants, sdNVP-exposed infants were significantly more likely to fail ART when initiated on NVP-based regimens. More recently, Pediatric AIDS Clinical Trials Network (PACTG) P1060 trial, a large randomized controlled trial conducted in six African countries, comparing a LPV/r-based ART regimen to a NVP-based ART regimen in HIV-infected

children between 6 and 36 months of age who previously received sd-NVP for PMTCT, showed that LPV/r-based ART was superior to NVP-based ART in preventing virological failure/discontinuation of ART (OR=18.6; P=.02). In this study, baseline NVP resistance was detected in 12% (18/148) of children studied and predicted ART failure in the NVP arm (P=.02 for the interaction between treatment and baseline NNRTI resistance).

NVP may be used in ART for sd-NVP-exposed children if the NVP-based ART is introduced after HIV replication is initially controlled with LPV/r-based therapy according to the NEVEREST study. In modified intent-to-treat analysis, more children who switched to NVP maintained <50 copies/ml through week 24 post-randomization compared to those continuing LPV/r (65.6% vs 49.5%, p=0.02). However, fewer children in the switch group than in the control group maintained <1000 copies/ml (84.9% vs 96.8% p=0.007). Similar findings were observed at week 156 post-switch [91]. Moorthy showed that levels at which NNRTI drug resistance mutations were present in pre-treatment plasma (≥25%, and therefore detected with standard clinical genotyping assays) at the start of the LPV/r-based ART in the infants in the NEVEREST study was predictive of ART failure with use of NVP-based ART [95].

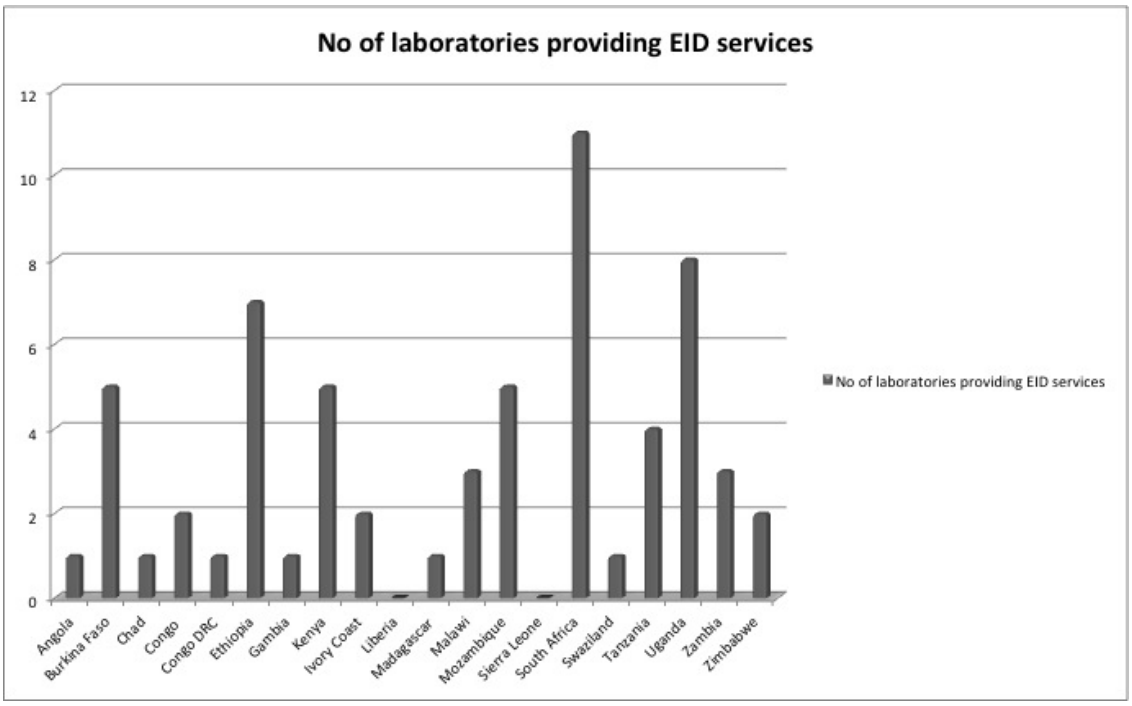
In summary, scale-up of PMTCT efforts following WHO guidelines should result in a decrease in pediatric HIV infection. However, the widespread use of NNRTIs for prophylaxis in children and pregnant/breastfeeding women or as part of ART regimens in women will lead to substantial increases in the proportion of children infected despite PMTCT with NNRTI-resistant HIV. Response to NNRTI-based first-line ART in HIV-infected children is compromised by prior exposure to NNRTIs for PMTCT and for maternal health, particularly in infants initiating ART immediately after diagnosis. Despite the revised 2010 WHO recommendation of LPV/r-based ART as the regimen for HIV-infected children with prior NNRTI exposure, in many countries children are started on NNRTI-based regimens regardless of previous NNRTI exposure, because of cost and feasibility. Even where an LPV/r-based regimen is available, some children initiating ART who may have previous NNRTI exposure may not have adequate documentation of exposure, or may have NNRTI-sensitive virus despite NNRTI exposure and not require PI-based ART. It is important to assess the proportion of children with HIVDR potentially associated with regimen failure.

Surveillance to assess HIVDR among HIV infected children <18 months is important and to date has not been implemented on a large scale due to cost and logistical constraints. However, the scale-up of

early infant diagnosis (EID) using dried blood spots (DBS) provides a unique surveillance opportunity to test remnant specimens for drug resistance.

WHO and CDC developed a surveillance method to assess initial HIVDR to specific ARVs among children <18 months of age and newly diagnosed with HIV in resource-limited countries. This surveillance activity is designed to be integrated into national surveillance strategies and repeated over time to capture evolving scenarios of PMTCT regimen changes and coverage. Results from these surveys will support decision-making on optimal selection of children's ART regimens.

Figure 1. Laboratories providing early infant diagnoisi (EID) services in countries (May 2010).

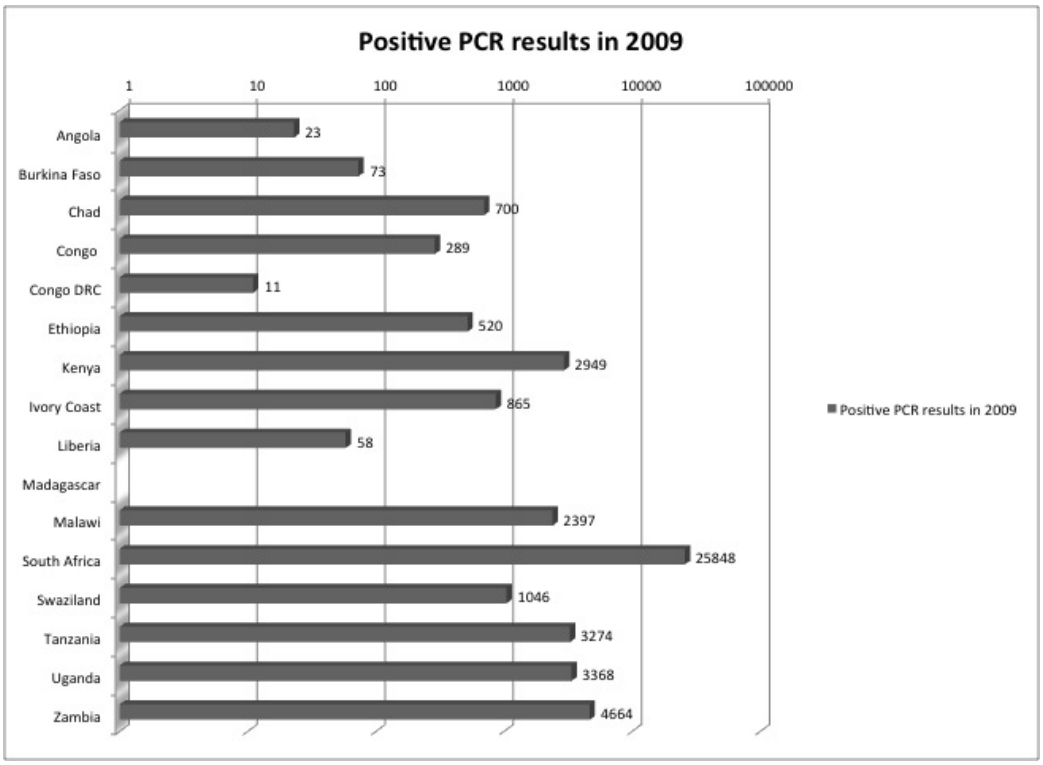


In some countries such as Madagascar, Angola, Burkina Faso, Congo DRC, Liberia, HIV positive tests in children <18 months of age is small (range 0-73) while in other countries a larger number of HIV diagnoses are made in this age group (range 289-25848) (**Figure 2**).

In 8/14 countries (57%), demographic and clinical data records for EID are sub-optimal. In 2009, implementation of a standard national form for EID was not available in most of the countries surveyed; this resulted in high heterogeneity of information, both for quality and type, within the same country. Among 70% of the countries, basic information such as age, gender, date of birth, date and

site of specimen collection is consistently reported. In some countries (South Africa, Kenya, Uganda, Swaziland and Mozambique), information on ARV exposure is recorded in EID forms accompanying DBS to the laboratories.

Figure 2. Total number of positive PCR results in children <18 months issued by national laboratories providing Early Infant Diagnosis (EID) services in 2009



DBS are often transported to EID laboratories within 4 weeks from collection. At least one remnant DBS is available in most countries after EID testing; however, long-term storage conditions differ across countries: DBS are stored at room temperature (Ethiopia, Kenya, Tanzania, Swaziland) or at -80 or -20 degrees (Angola, Burkina Faso, Uganda, Zimbabwe).

6.3.3 Methods

Because new EID methods using DBS are being implemented in many resource-limited countries and because of the suitability of DBS for HIVDR genotyping, DBS is the specimen type selected for this survey.

The survey method is retrospective and uses remnant DBS from HIV-infected children diagnosed with HIV <18 months stored at EID laboratories. Demographic and clinical information are abstracted from laboratory requisition forms accompanying DBS. Required and optional patient variables are presented in **Table 1**.

Table 1 Required and optional survey variables

Required variables
1. Date of birth (if not available, Age of child in months at time of blood draw)
2. Gender
3. Site name where DBS was collected
4. Site type where DBS was collected
5. Date of DBS collection
6. Date DBS frozen at -20 C or -70 C
7. Child receiving ART (not PMTCT) at time of specimen collection (yes/no)
8. Date of PCR testing
Optional variables
1. Exposure to breastfeeding at time of specimen collection
2. Duration of brestfeeding
3. ARVs (drug names) received by mother antepartum/intrapartum/postpartum/ during breastfeeding
4. ARVs (drug names) received by infant/child postpartum/during breastfeeding
5. Time of freezing DBS at -20C or -70C

DBS collected for EID and genotyped will originate from routine follow-up of HIV-exposed children through PMTCT programmes, Maternal and Child Health (MCH) or Antenatal (ANC) clinics, HIV testing of symptomatic children presenting to MCH, hospitals or other medical facilities, and from testing of children in provider-initiated testing and counseling (PITC) sites or Voluntary Counseling and Testing (VCT) sites.

As surveillance is conducted retrospectively using remnant DBS, specimens must have been stored and handled according to WHO recommendations. The relevant portions of the RT region of the *pol* gene of HIV will be sequenced using standard sequencing methods.

Survey inclusion criteria

Participant inclusion/exclusion criteria are described in **Table 2.**

Table 2 Participants inclusion and exclusion criteria

Inclusion criteria
• DBS tested HIV-positive by PCR from a child ≤ 18 months of age. • If DBS for PCR is collected from a child at different time points, these should be clearly labeled with a unique ID so that the child is not counted twice or more times. The most recent DBS specimen from the child is selected for genotyping. • At least one viable remnant spot is available (two-four DBS optimal). • DBS has been stored no longer than 30 days at ambient temperature, then stored at -20°C or -80°C with no thawing before genotyping.
Exclusion criteria
• DBS from children <18 months of age • Child is on ART at time of specimen collection

Laboratory and sample selection

In some resource-limited settings all EID DBS are tested by one national laboratory while other countries have many EID laboratories. When possible, all laboratories performing HIV EID will participate in pediatric HIVDR surveillance, and will contribute to the overall sample. If only a subset of laboratories participate, a simple random sample of laboratories will be chosen. Once a

laboratory is selected, HIV-positive DBS will be sampled using simple random sampling without replacement.

Sample size

The sample size calculation is based on the assumption that the "true HIVDR prevalence" is 50%; CI of +/- 6%; and power (1-β)=0.80. Prevalence of 50% is the most conservative assumption, yielding the largest sample size and most precise CI. Precise CI is especially important when prevalence is low. Sequence amplification success from DBS is assumed to be 80%.

Using the normal approximation to a binomial in PASS 2008 software (http://www.ncss.com), a 95% CI of 12% (+/-6%) for a prevalence of 50% requires a sample size of 267. Because the amplification rate is expected to be 80% the effective sample size is 267/.8 = 334. In countries where there is only one EID laboratory, DBS from eligible children are selected until a sample size of N= 334 is reached.

In countries where DBS are obtained from more than one laboratory, the sample size must be adjusted to account for the impact of intra cluster correlation; therefore, a design effect multiplier of 1.5 will be used. Accounting for the design effect, the final effective sample size for the country is 334*1.5 = 501. In countries with more than one laboratory contributing specimens, the proportion that each contributes to the sample size must be calculated and multiplied by 501 to determine the number of specimens any one laboratory contributes. The proportion for each laboratory is calculated by taking the national total number of HIV-positive DBS for the target year, and dividing the number of positive DBS from each laboratory for that year by that number.

If countries have insufficient numbers of eligible specimens to reach the required effective sample size the country should genotype all eligible specimens.

Ethical considerations

Remnant DBS specimens will be tested anonymously and no personal identifiers will be abstracted; a “non-research” waiver will be requested from Institutional Review Boards/Ethics Review Committees.

Statistical analysis

The prevalence of HIVDR mutations leading to a classification of high, intermediate, or low levels of HIVDR for each ARV routinely used for pediatric ART as determined by the Stanford will be provided.

HIVDR prevalence will be estimated with 95% confidence intervals based on exposure to PMTCT (Yes/None/Unknown). If sample sizes are sufficient and patient data are available, separate analyses will be performed evaluating the association of HIVDR with specific PMTCT regimens.

6.4 Discussion

Despite the revised 2010 WHO recommendations, in many countries children are started on NNRTI-based regimens regardless of previous NNRTI exposure, because of cost and feasibility. It is important to assess the proportion of children who carry mutations potentially associated with NNRTI-based regimen failure. Even in countries where PI-based regimens are offered to children exposed to NNRTI, PMTCT NNRTI exposure may not be routinely recorded or may be incorrectly reported as “none” or “unknown” and may be started on NNRTI-based regimens.

NNRTI resistance is often found at higher rates in children in observational studies as opposed to clinical trials. Explanations include insufficiently strict methodology for accurate evaluations, varying periods of exposure, or “real-world” conditions such as sub-optimal adherence to ARVs. It is important to perform HIVDR surveillance and to study the implementation of the PMTCT program in the field to support optimal pediatric ART strategies. This survey will provide descriptive evidence of HIVDR in HIV infected children and may provide information on association of different PMTCT ARV exposures and HIVDR, which will inform future WHO PMTCT ARV guidelines. This protocol has some limitations. First, data on ARVs exposure for PMTCT are not available in most countries, thus limiting the ability to test association between ARV exposure and HIVDR emergence. Second, in many settings DBS may not be properly collected, transported to store thus, lowering amplification efficiency.

In conclusion, the expansion of PMTCT options may lead to changing patterns of HIVDR in children infected when PMTCT regimens fail. This survey will provide information about HIVDR risks related

to pre-treatment ARV exposures, when information regarding exposure is available and provided that groups exposed to different regimens are sufficiently large. If current regimens or current record-keeping are non-optimal, results may provide opportunities for corrective action. If information regarding previous ARV experience is reported as “unknown” for many children and high levels of HIVDR are detected in this group, recommendations such as targeted early virologic monitoring (where feasible) and baseline genotypic testing (if possible) may be explored. The evaluation of HIVDR prevalence to specific ARVs will support decision-making about pediatric ART regimens in countries and globally. The survey may also provide information for review of WHO PMTCT guidelines.

Table 3. Information collected on national Early Infant Diagnostic forms as of May 2009

Countries	Infant info						Sample			Mother info						
	Patient ID	**Sex**	**Age**	**Date of birth**	**Infant prophylaxis**	**Clinical condition**	**Date of collection**	**1st or 2nd PCR1**	**Point of entry2**	**HIV status**	**On HAART**	**Pre-partum PMTCT**	**Intra-partum PMTCT**	**Post-partum PMTCT**	**Breastfeeding**
Angola							x	x							
Burkina Faso	x	x	x	x	x*		x		x		x*	x*	x*	x*	x*
Congo DRC	x	x	x	x					x						
Ethiopia	x	x	x	x			x	x	x						
Kenya	x	x	x	x	x		x			x	x				
Ivory Coast	x	x	x	x	x*	x*	x	x	x	x	x*	x*	x*	x*	x*
Liberia	x	x	x	x			x			x					
Malawi	x	x		x			x		x						
Mozambique	x	x	x	x	x		x	x	x	x	x*	x*	x*	x*	x
Uganda	x	x	x	x	x		x	x	x	x	x*	x*	x*	x*	x
South Africa	x	x	x	x	x*		x	x	x	x	x*	x*	x*	x*	x
Swaziland	x	x	x	x	x*	x	x	x	x	x	x*	x*	x*	x*	x
Zambia	x	x	x	x	x		x	x	x	x					x*
Zimbabwe	x	x	x	x	x	x*	x	x		x			x*		

6.5 Acknowledgements:

This protocol was developed and endorsed by the WHO HIVResNet Pediatric advisory panel whose members included pediatricians and HIV experts from a number of institutions, including PEPFAR/CDC (Dr Elaine Abrams**,** Dr Helen Dale, Dr. Carlo Giaquinto, Dr Sylvia Ojoo, Dr. Nigel Rollins, Dr. Paula Vaz). NIH K23 AI074423-05 (MRJ)

[1] Specifications to differentiate 1° and 2° PCR for the same infant are given.

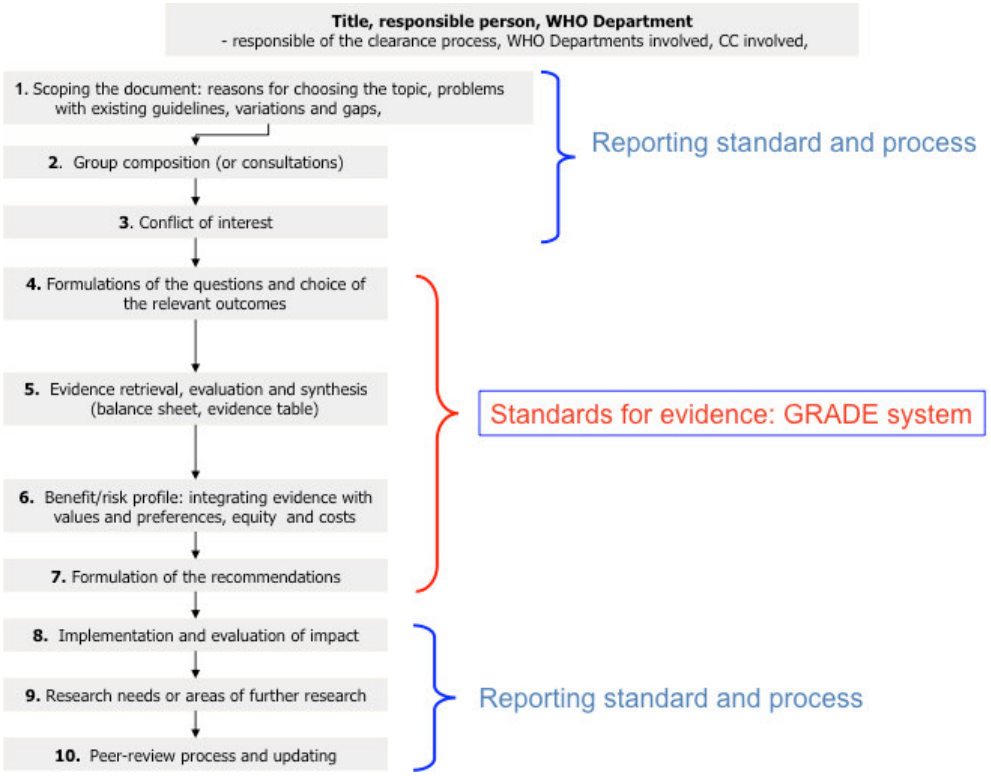
[2] Such as: PMTCT site, paediatric ART site, paediatric ward, vaccination site/MCHC

X = basic information collected, e.g. Infant prophylaxis Y/N; X* = more complete information is collected, e.g. Infant prophylaxis Y/N and type of ARVs

7. Research activities IV. Translating Evidence into Policies

Systematic and transparent methods to develop clinical, public health, and policy recommendations, are now used by several organisations [129, 130]. WHO's Cabinet recognised the need for using systematic and transparent methods to develop recommendations in 2003 and an official endorsement was given through the release of the guidelines for WHO guidelines [55]. Consequently the WHO process of guidelines revision was updated in 2007 and wide efforts are being made since then to standardize methods of assessing evidence and developing recommendations using the GRADE approach [131], in order to report openly and transparently these processes for those who may wish to act upon the recommendations.

The GRADE approach was developed by the GRADE Working Group, which began as an informal collaboration of academics and methodologists with an interest in tackling the shortcomings of the present evidence grading systems [92]. The GRADE system was chosen as it enables more consistent judgments, and communication of such judgments can support better informed choices in health care. Box 1 shows the steps in developing and implementing guidelines from prioritising problems through evaluating their implementation [56].



Although early initiation of cART may be beneficial for young children, lifelong treatment is problematic, given the limited availability of appropriate drugs, long-term cART toxicity, and difficulties with adherence, risk of viral resistance, and cost of such a strategy. The optimisation of 1st-line regimens in infants is critical. Moreover, currently only 6 drugs are available in appropriate infant formulations and treatment options are limited, especially in countries where expensive drugs remain unavailable.

The release of new data on the best options for initiation of ART in HIV-infected infants who have not been exposed to NNRTIs for maternal treatment or PMTCT has generated considerable discussions on whether international standards for treatment should be revised. This area is of particular interest because although access to cART for children has expanded considerably over the last few years, children still lag behind adults in terms of ART coverage. A better understanding of the most effective and appropriate treatment for infants is urgently needed to inform global policy.

WHO, currently recommending an NNRTI-based first line regimen for those HIV-infected infants not previously exposed to NNRTI, is now evaluating the implication of new data on LPV/r-based regimen in all infant starting ART regardless of NVP exposure. The decision-making process is occurring in the context of a new phase for the global HIV care and treatment scale-up evolving from the '3 by 5' initiative. Despite the achievements of the past decade in reducing HIV-related deaths, the global response to HIV is facing huge financial and technical challenges in achieving universal access [53].

The first part of this section was developed to discuss the policy implications of the evidence appraised in previous chapters concerning the optimal ART regimen for HIV-infected infants starting early treatment. With the aim of translating evidence into policy, the GRADE process was applied to formulate recommendations, which could inform the future WHO paediatric guidelines revision.

This work was facilitated by the expertise in guideline development that I acquired over the past 4 years while collaborating with the HIV and TB departments of the World Health Organisation. Further studies conducted at the London School of Hygiene and Tropical Medicine as part of the Public Health in Developing Countries MSc course have also contributed to increase my awareness of the challenges in implementation and to acquire a more comprehensive approach to health

issues in resource limited settings. A policy paper on this work was presented as final MSc project report and has been contributing to the development of a position paper on this topic.

The second part of this section was instead developed to highlight the limitations and the challenges of applying the GRADE framework while identifying recommendations for a public health approach, particularly for diagnostic interventions.
The process followed during the revision of the diagnostic guidelines for HIV infection in children was reconsidered and the issues around the methodology used highlighted. It was recognized that the GRADE framework may not be sufficient in certain contexts: focusing on accuracy to assess patient important outcomes may result in failure to adopt robust and useful technologies that the health systems is then unable to support. In the context of WHO the GRADE system is useful in assessing evidence but in developing recommendations directed at public health interventions for use and adoption into a range of contexts, additional standardized assessments, models and scenarios may be useful. A modified approach with a health systems lens was finally proposed.

I led the preparation of the manuscript in close collaboration with senior colleagues at WHO. The contribution to this work was facilitated by my direct experience on the application of GRADE for diagnostic interventions which occurred during the revision of the WHO guidelines [17] for diagnosis in infants and children, which I conducted across 2008 and 2009. A manuscript is under submission to the British Medical Journal.

7.1 First line antiretroviral treatment for HIV infected infants in low and middle income countries: a public health approach

London School of Hygiene and Tropical Medicine

PROJECT REPORT
MSc Public Health in Developing Countries
M. Penazzato

7.1.1 Executive summary

Early initiation of combination antiretroviral therapy (cART) is shown to be a life saving intervention for HIV infected infants and is currently recommended as international standard of care. However, lifelong treatment is problematic, given the limited availability of appropriate drugs, long-term cART toxicity, difficulties with adherence, risk of viral resistance, and cost of such a strategy. Currently infant formulations and treatment options are limited, especially in low and middle-income countries where expensive drugs remain unavailable. Efficacy of ART in young children is shown to be affected by maternal transmission of drug-resistant virus, arising either from multi-drug exposure or exposure to prevention of mother-to-child transmission (PMTCT) interventions such as single-dose nevirapine (sd-NVP). Scale-up of ART in resource-limited settings is likely to increase the prevalence of resistant transmitted virus over time and the occurrence of drug resistance needs to be carefully considered in recommendations for 1st-line treatment regimens.

Results from trials comparing PI versus NNRTI- based ART in children [33, 34], have only recently become available reporting conflicting results particularly in children not exposed to NNRTIs. This has generated considerable discussions on whether international standards for treatment should be revised. This area is of particular interest because despite the access to cART has been expanded considerably over the last few years, children still lag behind adults in terms of coverage. A better understanding of the most effective and most appropriate treatment regimens for infants is urgently needed to inform global policy decisions.

The question of what cART to start was addressed by the P1060 trial team in two different RCT comparing a NVP-based regimen with a LPV/r-based regimen in young children below 3 years in both NNRTI-exposed and unexposed children. It is well recognised that a substantial proportion of infants and young children exposed to sd-NVP at birth develop NNRTI resistance and that the efficacy of NVP-based regimens is compromised. Unexpectedly, sd-NVP-unexposed children also showed a higher failure rate if randomised to a NVP, compared to LPV/r, regimen. Overall, comparison of the data presented here shows that results from the two cohorts were consistent and that, in meta-analysis, no heterogeneity was found across the two studies. A systematic evaluation of the quality of the evidence was conducted and overall good quality of evidence was found to support the superiority of a LPV/r-based regimen for all HIV-infected infants regardless of their exposure to NNRTIs.

High-quality data from two RCTs suggest a benefit to LPV/r; however, the reasons for this finding (in non-NVP-exposed children) are not completely understood, and there are currently disadvantages to LPV/r over NVP in infancy, including cost, palatability, cold chain requirements and formulation. The GRADE framework was used and a risk and benefits assessment of extending the use of LPV/r-based regimen to every HIV-infected infants showed that: benefits are likely to out weight risks, particularly in terms of toxicity and resistance profile; costs can be contained through price negotiation and new formulation becoming available; LPV/r is not likely to be accepted by the intervention recipients (patients, HCW and health system); the feasibility is largely affected by issues around procurement and supply which can only be overcome by different formulations which are still unavailable.

In conclusion, it is advisable that international guidelines are only revised when better formulation will become available and the safety of new strategy for treatment sequentiation will be consolidated. Meantime, further evidence should be sought to complete the evaluation of non-virological health outcomes and a proper cost-effectiveness should be conducted.

7.1.2 Introduction: The Public Health Importance of first-line antiretroviral treatment in HIV-infected infants

The number of children under the age of 15 years living with HIV increased from 1.6 million (range, 1.4 million-2.1 million) in 2001 to 2.5 million (range 1.6 million-3.4 million) in 2009, and over 1000 newly infected infants continue to be born daily [1]. In the absence of combination antiretroviral therapy (cART), over 50% of HIV-infected infants progress to AIDS and death by 2 years of age. The introduction of cART has dramatically changed the natural history of HIV infection in children [2, 3], and now, in well-resourced countries, over 90% HIV-infected children reach the age of 10 years [4]. However, in 2009, only 28% (range 21%-43%) of the 1,270,000 (range 830,000-1,700,000) children estimated to need cART in low- and middle-income countries had access to it. Paediatric deaths account for 14% of all HIV-related mortality globally [108].

The natural history of perinatal HIV infection differs from that of primary infection in adults. Rapid disease progression is a hallmark of HIV infection during the first 2 years of life, especially in resource-limited settings. Early initiation of antiretroviral treatment in HIV-infected infants, irrespective of clinical immunological and virological condition has shown to increase survival and reduce disease progression. These benefits were confirmed in several observational studies and a randomised trial[22], which together support the value of early cART in different settings. However, the optimal regimen to start in these infants is still unclear and this paper aims to inform this decision with a public health approach.

Although early initiation of cART may be beneficial for young children, lifelong treatment is problematic, given the limited availability of appropriate drugs, long-term cART toxicity, and difficulties with adherence, risk of viral resistance, and cost of such a strategy. Although 25 antiretroviral drugs are licensed worldwide for the treatment of HIV-infected adults, several are either unlicensed or do not have appropriate formulations for very young children. Currently only 6 drugs are available in appropriate infant formulations and treatment options are limited, especially in countries where expensive drugs remain unavailable. An appropriate selection of two non-nucleoside reverse-transcriptase inhibitors (NRTI) can only be combined with a non-nucleoside

reverse-transcriptase inhibitor (NNRTI) such as nevirapine (NVP) or a boosted protease inhibitor (PI) such as lopinavir/ritonavir (LPV/r).

The optimisation of 1st-line regimens in infants is critical. Two recent trials, with contradictory results, investigated the effectiveness of different 1st-line cART regimens in children unexposed to NNRTI as part of prevention of mother-to-child transmission (PMTCT) interventions. In the PENPACT-1 trial, children from Europe, the USA and South America, aged one month to 18 years were randomised to start PI or NNRTI-based cART [33]. At four years, >80% of children in both arms had viral load <400 copies/ml with no differences in CD4 responses. By contrast, the P1060 trial, conducted mainly in African children aged 2-36 months and unexposed to NVP-based ART for PMTCT showed a higher rate of treatment failure in those starting NVP-based compared with LPV/r-based cART.

The prevalence of transmitted drug resistance in perinatal HIV infection increased by 58% between 1998 and 2002 in western countries ; similarly, the scale-up of cART is likely to increase the prevalence of resistant transmitted virus in resource-limited settings. Infants who have HIV infection and are NVP-exposed through infant or maternal treatment or prophylaxis have demonstrable viral resistance, compromising the response to NVP-containing 1st-line cART regimens. Evidence of the impact of NVP-resistance acquisition on treatment response lead to the prompt update of WHO treatment guidelines which currently recommend the use of LPV/r-based regimen in infants exposed to NNRTIs as part of PMTCT interventions [112].

The release of new data on the best options for initiation of ART in HIV-infected infants who have not been exposed to NNRTIs for maternal treatment or PMTCT has generated considerable discussions on whether international standards for treatment should be revised. This area is of particular interest because although access to cART for children has expanded considerably over the last few years, children still lag behind adults in terms of coverage. A better understanding of the most effective and most appropriate treatment regimens for infants is urgently needed to inform global policy decisions.

WHO, currently recommending an NNRTI-based first line regimen for those HIV-infected infants not previously exposed to NNRTI, is now considering to revise guideline

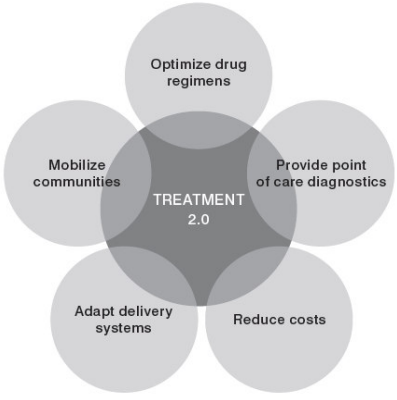


Figure 17. Treatment 2.0 priority work areas.

recommending a LPV/r-based regimen for any infant starting treatment. The decision-making process is occurring in the context of a new phase for HIV care and treatment scale up evolving from the '3 by 5' initiative. Despite the achievements of the past decade in reducing HIV-related illness and death, the global response to HIV is dealing with huge financial and technical challenges in achieving universal access. A strategy called "Treatment 2.0" has been developed to meet these challenges by improving the efficiency and impact of HIV care and treatment programmes in resource-limited countries [53](Figure 17). Key pillars are simplification, innovation, efficiency, effectiveness and cost-effectiveness, accessibility, affordability, decentralisation and integration, equity, and community mobilization.

A global plan 'Countdown to Zero' has been developed by UNAIDS and the US President's Emergency Plan for AIDS Relief (PEPFAR) and was launched in June 2011 making a commitment by 2015 to eliminate new HIV infections among children and to keep their mothers alive, in an effort to save millions of lives across the developing world, particularly in Africa. A more energetic expansion of PMTCT programmes will be forgone with a significant impact on the number of infants acquiring the infection and initiating cART. A shift to more efficacious regimens for PMTCT is also expected (Figure 18), to reduce infections as well as to contain the development of drug resistance compromising the treatment option for infants.

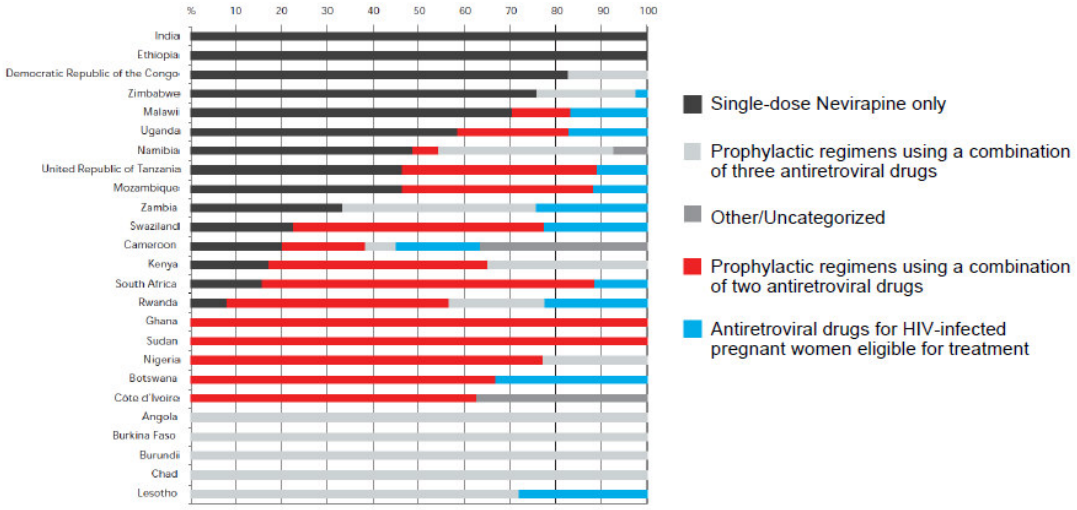


Figure 18 Distribution of the prophylactic regimen for the PMTCT (Source: Country Progress Reports 2010 [1])

In this context, deciding whether an update of treatment recommendation by WHO is advisable requires to conduct a comprehensive evaluation and risks, benefits, feasibility and costs implications of a LPV/r-based cART 1st-line regimen for every infant initiating treatment (regardless

of NNRTI exposure) have to be carefully evaluated. This policy paper will primarily revise the evidence supporting the use of a LPV/r–based regimen in infants and will subsequently discuss the challenges that this change would offer in terms of clinical management, feasibility and resource allocation. Finally, a proposed recommendation will be identified and ranked to inform WHO paediatric expert group consultation.

7.1.3 Aim and Objectives: Moving from Evidence to Policy

The overall aim of this paper is to determine whether the evidence recently acquired on the optimal regimen to be used in HIV-infected infants requires updating of WHO recommendations so that standard of care for HIV-infected infants should be a LPV/r-based regimen regardless of NNRTI exposure.

The specific objectives are:

1. Review existing literature and appraise the quality of evidence (LPV/r-based regimen versus NVP-based regimen as first line ART in HIV-infected infants).
2. Identify risks and benefits of expanding LPV/r-based regimen to every infants starting cART.
3. Explore values and preferences of LPV/r-based cART regimen in recipients.
4. Explore feasibility issues that countries may encounter in expanding the use of LPV/r-based regimen for HIV-infected infants.
5. Evaluate costs implications of moving from NVP to LPV/r-based cART.
6. Identify and rank proposed recommendation to inform WHO paediatric expert group consultation.

7.1.4 Methods

The GRADE framework was used to achieve an overall evaluation of the intervention and to rank the final proposed recommendation. This approach was chosen to harmonize the work with existing international standards for evidence evaluation and guidelines development [55].

The GRADE approach also allowed including estimations of the balance between risks and benefits, acceptability, cost and feasibility. For this reason this framework was considered instrumental to consider how values and preferences may differ with regards to desired outcomes and if uncertainty exists about whether the intervention represents a wise use of resources.

A. Systematic Review

A specific PICO question was initially identified and a systematic review was performed to evaluate the evidence assessing whether LVP/r-based regimen should be preferred to NVP-based regimens in HIV-infected infants starting treatment early.

P*opulation*: HIV-infected infants
I*ntervention*: LPV/r based cART regimen regardless of NNRTI exposure
Comparison: NVP based cART regimen
O*utcomes*: mortality, disease progression, increase of CD4 percentage from ART initiation, virological suppression or virological failure, change in growth parameters from baseline values following cART initiation and drug-related adverse events [132]

The first part of the review, focused on evidence produced by RCTs, was conducted according Cochrane review standards for literature search and paper selection, as part of a Cochrane review under development (chapter 4) [62]. Findings were subsequently corroborated in a wider search by including observational studies and updating the literature search conducted in 2008 during the revision of WHO recommendations for HIV-infected infants [133]

Search Strategy

The first part of the search was performed in consultation with the Cochrane HIV/AIDS Trials Search Co-ordinator (see chapter 1).

The second part of the search aimed to expand the literature review to observational studies addressing the question being considered. For this purpose, the search conducted to support the revision of WHO recommendations for HIV-infected infants [133]in 2008 was updated and articles were retrieved from Pubmed, EMBASE by applying the same search criteria between February 2008 to July 2011. Abstract from the World AIDS Conference, International AIDS Society conference (IAS) and Conference on Retroviruses and Opportunistic Infections (CROI) were also screened.

Selection Criteria

Selection criteria were predetermined on the basis of the current research question and in light of the existing strandards for ART in the paediatric population. In line with Cochrane procedures two of the authors independently screened records identified by the search. Studies were identified if they met the following eligibility criteria:

- Children less than 1 year included in the study population
- Treatment naive patients starting a cART regimen (mono an dual therapies were excluded)
- Comparison between treatment regimen reported
- Outcomes stratified by age (</>12 months at treatment initiation)

Studies were only rejected on initial screening if they did not include children less than 1 year of age or did not contain at least one comparison of ART regimen choice. Moreover, studies were excluded from the final quantitative synthesis if virological or immunological measurements were not available, if main outcomes were not stratified by age (less than 12 months) or if treatment regimens included drugs not in the market anymore (i.e. Nelfinavir).

Data collection and analysis

Data were independently extracted from the RCTs selected on patient characteristics, interventions and outcomes onto predesigned forms. Authors independently assessed these data, with any disagreement resolved by consensus with a third review author (for details see Chapter 4 pg 33).

Observational data were only analysed if more than one study was retrieved and otherwise just qualitatively described.

B. Grading Framework

Identify and rank the strength of recommendations in the context of public health requires a comprehensive evaluation which is based on evidence and allow to develop policies which are accepted, feasible and lead to cost-effective allocation of resources; a framework reflecting the relevance of this concepts was used (Figure 19).

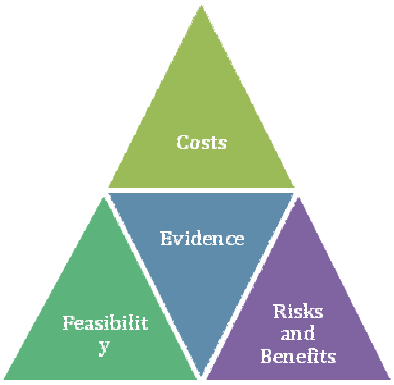


Figure 19 GRADING conceptual framework.

Quality of evidence appraisal was conducted by following the GRADE working group recommendations and the GRADEpro software was used to summarise findings.

The risk of bias was assessed through the Cochrane risk of bias tool [61] in order to identify possible problems in study design, such as for RCTs, lack of blinding or allocation concealment, incomplete reporting, selective outcome reporting, or use of invalidated outcomes measures.

Inconsistency was evaluated by assessing the similarity of estimates of effect across studies and potentially highlighting important unexplained differences in the results, which could decrease the confidence in the estimate of effect for a specific outcome. Differences in the direction of effect, the size of the differences in effect, and the significance of the differences were the guiding principles to assess inconsistency.

Uncertainty was also assessed by considering whether indirectness (the extent to which the patients, interventions, and outcome measures are similar to those of interest of the intervention under evaluation) was present and if compelling reasons to expect important differences in the size of the effect in the population target for the intervention, were found. Additional considerations were done around the precision of the estimates being provided by studies and the likelihood of reporting bias [92].

Risks and benefits were addressed on the basis of data available in the public domain regarding safety, formulation and dosing, drug interaction and resistance profile affecting subsequent treatment options.

Values and preferences, including reasons for which the recommended course of action (starting all HIV-infected infants with LPV/r based regimen) is likely or unlikely to be accepted by the patient,

health care workers or health systems were assessed by considering published literature from peer-reviewed journals and relevant conferences in this field.

Feasibility issues in countries were assessed by exploring PMTCT coverage, EID services, and drug availability at national level. Updated estimates were obtained from official reports published by UNAIDS and WHO. Moreover, update information regarding the ongoing drug development and access to ARVs where obtained from the WHO.

Costs implications were evaluated exploring the possibility to perform an economic evaluation. Drug costs were obtained with from the WHO, Medicine sans frontiers (MSF) and Clinton Health Access Initiative (CHAI).

7.1.5 Results

A. Systematic Review

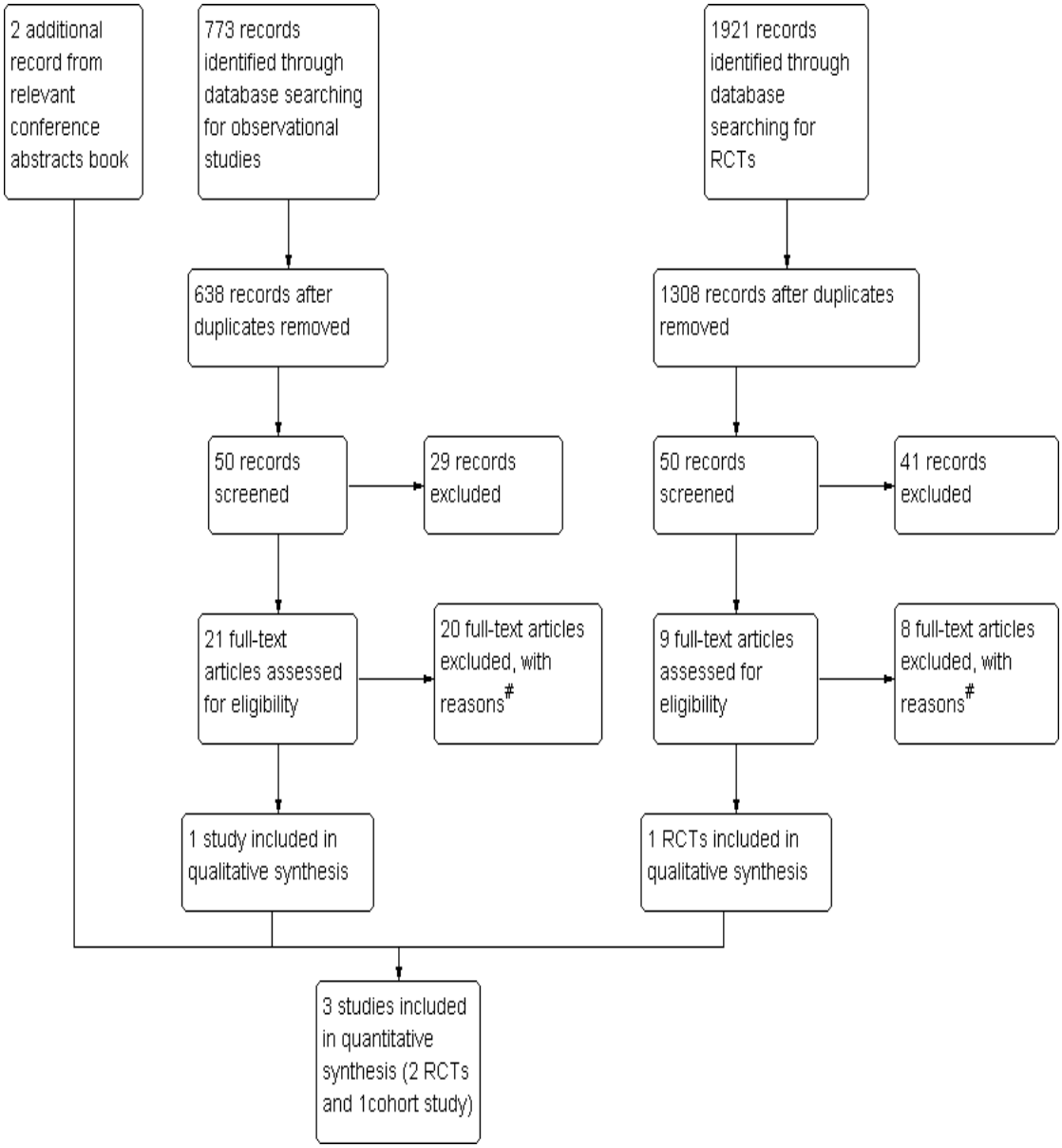
Medline/Pubmed yielded 697 records, CENTRAL 870, EMBASE 354 for a total of 1921 records. Duplicates were removed and out of 1308, 1 study was found eligible . One additional study [134] was retrieved from conference abstract book and two RCTs were identified (Figure 20)

Pubmed and EMBASE yielded 773 records when expanding the search to observational studies published from 2008 onwards; after duplicates were removed 638 titles were screened obtaining 50 articles. Twenty-one papers were selected by abstract. After full-text evaluation 1 study was identified in qualitative analysis and an additional study was then retrieved after further search on conference abstract-books.

Randomized control trials:

P1060 is an RCT of initial therapy with zidovudine and lamivudine plus either NVP or LPV/r in 123 HIV-infected children aged 6-36 months, in six African countries. Children enrolled in COHORT-1 had all been exposed to sd-NVP to prevent MTCT. The primary end point was treatment failure by study week 24. The secondary endpoints were virologic failure or death, immunological response, growth and toxicity. Enrolment in this cohort was terminated early on the recommendation of the data and safety monitoring board (DSMB).

In P1060-COHORT-2 [134], parallel RCT of P1060-COHORT-1 , 288 children aged 2-36 months who had not been exposed to sd-NVP prophylaxis were randomised to NVP-based or LPV/r-based 1st-line cART. The primary endpoint, was virologic failure or discontinuation of treatment by study week 24; secondary endpoints were: virologic failure or death, immunological response, growth and toxicity. This study was also terminated early as the DSMB recommended un-blinding the study results in October 2010 when 24 week endpoints had been achieved by all participants.



 # Reasons for exclusion in 28 studies: 3 were not ART-naive, 2 with mono/dual therapy, 3 outcomes not stratified by age, 10 not providing direct comparison of cART regimen, 3 used nelfinavir-based regimens, 7 virologic/immunological outcome not provided.

Figure 20 Flow diagram of studies selection.

Risk of biases assessment was systematically performed for both studies (Figure 22). The generation of random sequence was computerized and performed centrally by the trial statistician in all the studies being included. Allocation of concealment was adequately conducted by using an electronic interface to be used at the time of randomisation. Participants were not blinded to arm allocation but the studies endpoints were unlikely to be affected by unmasking. Blinding of outcome assessment was not performed; however main endpoints assessment relied mainly on laboratory measurements, which are unlikely to be affected by unmasking. Incomplete outcome data were reported in details and intention-to-treat analysis was performed for either P1060-cohort-1 or P1060-cohort-2 so that attrition bias is unlikely. Selective reporting was considered unlikely as main outcomes were all addressed as pre-specified by the studies protocol. The primary outcome was pre-specified in the study protocols and provided in study reports or by the investigators, so selective reporting at least of this outcome is unlikely. Given the small number of studies, it was not possible to formally assess other reporting biases. However, given the extensive searches of the standard and grey literature and trial registers, and also contact with experts in the field, it is unlikely that publication bias is an issue in this review. P1060-cohort-1 and cohort-2 were both terminated early as recommended by the DSMB on the basis of the pre-specified criteria, however, other potential biases due to the early termination of the studies should be considered (Figure 21).

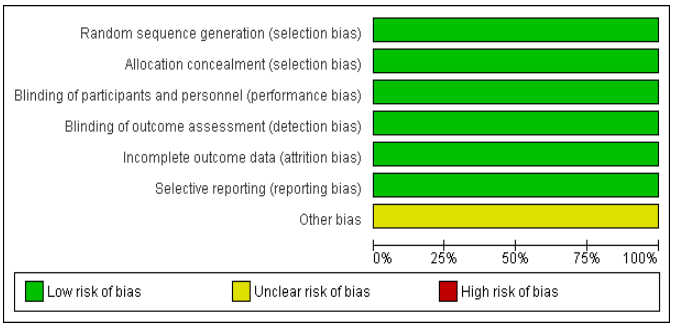


Figure 21 Risk of bias graph.

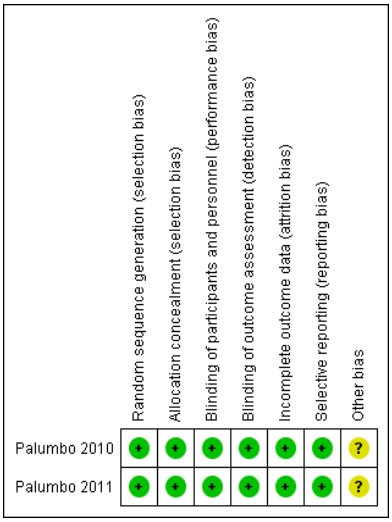


Figure 22 Risk of bias summary.

Results from the two RCTs were combined and analysed in meta-analysis (fig.7-12). Overall time to treatment failure was 2.01 (95%CI 1.47-2.77) times longer in children starting ART with a NVP-based regimen as compared to those starting on a LPV/r-based regimen (p<0.0001, Figure 23). These findings are consistent across studies (p=0.88, I^2=0%), suggesting that results are similar for NNRTI-exposed and unexposed children. There was no clear difference in effect by age group (subgroup heterogeneity: p= 0.97; I^2=0%) with a similar increase in time to treatment failure found in children starting NVP-based regimens regardless if they were older (HR=2.00 95%CI 1.32-3.03, p=0.001) or younger (HR=2.03 95%CI=1.24-3.32, p=0.005) than 12 months. The results were similar when a REM was used.

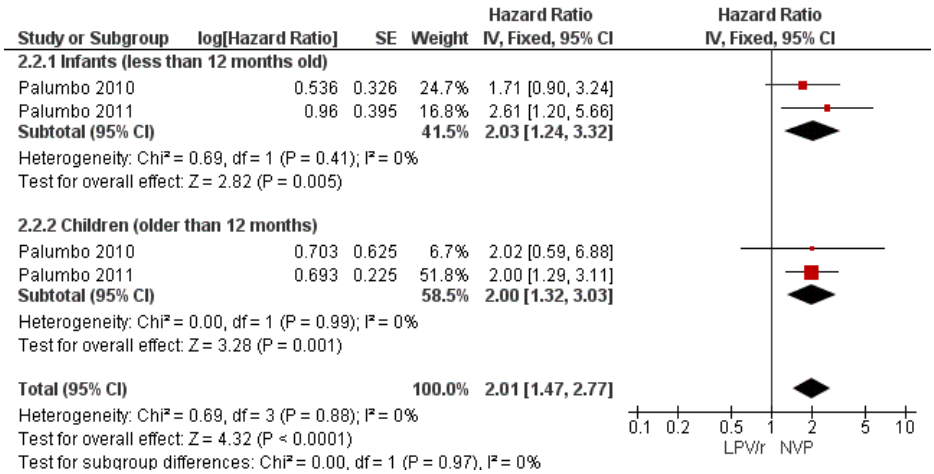


Figure 23 Treatment failure (virological failure or treatment discontinuation): Forest plot of comparison 2 NVP-based vs. LPV/r-based first line antiretroviral therapy.

Time to virological failure was overall 2.28 (HR 95% CI 1.55-3.34) times longer in children starting ART with a NVP-based regimen as compared to those starting on a LPV/r-based regimen (p=0.0005, Figure 24). Some modest heterogeneity was found (P = 0.23, I^2 = 31%) across studies suggesting that a difference may exist between NNRTI exposed and unexposed children. However, the random-effect model gives a similar result (HR=2.46, 95%CI 1.48-4.08) and the evidence of association (p<0.0001) between a NVP-based regimen and a longer time to virological failure (or death) remains very strong. In fact, the heterogeneity seems to be explained by a difference in effect by age group (subgroup heterogeneity: p=0.04, I^2=76.8%). The hazard ratio for virological failure (or death) in NVP-based regimen group compared to the LPV/r-based regimen group was larger in infants less than 12 months (HR 3.88, 95%CI 2.06-7.30, p<0.0001), as compared to older children (HR 1.67, 95%CI 1.03-2.70, p=0.04) with no differences between trials within these age-groups.

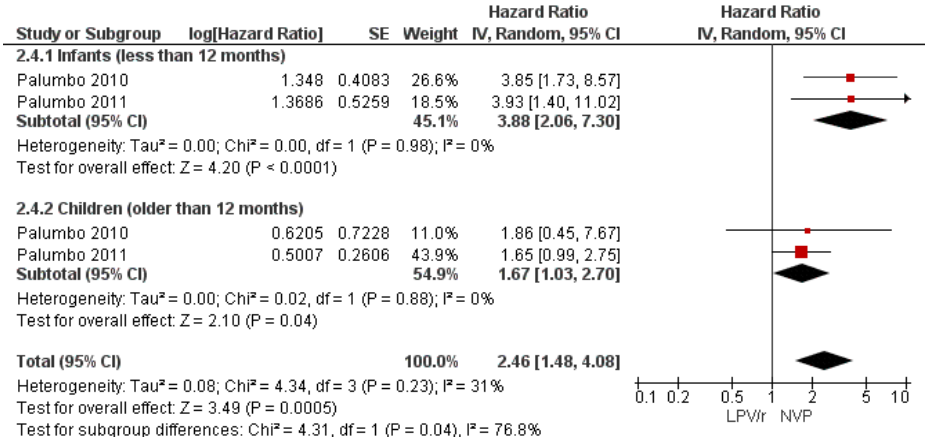


Figure 24 Virological failure or death: Forest plot of comparison 2 NVP-based vs. LPV/r-based first line antiretroviral therapy.

There was weak evidence of an association between treatment arm and immunological response (MD=1.56, 95%CI -0.29-3.41, p=0.10, Figure 25) and heterogeneity was limited (p=0.22; I^2=34%). The random effect model gave similar results (MD=1.79, 95%CI -0.70-4.28, p=0.16). These results provide some indication that the change in CD4% may be higher in the NVP arm as compared to the LPV/r arm.

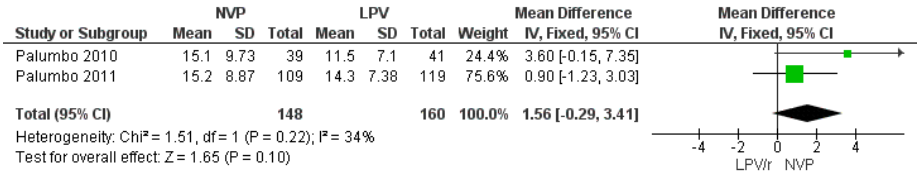


Figure 25 Change in CD4% from baseline: Forest plot of comparison 2 NVP-based vs. LPV/r-based first line antiretroviral therapy.

The change in weight z-score was larger in the NVP arm compared to the LPV/r arm (MD=0.37, 95%CI 0.08-0.65, p=0.01, Figure 26). These findings are consistent across studies (p=0.63; I^2=0%), suggesting that results are similar for NNRTI-exposed and unexposed children. The robustness of this findings were further confirmed with the random-effects model (MD=0.37, 95%CI 0.08-0.65, p=0.01).

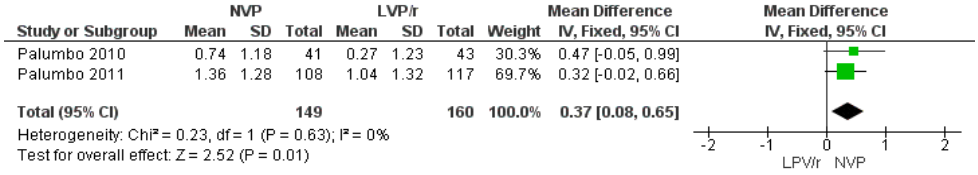


Figure 26 Change in weight z-score from baseline: Forest plot of comparison 2 NVP-based vs. LPV/r-based first line antiretroviral therapy.

Similarly a weak association was found between treatment arm and the change in mean height z-score whether the fixed (MD=0.26, 95%CI -0.01-0.53, p=0.06, Figure 27), or random effects model was used, being higher in the NVP group as compared to the LPV/r group. Heterogeneity detected across studies was modest (p=0.23, I^2=29%).

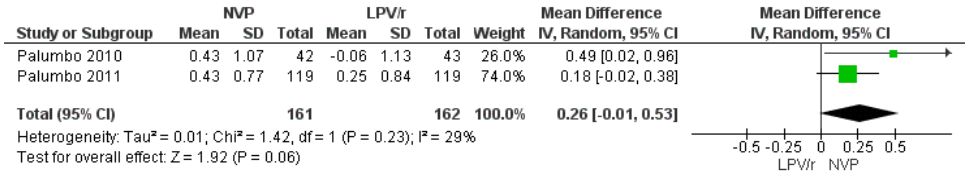


Figure 27 Change in height z-score from baseline: Forest plot of comparison 2 NVP-based vs. LPV/r-based first line antiretroviral therapy.

Adverse events associated with treatment were not significantly more frequent in the NVP arm with either the fixed or (RR=1.34, 95%CI 0.81-2.23, p=0.26) random effects model (RR=1.44, 95%CI 0.60-3.46, p=0.42, Figure 28). Some differences in effect across studies were found (heterogeneity: p = 0.12; I^2=58%).

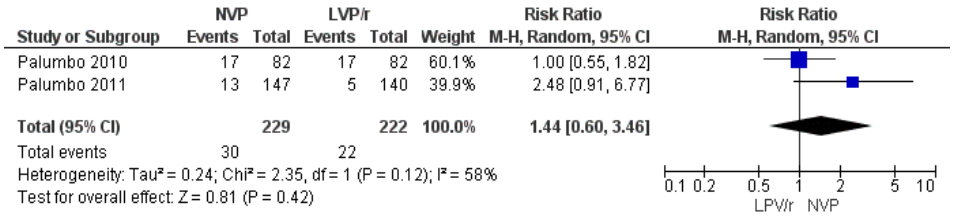


Figure 28 Adverse events: Forest plot of comparison 2 NVP-based vs. LPV/r-based first line antiretroviral therapy.

Observational studies:

Only one observational study meet the preselected criteria. In the EPPICC infant study data by 9 cohorts from Europe contributed data on HIV-infected infants born 1996-2008 and starting cART before 12 months. A total of 437 infants were followed for median 5.9 (IQR 2.3-7.6) years after starting cART; 30% had an AIDS diagnosis prior to cART initiation. After adjusting for relevant covariates, virological and immunological responses at 12 months varied by cART type ($p<0.001$ and $p=0.03$ respectively), with 4-drug NNRTI-based regimens being superior (virological response <400 copies/ml adjusted OR 3.00, 95% CI 1.24-7.23; mean increase in CD4 Z-score coefficient 0.64, 95% CI 0.10-1.17) to both 3-drug NNRTI-based and boosted PI-regimens which were similar. Rates of switching to second-line ART were lower among children starting 4-drug NNRTI-based and boosted PI-based regimens compared to 3-drug NNRTI regimens ($p=0.03$).

A cohort study conducted in Thailand was also initially evaluated but after consultation with the study authors the paper was excluded as the majority of infants were on a Nelfinavir-based antiretroviral therapy and did not meet pre-specified inclusion/exclusion criteria.

B. Grading the Evidence

Randomized control trials are considered to provide the highest quality of evidence, therefore for the purpose of this evaluation, only RCTs were assessed for quality of evidence appraisal [92]. For outcomes not addressed by RCTs, cohort studies meeting the pre-defined criteria were included.

All outcomes being reported were considered as important, however after separate ranking the highest importance was given to “treatment discontinuation” and “virological failure or death” which were considered as critical for the purpose of the quality of evidence evaluation.

No major risk of bias was detected from systematic evaluation (Figure 22). Both studies were terminated early and both were un-blinded, but considering the strict pre-specified criteria for trial discontinuation and the specific outcomes being considered as endpoints, is unlikely that interruption and un-blinding may have introduced serious bias.

Results were consistent across the two studies and no major indirectness was found as patients enrolled in the two studies represent a wide variety of patients, enrolled from different countries such as studies sites were in Malawi, South Africa, Uganda, Tanzania, Zimbabwe, Zambia and India. Even if enrolled in different cohorts, infants previously NNRTIs exposed or unexposed were both included.

Imprecision was identified to be a common flaw particularly for secondary outcomes, to assess which studies had low power.

Publication bias was considered as unlikely since online clinical trial register (ClinicalTrials.gov) was consulted: one additional trial of sd-NVP-exposed infants in Kenya was terminated early following the results of the P1060 trial (ClinicalTrials.gov reference NCT00427297) and no other similar RCTs were conducted elsewhere.

While the evidence of an advantage of one drug over the other was strong (high quality) for the most important outcomes (“treatment failure” and “virological failure or death”) it was weaker (low quality) for immunological response, growth parameters and drug-related adverse events, mainly due to lack of masking in outcome measurement and imprecision. Very low evidence is available for “virological suppression at 12 months” and for “time to switch to second-line therapy” as these outcomes were not evaluated in RCTs and addressed in only one observational study.

After systematic evaluation and placing high value to critical outcomes, the studies retrieved were considered to provide sufficient evidence to support the use of LPV/r-based regimen in HIV-infected infants starting first-line cART regardless of their previous exposure to NNRTIs.

Table 1 GRADE profile: Should LPV/r-based cART regimen vs. NVP-based regimen be used in HIV-infected infants?

Question: Should LPV/r-based cART regimen vs. NVP-based cART regimen be used in HIV-infected infants?
Bibliography: Palumbo 2010 , Palumbo 2011 [134], EPPICC 2011

Quality assessment							Summary of Findings				
Participants (studies) Follow up	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall quality of evidence	Study event rates (%)		Relative effect (95% CI)	Anticipated absolute effects	
							With Nvp based cART regimen	With LPV/r based cART regimen		Risk with Nvp based cART regimen	Risk difference with LPV/r based cART regimen (95% CI)
Treatment failure (CRITICAL OUTCOME; assessed with: Any interruption of study drugs (virological failure, toxicity,TB medication, death))											
451 (2 studies) 24 weeks	no serious risk of bias[1]	no serious inconsistency	no serious indirectness[2]	no serious imprecision	undetected	++++ **HIGH**[1,2]	57/229 (24.9%)	39/222 (17.6%)	**HR 0.50** (0.36 to 0.7)	**Study population**[3]	
										249 per 1000	**116 fewer per 1000** (from 67 fewer to 151 fewer)
										Low[3]	
										200 per 1000	**94 fewer per 1000** (from 55 fewer to 123 fewer)
										High[3]	
										300 per 1000	**137 fewer per 1000** (from 79 fewer to 179 fewer)
Virologic failure or death (CRITICAL OUTCOME; assessed with: RNA PCR<50 copies/ml)											

451 (2 studies) 24 weeks	no serious risk of bias[1]	no serious inconsistency	no serious indirectness[2]	no serious imprecision	undetected	++++ **HIGH**[1,2]	55/229 (24%)	26/222 (11.7%)	**HR 0.42** (0.25 to 0.7)	**Study population**[3]	
										240 per 1000	**131 fewer per 1000** (from 65 fewer to 174 fewer)
										Low[3]	
										200 per 1000	**111 fewer per 1000** (from 55 fewer to 146 fewer)
										High[3]	
										300 per 1000	**161 fewer per 1000** (from 79 fewer to 215 fewer)
Virological suppression (CRITICAL OUTCOME; assessed with: <400 copies/ml at 12 months from treatment initiation)											
123 (1 study) 5 years	no serious risk of bias	no serious inconsistency[4]	no serious indirectness[5]	serious[6]	undetected	+⊖ **VERY LOW**[4,5,6] due to imprecision	49/81 (60.5%)	30/42 (71.4%)	**OR 1.39** (0.62 to 3.13)	**Study population**	
										605 per 1000	**75 more per 1000** (from 118 fewer to 222 more)
										Low	
										500 per 1000	**82 more per 1000** (from 117 fewer to 258 more)
										High	
										800 per 1000	**48 more per 1000** (from 87 fewer to 126 more)
Immunological response (IMPORTANT OUTCOME; assessed with: Change in CD4% from baseline)											

451 (2 studies) 24 weeks	no serious risk of bias[1]	no serious inconsistency	serious[2,7]	serious	undetected	++⊖⊖ **LOW**[9] due to indirectness, imprecision	-	-	**Mean difference 1.56** (-3.41 to 0.29)	
Growth (weight) (IMPORTANT OUTCOME; assessed with: Change in weight z-score from baseline)										
451 (2 studies) 24 weeks	serious[1,8]	no serious inconsistency	no serious indirectness	serious	undetected	++⊖⊖ **LOW**[9] due to, imprecision	-	-	**Mean difference 0.37** (-0.65 to -0.08)	
Growth (height) (IMPORTANT OUTCOME; assessed with: Change in height z-score from baseline)										
451 (2 studies) 24 weeks	serious[1,8]	no serious inconsistency	no serious indirectness	serious	undetected	++⊖⊖ **LOW**[9] due to , imprecision	-	-	**Mean difference 0** (-0.53 to -0.01)	
Drug related adverse events (IMPORTANT OUTCOME; assessed with: NIAID scale (grade 3 or 4))										
451 (2 studies) 24 weeks	no serious risk of bias	serious[9]	no serious indirectness	serious	undetected	++⊖⊖ **LOW**[9] due to inconsistency, imprecision	30/229 (13.1%)	22/222 (9.9%)	**RR 1.44** (0.6 to 3.45)	**Study population** **131 per 1000** / **58 more per 1000** (from 52 fewer to 321 more) **Low** **50 per 1000** / **22 more per 1000** (from 20 fewer to 123 more) **High** **200 per 1000** / **88 more per 1000**

											(from 80 fewer to 490 more)
Switch to second line therapy (IMPORTANT OUTCOME; assessed with: change 3 or more drugs for any reason of 2 drugs for treatment failure)											
618 (1 study) 5.9 years	no serious risk of bias	no serious inconsistency[4]	no serious indirectness	serious[6]	undetected	+⊖ **VERY LOW**[4,6] due to imprecision	18/464 (3.9%)[10]	2/154 (1.3%)[10]	**HR 0.26** (0.06 to 1.19)	**Study population**	
										39 per 1000[10]	**29 fewer per 1000** (from 36 fewer to 7 more)
										Low	
										20 per 1000[10]	**15 fewer per 1000** (from 19 fewer to 4 more)
										High	
										100 per 1000[10]	**73 fewer per 1000** (from 94 fewer to 18 more)

[1] Both trials were stopped earlier by the DSMB according pre-specified criteria and although unlikely is not clear if this could have led to bias.[2] The two study populations are different in terms of NNRTI exposure. Cohort 1 (Palumbo 2010) patients had all been previously exposed to NNRTI while in Cohort 2 (Palumbo 2011) patients were all NNRTI unexposed. Therefore the treatment efficacy can be generalized to both subgroups of the target population.[3] The range was considered by looking at NNRTI exposed and unexposed groups [4] Only one study assessed this outcome (EPPICC 2011).[5] NNRTI exposed and unexposed-children were included in this cohort study (30% had been exposed to maternal ARV). Cohorts included in this study were gathered from Europe.[6] Low power to detect small differences as few children were started on a LPV/based regimen.[7] Patients enrolled in the two trials were severely immunosuppressed and is unclear whether the same effect would be detected in infants starting treatment according recommendation with good clinical and immunological conditions.[8] Outcome measurement was un-blinded and potential biases arising from this cannot be ruled out.[9] Palumbo 2010 showed no difference between study arms, while in Palumbo 2011 there were more adverse events in the NVP arm.[10] Rates per 100 person/year of follow up.

C. Risk and Benefit Assessment

Nevirapine has been extensively used in children in LIC/MIC, achieving an excellent as well as durable viral and immunological response. However, LPV/r-based regimens may offer additional benefits and yet concomitant risks, which need to be taken into account when projecting recommendation to a global scale.

Risks and Benefits

Key principles to assess the medical risks and benefits of moving to LPV/r-based regimen as first line treatment for any infants starting cART include: drug safety, dosing and formulations, drug interaction and the resistance profile affecting subsequent treatment options.

Drug safety

The use of LPV/r allows to avoid the higher incidence of rash observed for NVP in comparison to any other ARVs; NVP-related rash may be severe and life-threatening. A rare but potentially life-threatening risk of hepato-toxicity seems to be associated with the use of NVP and this makes the drug less suitable for treating children who are on other hepatotoxic medications, or drugs that can cause rash. Even though usually transient, NVP toxicity has been reported in up to 17% of the cases, higher with high CD4, therefore is a potential concern in the context of early start with good immunological conditions in infants.

PI-based regimens show a better safety profile in the short-term with minor gastrointestinal symptoms, which are usually transient. However, long-term complications are associated to PI-based regimen: higher prevalence of lipodistrophy [41] and metabolic outcomes including hypercholesterolemia [42] have been described in older children. Possible association with vascular and endothelial function changes was also reported but the clinical significance remains to be determined. These complications are poorly known in infants and young children, but should be considered in the context of life-long treatment.

Caution should be used when prescribing LPV/r to young infants. The use of LPV/r oral solution has been approved by the FDA for use in infants 14 days and older, but current guidelines do not recommend use in preterm babies in the immediate postnatal period. LPV/r oral solution contains 356.3 mg/ml ethanol and 152.7 mg/mL propylene glycol. Reduced hepatic metabolism and renal clearance in newborns can lead to accumulation of all 3 ingredients especially in preterm babies and cases of toxicity in neonates have been reported to FDA [44]. However, delay in early infant diagnosis make this concern less of an issue in LIC/MIC where infants are rarely diagnosed with HIV before their second of third month of life [108].

Dosing and Formulations

While NVP dosing and its use in infants is well established, data on the use of LPV/r is more limited. Studies in the U.S. [45] report that a high dose of 300/75 mg/m^2/dose of LPV/r is considered appropriate for most infants <6 months of age, however, a lower LPV concentrations was observed in the first months of life and there may be a rational to explore the effect of dose of LPV/r in very young infants, who affected by a limited absorption of the drug. For these reasons in countries where this is possible a frequent monitoring of young infants receiving LPV/r would be advisable, adjusting the dose for weight gain and maintaining maximal drug exposure during periods of rapid growth. This would be less feasible in countries were distance from health centres and health facilities overload would make frequent monitoring a challenge for families and health workers.

Lopinavir/r is administered twice a day similarly to NVP, therefore a change in recommendation would not reduce drug burden. While NVP is an established component of existing FDCs, no FDC formulation containing LPV/r has so far been developed for adults or children, and drug development in children still lack behind adults. Therefore, while infants starting on NVP-based may have the chance to switch to a FDC as getting older, those starting on a LPV/r-based regimen would have to continue on a syrup formulation until they are 4-6 years and they can swallow the paediatric tablets which cannot be crushed.

ARVs suitable for young infants should ideally be pleasant-tasting, of high concentration (requiring small volumes), with no food restrictions, be heat-stable and require administration no more than twice daily. LPV/r syrup requires refrigeration and intake with food. Melt Extrusion technology has helped to overcome these issues by improving stability and bioavailability of LPV/r formulations. Cipla pharmaceuticals, India, has recently developed a sprinkle formulation in 40/10 mg capsules appropriate for even the smallest children, as it allows the drug to be easily mixed in with food. Bioequivalence studies showed profile comparable to the branded product [135]. The pharmacokinetics of Cipla LPV/r (Lopimune) in paediatric sprinkles is currently under investigation in African infants as part of the CHAPAS 2 trial. The acceptability of its use and the formulation preferences reported by carers will also be investigated (source: Clinical Trial Unit, MRC, London).

Formulation	**<3 kg**	**3-4 kg**	**4-5 kg**	**5-7 kg**	**7-10 kg**	**10-12 kg**	**12-14 kg**	**14-20 kg**	**20-25 kg**
Sprinkle 40/100 mg	-	3	3	3	4	4	5	6	7
Syrup 80/20 mg/ml	1.5 ml	1.5 ml	1.5 ml	1.5 ml	2 ml	2 ml	2.5 ml	3 ml	3.5 ml
Tablets 100/25mg	-	-	-	-	2	2	2	3	3

Table 2 Number of tablets, sprinkles capsules and syrup mls by weight band (Source: CHAPAS 2 trial protocol)

Drug-drug interaction

Interaction with other medication is a challenge in cART management, both in adults and children. Concomitant anti-TB treatment occurs frequently considering the high prevalence of TB infection in HIV-infected patients, especially in Sub-Saharan Africa where HIV-infected children are at 20-fold higher risk for contracting TB than HIV-uninfected children. This is particularly true in children less than one year who are

recognized to be the group of children most at risk for TB infection, often presenting with severe clinical features such as disseminated TB.

Small studies in children, has suggested that that co-administration of rifampicin may not result in lowered NNRTI concentrations in paediatric populations and that prescribing the maximum dose of 200mg/m2 is should be a sufficient approach to avoid sub-therapeutic drug levels.

Co-administration of LPV/r with rifampicin results in a 90% to 99% reduction in the trough concentration (C_{min}) of lopinavir so that co-administration of rifampicin and LPV/r is not recommended. Administering double doses of co-formulated lopinavir/ritonavir [46] resulted in inadequate lopinavir concentrations in young children treated concurrently with rifampicin. However, a study conducted South African patients (aged 6 months-3 years) demonstrated that an adjusted dose regimen of LPV/r (LPV/r ratio of 1:1) achieved adequate lopinavir C_{min} in most HIV-infected children when co-administered with rifampicin-based anti-tubercular treatment [47].

Significant interaction with antimalarial drugs potentially used in the context of malaria treatment or IPTi has not been reported, however data in this specific population are still lacking.

Resistance and future treatment options

Robust evidence has shown that LPV/r, similarly to other boosted PIs, is featured by a higher genetic barrier to drug resistance resulting in good durability and potency [48, 49]. By contrast NVP presents a low genetic barrier and the possibility of extensive class cross-resistance which precludes sequential use of efavirez [42, 43]. This was further confirmed in South African NNRTI-exposed infants enrolled in the NEVEREST trial; virological failures occurred mostly in those switching to NVP during the first year of treatment, while infants continuing on LPV had episodes of failure later on, usually after the first year of treatment [95].

It is unfortunately acknowledged that the use of LPV/r in a 1st-line regimen may compromise the potential to construct a potent 2nd-line regimen. However, a study from the UK suggests that PI cross-resistance is very unlikely when resistance to

LPV/r occurs and potent drugs such as darunavir could still be used [136]; nevertheless PI other than lopinavir are still unavailable in most LIC/MIC.

Despite a study [137] looking at triple class failure arising from the initial regimen did not show any difference between PI-based and NNRTI-based regimen 1st-line regimens, in the PENPACT1 [138] trial PI-resistance was uncommon with virological failure and PI seemed to be protective against the development of NRTI-resistance, which was more common (10% more) in those initiating treatment with an NNRTI-based regimen, particularly in those switching at higher viral load levels. Lack of viral load monitoring results in switching to 2nd-line with high viral load levels therefore induction of NNRTI and NRTI-resistance reducing treatment option is a considerable concern in a context of limited resources and limited drug availability.

Second line NNRTI containing regimen is not recommended when cART is initiate with a PI-based regimen [85], however switching to a NVP-based regimen in infants who started a LPV/r-based regimen when virological suppression is achieved was shown to be safe and this could be a successful strategy to contain the challenges of a LPV/r-based 1st-line regimen in infants[51].

Values and preferences

Patients

To date a proper evaluation of the acceptability of LPV vs. NVP in this specific age group hasn't been conducted. Irrespective of the specific antiretroviral being used syrup formulations present significant challenges for children and their caregivers. Problems with the palatability of some antiretroviral syrup formulations have been reported, in some cases requiring administration through the use of gastrostomy tubes to improve adherence [139-141].

There are few studies investigating the acceptability of tablet versus syrup formulations of anti-retroviral drugs for carers and their children, including in resource-limited settings.

In the ARROW trial a sub-study [142] was conducted to investigate the acceptability of tablet versus syrup formulations for carers and their children, including in resource-limited settings. At the time of changing from syrup to tablet formulations,

79% of child primary caregivers reported problems, the most frequent being difficulties managing large numbers of bottles (46%), the weight of bottles (63%), and transport of bottles (57%). A child on ART weighting 10-12kg would typically require seven bottles of syrup at each 28-day visit and therefore considerably more with less frequent visits[142]. Keeping them confidential and the use of syringes for which different doses for individual drugs are required it's an additional challenge.

These issues are particular relevant for LPV/r, the palatability is really poor and infants have periods of confirmed suboptimal adherence with higher rates than in patients taking other agents [143, 144]. The quantity of syrup to be administered in an infant is minimal (Table 2) and measurement of 1.5 ml with a syringe can be very difficult for caregivers, especially when grandparents are those administering drugs.

Moreover, starting a NVP-based regimen that can later be substituted with an FDC formulation, which would reduce pill burden and make administration of drugs easier could be more acceptable, both for patients and their care givers.

Health care providers

Currently there is a complete lack of information regarding health care providers' preferences on first line therapy for infants. Simplification and harmonisation are usually the key principles for an acceptable intervention in HIV clinics. Considering the current recommendations for first line treatment in adults and older children, a LPV/r-based regimen would probably add some complexity in treatment management leading to a low acceptance of the intervention.

Health systems

To our knowledge, health system preferences have not been formally assessed so far, but certainly the implementation of the use of LPV/r as 1st-line for those NNRTI-exposed infants has been very slow in most of countries, suggesting that barriers for its use are considerable. Cold chain requirements, need of proper storage spaces and specific challenges in forecasting as well as along the entire procurement and supply chain makes recommending LPV/r for any HIV-infected infants poorly accepted at a national level, particularly for those countries who are already struggling in achieving adequate coverage of services such as immunisations or EID for HIV.

Feasibility

Successful PMTCT programmes, which include maternal cART or combined prophylactic regimens, reduces the risk of transmission to well under 5% although coverage remains disappointingly low in many countries. By 2010 only around half of HIV-infected pregnant women in low- and middle-income countries received ART for PMTCT, and in many countries, coverage was under 20%[108]. Without any PMTCT intervention 30% of the newborns would acquire HIV from the mother. From this overall figures, and assuming every other factor being irrelevant we can estimate that: 2.5% of infants born to HIV infected women would acquire the HIV despite PMTCT intervention, 15% would have acquired the infection as a result of not having access to PMTCT, leading to an overall 17.5% of infants born to HIV-infected mothers becoming HIV infected. According the existing recommendation[21] in this scenario only 14% (2.5%/17.5%) of the HIV-infected infants would need to start treatment with a LPV/r-based regimen, while the remaining would be started to an NVP-based regimen as unlikely to carry resistance to NNRTIs (Figure 29). Revising the recommendation would mean that more than 80% of HIV infected infants who are currently starting on an NVP-based regimen would have to start with a LPV/r-based regimen instead, highlighting the large impact which this decision could potentially have at a national and programme level. However, this impact is significantly affected by the degree of PMTCT scaling up (Figure 29) since, as effective interventions are scaled up, infections in infants born to HIV-infected mothers reduce significantly with a remarkable increase in the number of children being exposed to NNRTIs.

Moreover the current suboptimal implementation of EID programmes (only 15% of infants born to HIV-infected mothers receive virological testing [108]) leads to the majority of infants receiving early diagnosis having gone through PMTCT services, while most of HIV-infected infants unexposed to PMTCT interventions remain often undiagnosed until becoming symptomatic. This scenario results in the majority of HIV-infected infants currently starting treatment needing a LPV/r-based regimen due to their exposure to NNRTIs, and in practice reduces the impact of a revision of 2010 recommendations.

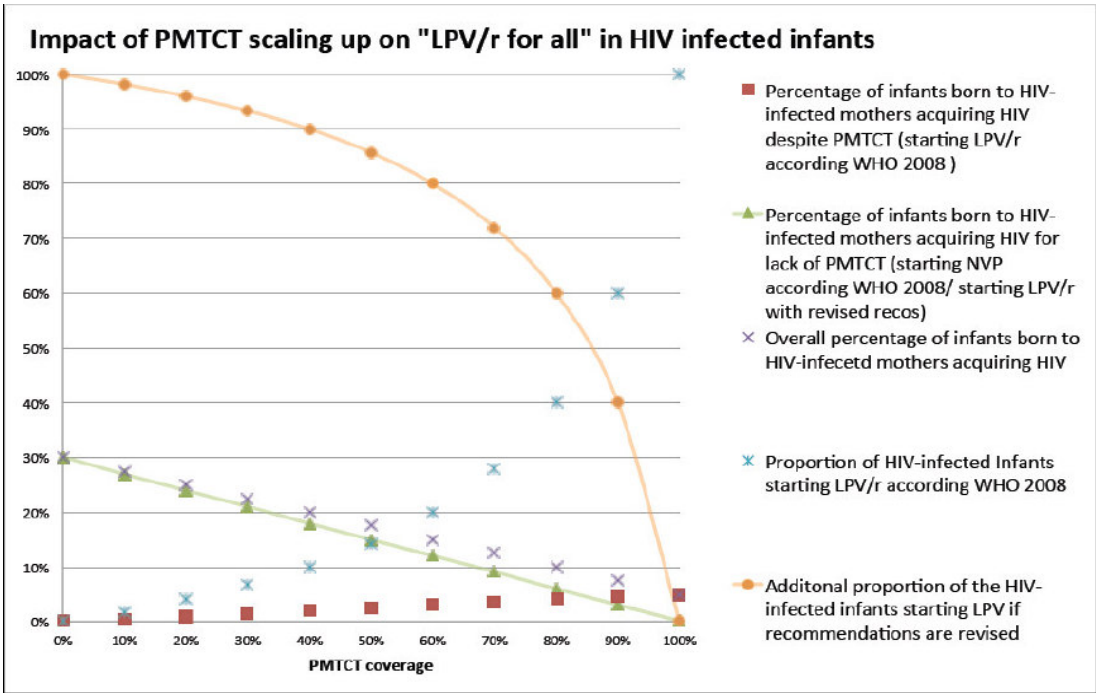


Figure 29 Impact of PMTCT scale up on the impact of revising recommendations.

The annual WHO 2010 survey on ARV use [145] conducted in 62 LIC and MIC showed that most countries have adopted WHO 2010 recommendations in their national guidelines; among 52 countries who provided information 30 countries have revised their guidelines in 2010, 8 countries are planning the revisions for 2011 or 2012. However, the implementation of early treatment initiation has been slow and difficult so that infants account for 25% of all new initiations and only 15% of infants are started on a LPV/r-based regimen. Overall, the uptake of PI in paediatric 1st-line cART was very limited by December 2009 (Figure 30). Only 28,200 children were receiving a PI-based regimen in first line, with a very limited number of countries reporting an uptake and South Africa accounting for 9 out of ten children on PI. LPV/r is in first line cART for infants only in Botswana, India, Kenya, South Africa (41% of children)[145].

The 2010 survey also showed that the pattern of use was different in the countries of the America region, with 39% of children receiving a PI-containing regimen on 1st-line [108].

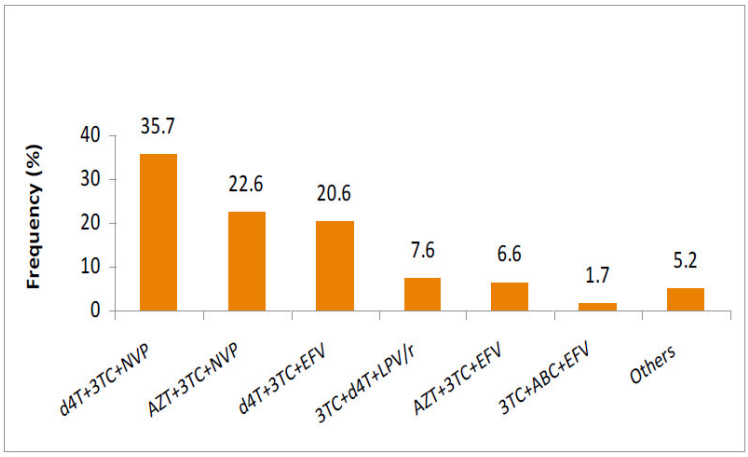


Figure 30 Paediatric 1st line regimen in LIC and MIC (except America region) Source: WHO survey 2010 on ARV use, December 2010.

The main feasibility issues around the use of LPV/r in infants relates to the *procurement and supply* chain. The only product suitable for infants and currently prequalified by WHO is the oral solution produced by Abbott Laboratories in the UK. This product is significantly heat-unstable (
Table 3) and has to be stored at 2 to 8°C, cold chain is therefore a requirement for the entire supply chain up to the patients. According the manufacturers outside labelled storage temperatures there is extreme variability in the pharmacological activity of the product, which significantly undermine the effectiveness of treatment.

Temperature/ Time Allowed outside Recommended Storage	Allowed Temperature Range	Allowed Time Duration				
		Manufacturer	Internal customer	Intermediate Customer		End Customer
		GPO Distribution	Affiliate	Wholesaler/ Distributor/ Government	Pharmacy/ Physician/ Hospital	Patient
	-15 to 1 ℃ (5 to 35 ℉)	2 days	1 day	1 day	1 day	1 day
	2 to 8 ℃ (36 to 46 ℉)	Until expiry				
	9 to 25 ℃ (47 to 77 ℉)	1 week	1 week	1 week	1 week	42 days (or per product labels)
	26 to 30 ℃ (78 to 86 ℉)	4 days	1 day	1 day	1 day	1 day

Table 3 Kaletra oral solution outside labelled storage temperature conditions (Source: Abbott Laboratories, Global Pharmaceuticals operations, Global distribute

This is the only product requiring cold chain among available drugs in developing countries and a dedicated supply system has therefore to be planned, when scaling up early treatment for HIV-infected infants.

In Sub-Saharan Africa, significant challenges are also faced by carers, who struggle to find cold places to store drugs in their household. This ultimately compromises the effectiveness of therapy and raises inequities between the rich and the poor, between urban and rural areas.

Issues in procurement and supply affect significantly the service delivery system, compromising the ability to decentralize HIV treatment and care. South Africa started the implementation of LPV/r-based regimen 1st-line cART in less than 3 years old in 2007, benefiting from Global Fund support; no procurement and distribution problems were observed in the Western Cape thanks to good communication between clinician local pharmacists and provincial pharmacists. By contrast, problems in other provinces with poor pharmacy management in primary care settings were noted (personal communication Louise Kuhn: WHO expert consultation December 2010).

Similar difficulties are therefore expected for those countries, which are currently decentralizing HIV treatment and care and recommending LPV/r-based regimen for every infant may jeopardize treatment decentralization in those countries where delivery system is still centralized to urban areas.

Cost implications

The cost of first line therapy for paediatric ART has reduced dramatically due to the availability of generic drugs ($50 a year on average in 2009 compared to about $20,000 a few years before)[18]. However, the newest recommendations including more effective drugs increase the median price of 1st-line regimens and this is particularly true for paediatric regimens (Figure 31) so that ARVs prices continue to challenge the scaling up of ART. The introduction of LPV/r in 1st-line for NNRTI-exposed infants has already increased significantly the price of 1st-line regimen and further expansion of the population for which LPV/r is used is likely to have a considerable impact on costs. Lopinavir/r is attributable of 70-80% of the total cost of the regimen when combined with stavudine and lamivudine.

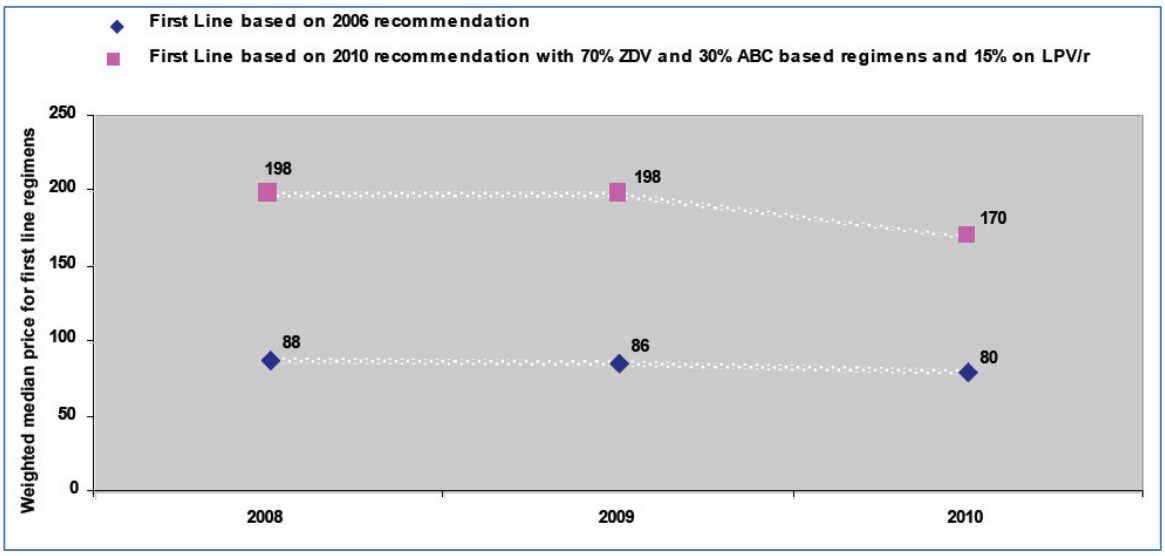


Figure 31 Weighted median price for first line paediatric regimens in LIC: comparisons 2006-2010 guidelines (source: Annual Consultation with Pharmaceutical Companies-WHO).

Currently only Abbot oral formulation is approved by the WHO Prequalification Programme and by the U.S. FDA, costing about 164$ for LIC/MIC and 400$ for HIC (at least four times the lowest price of NVP oral solution). A generic product has recently been submitted for WHO prequalification by Cipla (India) and is currently recommended for procurement by Expert Review Panel (ERP) of The Global Fund (see 7.1.9 1,2-pg. 180).

Favorable prices have also been obtained though CHAI mediation. CHAI price negotiation benefits of agreements with eight manufacturers of ARV formulations, active pharmaceutical ingredients and/or pharmaceutical intermediates. The CHAI prices are available to 70 countries participating in the CHAI Procurement Consortium and apply to procurements by national governments that are members of the consortium, or organizations procuring on behalf of member governments, to support public care and treatment programs.

Indirect costs should also be considered in this scenario, since assuring an adequate supply of LPV/r oral formulation requires a functioning cold chain and larger to store the drugs, which have also to be budgeted for by programmes. Despite potentials for costs containment in the near future in line with the trend so far achieved, the high

cost of LPV/r-based regimen is still a key barrier to implementation and scaling up.
A proper cost-effectiveness analysis to address the balance between benefits obtained and costs expected has not been conducted yet. A similar assessment was conducted in pregnant women enrolled in the OCTANE trial, however significant differences are expected and a paediatric 'ad hoc' evaluation is needed.

In conclusion, the risk and benefits evaluation shows that: the use of LPV/r for any infants starting cART is supported by high quality of evidence for the most important outcomes; benefits out weight risks at least in the short-term, particularly in terms of toxicity and resistance profile; costs can be contained through price negotiation and new formulation becoming available; LPV/r is not likely to be accepted by the intervention recipients (patients, HCW and health system); the feasibility is largely affected by issues around procurement and supply which can only be overcome by different formulation which are still unavailable.

Table 4 Risk and benefits assessment for the use of LPV/r based regimen in HIV-infected infants regardless NNRTI exposure.

Recommendation: LPV/r regimen should be used as first-line treatment in any HIV infected infants starting cART regardless of NNRTI exposure.		
Population: HIV infected infants		
Intervention: LPV/r based regimen as first line cART		
Factor	**Decision**	**Explanation**
Quality of evidence	Yes	Strong quality of evidence for treatment failure and virological failure
Benefits or desired effects	=	Avoid NVP toxicity Less resistance to NRTIs When virological treatment due to LPV/r resistance development, DRV can still be used as cross-resistance is limited
Risks or undesired effects		Long- term metabolic toxicity still unclear No FDC available Low adherence for poor palatability Lack of second line options in RLS Interaction with TB drugs which however could be overcome by RTV double boosting
Values and preferences	No	Poor palatability so poor acceptance by patients and carers. Add complexity to treatment management as non-harmonised with older children treatment. Cold chain and storage requirements makes is less acceptable by the health system.
Feasibility	No	Cold chain Storage requirement Difficult forecasting and likely stock out of drugs Lack of harmonisation with the rest of the population Barrier to decentralisation of HIV treatment and care
Costs	=	Still a barrier but potentials for drug price negotiations and further decrease.

7.1.7 Discussion and Conclusions

Evidence

Immediate ART, at least in young infants, reduces morbidity and mortality and may improve neurodevelopmental outcome. Data from the trials presented show that good virological and immunological outcomes are achievable in early life and that choice of first line treatment is critical in the context of a long-life treatment.

The P1060 trial team addressed the question of what ART to start, comparing a NVP-based regimen with a LPV/r-based regimen in young children below 3 years. Two parallel trials recruited both sd-NVP-exposed children (cohort 1) and sd-NVP-unexposed children (cohort 2 [134]). It is well recognised that a substantial proportion of infants and young children exposed to sd-NVP at birth develop NNRTI resistance, and early data suggested that this may compromise the efficacy of NVP-based regimens. This concern was further consolidated by the P1060-cohort-1 data, which showed a significantly higher failure rate among sd-NVP-exposed children randomised to an NNRTI, compared to LPV/r, regimen. Unexpectedly, sd-NVP-unexposed children in cohort-2 also showed a higher failure rate if randomised to a NVP, compared to LPV/r, regimen. Methodologically, the P1060 trial was robust and investigators were as rigorous as possible in ensuring correct allocation of NVP exposure status, although it is difficult to completely rule out the possibility that some infants in cohort 2 were NVP-exposed.

The strength of association between starting ART with NVP and having a higher risk of virological failure in infants (less than 12 months) was much larger than the risk observed in older children, suggesting that NNRTI resistance may have an attenuated effect in older children, who benefit from a "wash out" period; these findings would be consistent with data obtained from the NEVEREST cohort where detection of NNRTI-resistant strains was negatively correlated with increasing age at treatment initiation.

Overall, comparison of the data presented here shows that results from the two cohorts were consistent and that, in meta-analysis, no heterogeneity was found across the two studies. It remains unclear why LPV/r should be superior to NVP even in children not previously exposed to sd-NVP for PMTCT, although there are several theoretical explanations. First, infants may be disadvantaged by a NVP-based regimen because of the low genetic barrier to resistance in the context of high viral loads during early life. Although dosing of NVP was increased during the course of the P1060 trial following release of new WHO guidelines, the Authors did not find evidence for a dose effect to explain the observed failure rate among NVP-treated children. Second, it has been proposed that the use of lead-in dosing, whereby NVP is given only once-daily for the first 2 weeks, may lead to under-dosing and thereby facilitate NNRTI resistance and virological failure. Third, it is possible that infants without documented NNRTI exposure were, in fact, exposed to NVP, although the Authors made every effort to ensure that exposure history was accurate for each infant. Studies in South Africa have indicated that levels of transmitted resistance remain low despite treatment scale-up, so acquisition of NNRTI resistance mutations through this route seems unlikely. Although population sequencing was conducted in a subset of the P1060 study population, more sensitive allele-specific resistance assays are ongoing to rule out the possibility of undocumented NVP exposure.

High-quality data from two RCTs suggest a benefit to LPV/r; however, the reasons for this finding (in non-NVP-exposed children) are not completely understood, and there are currently disadvantages to LPV/r over NVP in infancy, including cost, palatability, cold chain requirements and formulation. An alternative approach to long-term LPV/r, which was tested in the NEVEREST trial [51], could be to start ART with a LPV/r-based regimen and switch to NVP once virological suppression is achieved.

Feasibility and Equity

Optimal paediatric 1st-line therapy needs to consider a balance between affordability, availability, toxicity and sustainability. Where possible, ARTs for children should be harmonized with recommended ART for adults to facilitate programmatic management, secure drug production and achieve cost reduction.

Particularly when LPV/r-based regimens are used, syrups, are bulky, difficult to transport and store, complex to administer by caregivers and expensive, resulting in complex regimens not aligned with adult programmes.

A proper cost-effective analysis is currently difficult to conduct since health consequences; including treatment sequencing and impact of long-term toxicity are poorly known in the paediatric population. However, exploring in details the cost-implications, using strong assumptions developed around data from LIC/MIC and performing sensitivity analysis to allow for differences and uncertainty around assumption and parameters will be of absolute importance in the next future. A strong economic evaluation would in fact inform and facilitate decisions around the adoption of international standards at a national level.

The sustainability of providing LPV/r to all HIV-infected infants has also to be considered; however it will be difficult to estimate since significant changes are expected, particularly in paediatric treatment financing, drug price negotiations and effective PMTCT scaling up. Despite its relevance, action should be taken regardless the lack of reliable estimates.

In the current context of limited funding, providing LPV/r only to those who currently have access to it would require careful consideration of equity. Assuming every HIV-infected infant is diagnosed early, the revision of current recommendation may adversely affect the likelihood of achieving universal coverage. Access to LPV/r would likely to be easier in urban areas where access to health services is higher, drug storage easier and transport costs reduced. Countries with larger inequalities between urban and rural area would be more affected by a change in recommendation, and only few well-organized and well-funded national programmes would currently be able to implement these change in both rural and urban areas equitably.

Further considerations would include whether scaling-up of LPV/r may reduce access to cheaper cART drug regimens for those most in need; whether sufficient capabilities are in place to ensure drug availability and conduct accurate forecasting; may lead to a disconnect between paediatric and adult care affecting retention in

care particularly in the poorest areas; and finally whether this approach may hamper decentralisation of treatment and care.

Despite these considerations it is vital to implement the most effective treatment for those most in need. Once optimal treatment standards have been defined the international community should strive to guarantee that universal access to the best possible quality of care is achieved as soon as possible.

7.1.8 Recommendations

Despite the evidence supports the used of LPV/r-based, benefits out-weight risks and cost implication could be addressed by increasing funding and reducing ARV price, the acceptability and feasibility of the intervention remain a huge barrier. It is therefore recommended that international guidelines should only be revised when better formulation will become available and the safety of new strategy for treatment sequentiation will be consolidated. Meantime, further evidence should be sought to complete the evaluation of non-virological health outcomes. A proper cost-effectiveness analysis to formally assess the cost-implication and allow countries to plan resource allocation is urgently needed.

7.1.9 Annexes

Annex 1. Lopinavir/r formulations price.

	Daily dose	Abbott		Aurobindo (CF)	Cipla (CF)	Hetero	Matrix (CF)
		Category 1 countries	Category 2 countries				
Who can access this price?		See annex 2 & annex 8		See annex 2			
LPV/r 80/20mg/ml oral solution	4 ml	**164 (0.112/ml)**	**400 (0.274/ml)**		256 (0.175/ml)		
LPV/r 100/25mg tablet (heat-stable)	3	**153 (0.140)**	**376 (0.343)**	**175 (0.160)**			**164 (0.150)**
LPV/r 133/33mg soft-gel capsule (non heat-stable)	6	**500 (0.228)**	**1000 (0.457)**		633 (0.289)		
LPV/r 200/50mg tablet (heat-stable)	4	**410 (0.281)**	**1000 (0.457)**	**438 (0.300)**	**499 (0.342)**	493 (0.338)	**402 (0.275)**

* The price in brackets corresponds to the price of one capsule / tablet / ml of oral solution. Products quality-assured by US FDA or WHO prequalification (as of May 2011) are in bold.

**(CF) The Clinton Foundation has negotiated with this manufacturer for reduced prices on some formulations for countries in their consortium.

Annex 2. Countries categories for price negotiation (Source: Abbott's Access to HIV Care Program)

Category 1 countries:

Africa and Least-developed countries:

Afghanistan; Algeria; Angola; Bangladesh; Benin; Bhutan; Botswana; Burkina Faso; Burundi; Cambodia; Cameroon; Cape Verde; Central African Republic; Chad; Comoros; Congo- Brazzaville; Côte d'Ivoire; Congo (Democratic Republic); Djibouti; East Timor; Egypt; Equatorial Guinea; Eritrea; Ethiopia; Gabon; Gambia; Ghana; Guinea; Guinea-Bissau; Haiti; Kiribati; Kenya; Laos; Lesotho; Liberia; Libya; Madagascar; Malawi; Maldives; Mali; Mauritania; Mauritius; Morocco; Mozambique; Myanmar; Namibia; Nepal; Niger; Nigeria; Rwanda; Samoa; São Tomé and Principe; Senegal; Seychelles; Sierra Leone; Solomon Islands; Somalia; South Africa; Sudan; Swaziland; Tanzania; Togo; Tunisia; Tuvalu; Uganda; Vanuatu; Yemen; Zambia; Zimbabwe.

Category 2 countries:

Low-income economies (excluding Africa and the LDCs as defined by the UN) India; Kyrgyzstan; Mongolia; Pakistan; Papua New Guinea; Tajikistan; Uzbekistan; Vietnam.

Lower middle-income economies (excluding Africa and the LDCs as defined by the UN) Albania; Armenia; Azerbaijan; Belarus; Bolivia; Bosnia and Herzegovina; Brazil; China; Colombia, Dominican Republic; Ecuador; El Salvador; Fiji; Georgia; Guatemala; Guyana; Honduras; Indonesia; Jamaica; Jordan; Kazakhstan; FYR-Macedonia; Marshall Islands; Micronesia; Moldova; Montenegro; Nicaragua; Paraguay; Peru; Philippines; Serbia; Sri Lanka; Suriname; Syria; Thailand; Tonga; Turkmenistan; Ukraine.

Annex 3. The Clinton Health Access Initiative (CHAI) — Antiretrovirals price list.

PAEDIATRIC PRODUCT		CEILING PRICE (USD)			SUPPLIER			
Name and strength	Packaging	Per Year	Per Pack	Per Unit	Aurobindo	Cipla	Matrix	Strides
3TC (50mg/5ml)*	HDPE bottle 240ml	$28	$1.85	$0.008	✔[2]			
ABC (20mg/ml)*	HDPE bottle 240ml	$203	$13.50	$0.056	✔[2]	✔[1]		
ABC 60mg	HDPE bottle 60 tablets	$125	$5.20	$0.087	✔[2]		✔[1,2]	
ABC (60mg) + 3TC (30mg)	HDPE bottle 60 tablets	$151	$6.30	$0.105	✔[2]		✔[1]	
AZT (50mg/5ml)*	HDPE bottle 240ml	$63	$2.10	$0.009	✔[2]			
AZT (100mg)	HDPE bottle 100 capsules	$34	$4.75	$0.048	✔[2]	✔[1,2]		
AZT (60mg) + 3TC (30mg)	HDPE bottle 60 tablets	$78	$3.25	$0.054	✔[2]		✔[1]	
AZT (60mg) + 3TC (30mg) + NVP (50mg)	HDPE bottle 60 tablets	$102	$4.25	$0.071			✔[1,2]	
d4T (1mg/ml)*	HDPE bottle 200ml	$47	$1.30	$0.007	✔[2]			
d4T (15mg)	HDPE bottle 60 capsules	$16	$1.35	$0.023	✔[2]			
d4T (20mg)	HDPE bottle 60 capsules	$8	$1.40	$0.023	✔[2]			
d4T (6mg) + 3TC (30mg)	HDPE bottle 60 tablets	$46	$1.90	$0.032		✔[2]		
d4T (12mg) + 3TC (60mg)	HDPE bottle 60 tablets	$40	$3.30	$0.055		✔[2]		
d4T (6mg) + 3TC (30mg) + NVP (50mg)	HDPE bottle 60 tablets	$55	$2.30	$0.038		✔[1,2]		
d4T (12mg) + 3TC (60mg) + NVP (100mg)	HDPE bottle 60 tablets	$52	$4.30	$0.072		✔[1,2]		
EFV (50mg)	HDPE bottle 30 tablets	$27	$2.24	$0.075	✔[2]		✔[2]	
EFV (200mg)	HDPE bottle 90 capsules	$34	$8.55	$0.095	✔[2]		✔[2]	
EFV (200mg)	HDPE bottle 90 scored tablets	$38	$9.60	$0.107				✔[1,2]
LPV/r (100/25mg)	HDPE bottle 120 tablets	$214	**$17.85**	**$0.149**	✔[2]		✔[1]	
LPV/r (80 +20 mg/ml)*	HDPE bottle 300ml	$240	**$48.00**	**$0.160**		✔[3]		
NVP (50mg/5ml)*	HDPE bottle 240ml	$59	**$1.95**	**$0.008**	✔[2]			

1) Approved by the WHO Prequalification Programme; (2) Approved by the U.S. FDA or other SRA; (3) Submitted to the WHO, U.S. FDA or other SRA for review and recommended for procurement by Expert Review Panel (ERP) of The Global Fund; (4) Submitted to the WHO, U.S. FDA or other SRA for review but not yet recommended by ERP.

7.2 The challenges of applying the GRADE approach in the context of assessing EID strategies for public health action.

Penazzato M[1], Essaje S[2], Crowley S[3]

[1]Paediatric Department, University of Padova
[2]Treatment and care unit, HIV department, World Health Organisation
[3] Health & Nutrition, UNICEF South Africa

7.2.1 Introduction

The GRADE approach offers a consistent and systematic way to assess evidence and make judgements about the strength with which a recommendation can be made for a range of health interventions[92].It is increasingly being used by a wide range of policy makers.

For WHO, wide efforts are being made to standardize methods of assessing evidence and developing recommendations using the GRADE approach [146, 147], and to transparently report these processes for those who may wish to act upon the recommendations. It remains unclear how to use the GRADE approach in the context of assessing diagnostic tests and strategies [57]. This gap is important, as WHO is expected to make recommendations for member states that include countries with limited health system capacities and a variety of epidemiological patterns and economies. We therefore piloted a GRADE approach to assess tests used for diagnosis of HIV in infants. Here we report on benefits and constraints of the GRADE approach in developing recommendations for such use in the context of public health recommendations.

7.2.2 Methods and process

As part of the process to review and update recommendations for treating infants with HIV infection, we needed to assess which methods were most suitable for diagnosing HIV infection in infants, and the optimal testing schedule for infants exposed to HIV. The majority of these HIV exposed infants are in sub Saharan Africa.

Currently definitive methods for diagnosing HIV in infancy require costly virologic assays that can only be performed in specialized and well-capacitated laboratories [133, 148]. Interpreting test results is complicated by the fact that it may take days to weeks for HIV to be detectable following infection, and for women who choose the breastfeed, post partum transmission may occur across the entire breastfeeding period[149]. Existing WHO recommendations stipulate initial HIV positive test results should be confirmed by repeat testing wherever possible, but that repeat testing should not delay life saving treatment interventions. However it is also recognized that in many low income countries, performing even one virologic tests is demanding[6].

7.2.3 Performing the quality assessment

The guideline group defined the critical outcomes of diagnostic testing for infants: accuracy was considered the most important parameter, although laboratory experts had concerns relating to reproducibility of the test. The final profiles examined sensitivity, specificity, negative and positive predictive values for the use of tests performed at 4-6 weeks. This is the time when WHO recommends that a blood sample is obtained for virological testing in HIV exposed infants[6] because it coincides with the first full expanded immunization programme visit. A simple model developed to quantify the trade off between missed diagnosis and increasing HIV-attributable mortality in infected infants also supports this as the optimal time to test for HIV in exposed infants. We developed grade profiles of the quality of evidence for the different assay methods available and assessed accuracy on plasma, whole blood or whole blood collected onto filter paper (dried blood spots or DBS). The latter is a simple method to obtain infant samples, which can be easily transferred to specialized laboratories.

7.2.4 Defining high quality diagnostic studies

A systematic search for all published studies in peer-reviewed journals within the public domain was conducted, but only studies that met criteria outlined in the

QUADAS [150] (box 1) and STARD[151] checklists were considered valid for the purposes of assessing the quality of evidence. Key criteria include: comparison with the recognized 'gold standard'; studies with consecutive enrolment of patients; inclusion of infants with an uncertain diagnosis, and studies containing infants who had been exposed *in utero* to HIV (our population of interest). In considering assays performed on dried blood spots, conference abstracts were included and additional data requested from authors. Studies that fulfilled the above criteria were considered to start from a high level of evidence, and were subsequently reviewed for study quality, consistency and directness. Several observational studies could not be considered as they failed to meet our criteria for diagnostic accuracy. Several studies were also excluded as they were performed in patients known to be HIV infected, rather than for diagnostic purposes in populations where HIV infection is unlikely.

Box 1

QUADAS Checklist for Diagnostic Accuracy Studies

Item		Yes	No	Unclear
1.	Was the spectrum of patients representative of the patients who will receive the test in practice?	( )	( )	( )
2.	Were selection criteria clearly described?	( )	( )	( )
3.	Is the reference standard likely to correctly classify the target condition?	( )	( )	( )
4.	Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	( )	( )	( )
5.	Did the whole sample or a random selection of the sample, receive verification using a reference standard of diagnosis?	( )	( )	( )
6.	Did patients receive the same reference standard regardless of the index test result?	( )	( )	( )
7.	Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	( )	( )	( )
8.	Was the execution of the index test described in sufficient detail to permit replication of the test?	( )	( )	( )
9.	Was the execution of the reference standard described in sufficient detail to permit its replication?	( )	( )	( )
10.	Were the index test results interpreted without knowledge of the results of the reference standard?	( )	( )	( )
11.	Were the reference standard results interpreted without knowledge of the results of the index test?	( )	( )	( )
12.	Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	( )	( )	( )
13.	Were uninterpretable/ intermediate test results reported?	( )	( )	( )
14.	Were withdrawals from the study explained?	( )	( )	( )

Considering limitations and indirectness:

We used the QUADAS checklist to evaluate study limitations. The key limitation identified was lack of details related to execution of the test, and the assays and reagents used. This is of particular concern with nucleic acid amplification technologies, where "home brew" kits or other non-commercially available kits,

assay or reagents have been used, which may be of variable quality and consistency. This issue can only be overcome when national reference laboratories validate the assays being used; a proper quality assurance has been undertaken along side EID implementation, therefore this was not considered as a major limitation.

The assays may also have been evaluated on a limited HIV subtype repertoire, and may perform differently with different HIV subtypes[152]. However, while early studies had been performed in countries where sub-type B was most common, more recently a wider repertoire of subtypes have been assessed; nucleic acid amplification technologies being used showed a good performance with different subtypes, and the only concern remains for the subtype D for ultrasensitive p24 antigen assay[17] .

The GRADE group suggests that judging directness may be the greatest challenge in decision making for guideline panels. We found that the GRADE framework for decision-making lends itself to down grading of the evidence for features that could also be considered as downgrades within study design; and did not adequately capture all of the key domains important in considering diagnostic tests.

One specific challenge in considering HIV diagnostic tests for infants is that almost all accuracy studies have been conducted in high-income countries, where breastfeeding is not the usual feeding choice for women with HIV. Therefore because the reported rate of false positive and false negative test results did not include breastfeeding populations we developed a simple model to assist the guideline panel in assessing the implications for this on the timing of the testing. The fact that predominant HIV viral sub types differ globally and most of the valid studies were performed in the United States also raises issues of directness. Consistency of results was not a major problem across studies, although earlier studies generally reported lower sensitivity and specificity. No important inconsistencies were noted in sensitivity or specificity and the confidence intervals were not considered as a problem. However, it is noteworthy that the pooled analysis of results in a 2x2 table

provides in practice a non-inferiority comparison, and therefore cannot formally determine if an new assay is superior compare to the existing gold standard

Question: Should HIV infection in infants be diagnosed performing an HIV RNA NAT test at 4-6 weeks?							
Population group: infants exposed to HIV (i.e.<18 months infants born to an HIV positive mother)							
Intervention: HIV RNA NAT at 4-6 weeks							
Comparator: HIV diagnosed by HIV culture or DNA PCR or HIV antibody testing after 18 months of age							
No of studies	**Design**	**Limitations**	**Consistency**	**Directness or generalisability**	**Imprecise or sparse data**	**Other factor**	**QUALITY RANK**
Outcome: **SENSITIVITY** 97.39% (149/153) 95% CI [93.44-99.28]							
4 studies (153 infected over 434 infants)	VALID ACCURACY Cohort studies[1] **4**	No serious limitations[2]	No serious inconsistency	No serious Indirectness[3]	No serious imprecision		**HIGH QUALITY**
Outcome: **SPECIFICITY** 98.93% (278/281) 95% CI [96.91-99.78]							
4 studies (153 infected over 434 infants)	VALID ACCURACY Cohort studies[1] **4**	No serious limitations[2]	No serious inconsistency	No serious Indirectness[3]	No serious imprecision		**HIGH QUALITY**
Outcome: **POSITIVE PREDICTIVE VALUE** 98.03% (149/152) 95% CI [94.34-99.59]							
4 studies (153 infected over 434 infants)	VALID ACCURACY Cohort studies[1] **4**	No serious limitations[2]	No serious inconsistency	No serious Indirectness[3]	No serious imprecision		**HIGH QUALITY**
Outcome: **NEGATIVE PREDICTIVE VALUE** 98.58% (278/282) 95% CI [96.41-99.61]							
4 studies (153 infected over 434 infants)	VALID ACCURACY Cohort studies[1] **4**	No serious limitations[2]	No serious inconsistency	No serious Indirectness[3]	No serious imprecision		**HIGH QUALITY**

The cost and the complexity of the test and the need for specialized laboratory and specimen collection is most relevant in developing recommendations rather than determining the quality of the evidence, but high cost also contributes to indirectness, if many countries and laboratories would be unable to perform the tests being considered.

Two other areas for concern were identified in the assessment of quality; firstly the use of antiretroviral medicines as infant prophylaxis for prevention of mother to child transmission may interfere with detection of HIV RNA, as ARVs inhibit viral replication. Secondly in the breastfeeding mother on combination antiretroviral therapy, some ARVs may pass into breast milk and inhibit viral replication in the infected infant [153, 154]. However, the experts' panel felt confident to consider these as minor flaws as there is no evidence currently available to believe this is a concern.

1. For diagnostic assessment VALID ACCURACY studies provide as high a quality of evidence as RCTs when considering patient important outcomes. Therefore those studies providing data about the use of a recognized gold standard test in the population of interest (HIV exposed infants), whose diagnosis was uncertain and consecutively enrolled, were selected and considered to start from a high quality.
2. Assessed with QUADAS checklist.
3. However the HIV primers used in the studies selected were mainly for subtype B and different qualitative thresholds have been considered. Moreover there are concerns about the possible influence of different PMTCT regimens on the viral load detectability, even if currently there is no evidence to suggest this is a problem.
Using indirect evidence about impact on patient important outcomes is essential but not enough

The GRADE approach suggest that accuracy studies may not be a good proxy for patient -important outcomes and encourages guideline groups to assess the balance between desirable and undesirable consequences of diagnostic tests. GRADE also describes a simplified approach that considers true positives and negatives and false positives and negatives. In the case we are reporting on, this alone is unlikely to enable a guideline panel to arrive at a balanced judgment of the likely benefits for the diagnostic testing proposed. There are several other facets of the intervention that largely depend on the health system in which it will be used, that are required to determine likely benefit and whether the guideline panel can make a strong recommendation.

The WHO recommends antiretroviral treatment for all infants confirmed positive on diagnostic testing, hence the need to determine whether effective interventions are available is critical[112, 113]. For the infant who tests negative but is still breastfeeding difficult feeding choices remain. However the panel also needed to consider whether the efficacy of the intervention depended on results being provided in a timely fashion. In this instance the risk of death increases dramatically from 6 weeks (time of the testing) and so if the health system is not able to deliver the result to the mother or carer in a timely fashion, the benefits of the intervention are unlikely to be attained. Over 80% of infants with HIV developed severe disease in the first 6 months in a trial in South Africa[22] and a recent review and meta analysis confirm that infants starting ART with severe disease have worse outcomes[155].

Test under evaluation a 6 weeks	**Putative benefit**	**Sensitivity**	**Specificity**	**True positive (PPV)**	**True negative (NPV)**	**False positive**	**False negative**
HIV DNA (reference)	Can be done on DBS	92.86	99.09 Equal	95.59	98.50	0.7% 9/1202	1.2% 15/1202
HIV RNA	Also potentially provides information on prognosis	97.39 Improved	98.93 Slightly less	98.03	98.58	0.9% 4/434	0.6% 3/434
Ultra sensitive p24 antigen	Less lab requiremen t	95.62 Less	99.42 Equal	98.57	98.19	0.5% 6/1473	1.3% 19/1473
Likely patient important outcome				Need ART	Can consider stopping breastfe eding	Start ART	May die without ART

In most settings the actual specimen is collected at a site distant to the laboratory, and at a site where the intervention (ART) is not delivered, and so results may not result in referral or receipt of care, despite an accurate test being performed and a timely result being delivered. There are no reported studies that examine whether the diagnostic testing strategy leads to improved outcomes, and accuracy studies provide no sense of likely patient-important outcomes. The Clinton Health Access

Initiative (CHAI) and UNICEF have reported on programme evaluations of referrals to care after testing for infants and shown that in many settings up to 50% of positive infants never receive their test results, either because of test delays, loss to follow-up or death. Fewer still are initiated on to lifesaving ART [156]. This is not captured in the balance tables suggested by the GRADE panel.

7.2.5 Moving to recommendations

The GRADE working group propose that the strength of a recommendation can be determined by the extent to which we can be confident that the desirable effects of an intervention outweigh the undesirable.
Recommendations that are strong mean that in most situations the benefits of the diagnostic test are worth the risks or additional incremental costs. Weak recommendations are made when patients' and clinicians' values and preferences will vary, or when the balance between desirable and undesirable consequences of the diagnostic is equivocal [131]

We would argue that in developing recommendations on the use of diagnostic tests or testing strategies, an assessment of the capacity of the health systems to perform the test for those in whom it is indicated and to respond to the test with interventions is necessary before being able to achieve realistic appraisal of patient important outcomes. Thus consideration of the patient important outcome requires that the intervention is feasible and that the health system into which it is being recommended for introduction has to have the capacity or can afford to provide the interventions in a timely fashion and in ways likely to influence the patient-important outcomes.

We have therefore modified the GRADE balance sheets and terminology of the recommendations to help policy makers and programme managers interpret the recommendations and to ensure the guideline panel considers the feasibility of the diagnostic tests and follow up actions being feasible and taken (box 2).

Box 2 Adapted risk benefits balance sheets

Factor	Implications for making recommendations	Infant HIV testing
Quality of the evidence	Higher the quality of the evidence the more likely a strong recommendation can be made	Moderate quality for all methods RNA testing offers lowest reported false negative rates and highest sensitivity
Balance between desired and undesirable effects	Larger the gap or gradient between these then more likely a strong recommendation will be made	Tests providing more false negative is most likely to result in harm
Values, preferences	If there is a great deal of variability or strong reasons that the recommended course of action is unlikely to be accepted by the patient, health care worker or health system then it is more likely a weak recommendation will be made.	Patient- prefers DBS Health worker prefers DBS Laboratory prefer to process plasma specimens
Cost	Higher the cost both financial and in terms of infrastructure, equipment or requirements, and more resource intensive requirements, then less likely to make a strong recommendation	DNA costs about half RNA Assay reagents and other consumables costs similar
Feasibility	Is the intervention possible and practical in the settings where greatest impact is likely to be attained or is being sought, the more feasible the intervention is the more likely a strong recommendation should be made.	All assays have evidence of accuracy using dried blood specimens - which allows use in PMTCT context Neither assays licensed for use as diagnostic test RNA is licensed for viral load
Balance	Most likely impact on patient-important outcomes with point of care test and immediate results- none available. Most desirable to minimize false negatives in counties forced to rely on one test strategies Feasibility assessment suggest DNA favourable Risk benefits suggest RNA favourable	
Summary	Considerable concern about all available tests if used within one test strategy Strong - recommend HIV-RNA or HIV DNA on plasma or DBS	

The guideline group felt that making explicit decisions about feasibility forced the group to consider factors not contained in assessment of cost. In most countries where the burden of HIV in infants is considerable, a single virological test strategy for HIV exposed infants has proved costly but increasingly feasible to institute (UA report 2007-2008). It has however proved difficult to assure the quality of the testing and even more difficult to ensure infants and mothers receiving test results benefit from early testing.

Particularly for diagnostic tests the context and health system determine the likely patient-outcomes, and where access to the required interventions are independent of the performance of the diagnostic test performance, focusing on accuracy to assess patient important- outcomes may result in failure to adopt robust and useful technologies or adoption of technologies that the health systems is then unable to support. We therefore provide a modified GRADE approach that uses a health systems lens.

MODIFIED GRADE RECOMMENDATION FOR PUBLIC HEALTH INTERVENTIONS

Strong	**Conditional**	**Research**
Implications: The panel is confident that the desirable effects of adherence to the recommendation outweigh the undesirable effects.	**Implications:** The panel concludes that the desirable effects of adherence to a recommendation probably outweigh the undesirable effects, however, it is only applicable to a specific group, population or setting, or new evidence may result in changing the balance of risk to benefit, or the benefits may not warrant the cost or resource requirements.	**Implications:** Further research is required before any recommendations can be made.
Policy makers: Policy makers can be confident that the recommendation can be adopted as policy in most situations.	**Policy makers**: Policy makers need to consider that in specific situations this recommendation should be followed and identify and develop directives that outline those specific situations, or policy makers need to determine if the potential benefits warrant the health system costs, or policy makers need to recognize that there is currently insufficient evidence to make a strong recommendation and that further research is likely to have a potential for reducing uncertainty about the effects of the intervention.	**Policy makers**: Research is needed before any recommendations or policy directive can be developed.

7.2.6 Observations on the process

In assessing whether a test should be considered our expert panel primarily wished to review the accuracy of the diagnostic tests. In moving to when and how it should be used and in making recommendations they felt it was important to consider the health systems outcomes. The experts were very concerned by using terms such as weak for recommendations that were based upon high or moderate quality of evidence but were largely unfeasible due to health system constraints. The group therefore felt that the term conditional better described recommendations where uncertainties about the balance of desirable and undesirable effects made strong recommendations impossible.

7.2.7 Conclusions

GRADE does offer a comprehensive, systematic process to appraise evidence and develop recommendations, and can also be used in assessing diagnostic tests and strategies. For use in context of WHO recommendations about public health interventions for adoption in low and middle income countries assessing diagnostic tests relying on patient important outcomes and inferring where there are no RCTs is insufficient. To assess public health benefit, health system factors that influence patient-important outcomes have to be examined. In considering diagnostic tests the timing and place of testing and return of results is important. In the context of WHO the GRADE system is useful in assessing evidence but in developing recommendations directed at public health interventions for use and adoption into a range of contexts, additional assessments, models and scenarios may be useful.

As the context and health system determine the likely patient outcomes, particularly for diagnostic tests, and where access to the required interventions are independent of the performance of the diagnostic test performance, focusing on accuracy to assess patient important outcomes may result in failure to adopt robust and useful technologies or adoption of technologies that the health systems is then unable to support. We therefore provide a modified GRADE approach that uses a health systems lens.

8. Discussion

This final chapter provides some conclusive comments about this research project investigating the optimisation of HIV-infected infants' management. In this chapter the main findings are summarised, key messages presented and additional research needed outlined. Conclusive remarks address how the objective of the PhD has been achieved through the formal teaching and by carrying out this research project.

8.1 Key messages

Early ART in infancy

A systematic revision of the evidence (Chapter 4) has confirmed that there are unequivocal benefits of early treatment initiation in infancy. The uptake of the research findings on this matter has been quick and effective so that recommendations for treatment initiation in children <2 years of age have already been updated in all settings.

Findings from the EPPICC analysis (Chapter 5), presented here, confirm that outside trial settings, an effective treatment response can be achieved in infants who start ART early in life, with the majority remaining on first-line ART after five years.

Early initiation of treatment is further supported by ongoing work on modelling long-term CD4 recovery dynamics which demonstrates that delaying ART during childhood will impair the expected CD4 concentration on entering adulthood [157]. Immunosuppression at ART initiation not only damages the potential for immune recovery, but also affects the chances to undergo safe treatment interruptions as shown by the PENTA 11 trial [158].

Despite the undisputed benefits of early initiation of treatment, the programmatic impact of adopting early therapy according the new 2010 WHO paediatric guidelines is still unknown and there are concerns it will significantly increase the workload in already overburdened programmes. In this report the potential scenario that programmes may encounter after implementation of the new recommendations

was described (Chapter 6.1) and an overall increase of 17% of children in need for treatment was observed. Remarkable increase was seen at enrolment in the programme when the number of treatment-eligible patients increased by 65%.
Unfortunately, despite the update of national guidelines and the endorsement of WHO recommendations by most countries, the implementation of early ART remains challenging and only a very small proportion of infants are started on treatment globally. Additional efforts are still needed to ensure infants and young children are initiated on treatment in a timely manner and in this context, strengthening of early infant diagnosis services appears critical.

Early infant diagnosis

The development of technologies which can use DBS has made virological testing possible even in remote areas. However, the optimisation of the use of this technologies and the trade-off between sensitivity and costs make the development of diagnostic strategies in different settings very challenging. Part of this thesis (Chapter 6.2) has highlighted the main barriers in achieving an effective implementation of EID services, such as long turnaround time, delay in results being returned to patients, high lost to follow up and lack of linkage to HIV care and treatment. In addition, it was noticed that cost and performance of virological tests are influenced by multiple factors such as HIV prevalence (positive and negative predictive value), which ultimately impacts the number tests undertaken.

The recognition that in resource limited settings deviation from the expected course of action may be unavoidable leads to adopt a pragmatic approach and discuss the cost-effectiveness of the EID algorithm currently recommended by the WHO. This rational inspired the development of an economic evaluation study proposal which was here presented and aims to explore the cost implications of different diagnostic strategies. The differences between all the possible entry paths, such as HIV prevalence in the target population, need for service decentralization, context-specific barriers and different cost implications may support the recommendation of alternative EID strategies and inform how to allocate best the given budget a country

is provided. In this context, it can be argued that the resources spent with the current testing algorithm (including confirmatory test) in a PMTCT service where effective interventions would bring MTCT down to 2-5%, would be better spent to further expand access to PMTCT interventions and decrease HIV transmission. By contrast, ensuring appropriate and active testing in entry paths such as paediatric wards and malnutrition clinics where HIV prevalence is much higher virological testing would yield a bigger number of positive results leading to larger benefits.

However, these considerations should be balanced out by the recognitions that, the linkage with PMTCT services has been shown to effectively enhance retention in care and ensure a successful EID cascade. Moreover, decentralisation of EID strategies, while enabling an expansion in access to testing where PMTCT interventions are limited or less effective (sd-NVP) and HIV transmission higher, may be of limited effectiveness when not coupled with decentralisation of HIV care and treatment.

Hence, formally assessing how these factors combines in real life and in different settings is critical to plan a more effective use of resources to concomitantly reduce transmission and ensure a timely HIV diagnosis in every setting.

The challenges faced by EID implementation and the debate around the cost-effectiveness of current strategies reinforce the need of using a pragmatic and comprehensive framework when developing recommendations for global use. The difficulties encountered during the revision of WHO diagnostic guidelines for HIV infection children were also explored and presented in this report (Chapter 7.2).

For diagnostic tests the context and health system determine the likely patient outcomes, and access to the required interventions are independent of the performance of the diagnostic test performance. Focusing on accuracy to assess patient important outcomes may therefore, result in failure to adopt robust and useful technologies or adoption of technologies that the health systems is then unable to support. Moreover, despite the fast development of newer and more precise technologies which remarkably improve the accuracy of these tests, adoption of new procedures and review of existing strategies in the field require

time and resources. This, ultimately impacts in the inability of quickly uptake new scientific discoveries and maintain the country strategies updated.

First-line ART

Once infants and young children are successfully diagnosed in a timely manner and treatment is initiated, issues around choice of first-line ART including potential treatment sequencing require careful consideration. Methodologically, the P1060 trial was considered to be robust (Chapter 4.1) even if concerns on the limitation of using combined endpoints were raised. The use of a meta-analytic approach allowed to compare the data from the two P1060 and to highlight a substantial homogeneity in results. This raises further questions on the interpretation of cohort 2 results and feed the current debate around the optimal first-line ART for infants.

Infants may be disadvantaged by a NVP-based regimen because of the low genetic barrier to resistance in the context of high viral loads during early life. This may also be enhanced by the use of lead-in dosing. However, the CHAPAS 1 trial showed no difference in virological suppression after 48 weeks between children starting full-dose NVP versus lead-in dose [36].

The age effect in both cohorts and the lack of difference between cohort 1 and 2, could be explained by the possibility that infants without documented NNRTI exposure were, in fact, exposed to NVP or had acquire an NNRTI-resistance virus. The pressures to be enrolled in a study may have affected the reliability of the information regarding NVP exposure as seen in other trials (ie. DART trial- Di Gibb personal communication). Moreover, robust paediatric data assessing the frequency of NNRTI-resistance virus circulation at a population level is still lacking.

In this context, it is important to assess the proportion of children who acquires mutations potentially associated with NNRTI-based regimen failure as NNRTI resistance is often found at higher rates in children in “real-world” conditions as opposed to clinical trials. This will be particularly relevant in the current scenario of several countries rolling out maternal ART to prevent MTCT (Option B) [128], even

though, the reduction in transmission may ultimately reduce the absolute circulation of resistant viruses.

For this purpose an HIVDR surveillance study was developed (Chapter 6.3) in collaboration with WHO and CDC. This survey is expected to provide descriptive evidence of HIVDR in HIV infected children under 18 months of age. Despite some obvious limitations, such as limited data availability and suboptimal handling of DBS samples, the evaluation of HIVDR prevalence to specific ARVs could inform decision-making about pediatric ART regimens to adopt within countries.

Policymakers are currently expected to deliberate over the findings of the P1060 trial and consider the implications of recommending PI-based therapy for all infants. Despite an almost complete endorsement of the WHO recommendations for LPV/r in NVP-exposed HIV-infected infants, LPVr is used in 12% of all first line regimens in children – increased from 9% in 2009. To date, the vast majority (97%) of children using LPV/r in first line are in South Africa, with the remaining in a small number of countries in Africa and Europe.

A systematic evaluation of risks and benefits of rolling out LPV/r-based regimen as a first-line therapy for any infant and young children starting ART was here presented (Chapter 7.1) highlighting extensively issues around toxicity, treatment sequencing, risk of drug interactions, cost implications, acceptability and feasibility. LPV/r is much more expensive than NVP and, even though new formulations are under development, is currently only available as an unpalatable liquid formulation.

The harmonisation with recommended ART for adults is one of the “Treatment 2.0” pillars to facilitate programmatic management, secure drug production and achieve cost reduction. Unfortunately, the specific cost implications of a potential change in policy are still unknown and further factors should be considered, including: whether scaling-up of LPV/r may reduce access to cheaper cART drug regimens for those most in need; whether sufficient capabilities are in place to ensure drug availability and conduct accurate forecasting; may lead to a disconnect between paediatric and adult care affecting retention in care particularly in the poorest areas; and finally whether this approach may hamper decentralisation of treatment and care.

In this context of limited resources and limited scientific evidence, alternative strategies should be considered. The findings from the EPPICC cohort analysis (Chapter 5) suggest that an induction-maintenance approach to first line ART using NVP with 3 NRTIs may be superior and if results of the ARROW trial confirm this observation an effective alternative may become available. An additional alternative to long-term LPV/r, is the NEVEREST [51] strategy where ART is initiated with a regimen containing LPV/r switching to NVP once virological suppression is achieved. Trials are ongoing to further assess this approach in infants and young children (MONOD, NEVEREST-3).

Key messages		
	Evidence	**Challenges**
Early ART	• Reduces mortality and disease progression • Improves neurodevelopmental outcomes • Achieves good and sustained virological response • Preserves immune function • Allows safer treatment interruptions.	• Requires early diagnosis • Remarkable increase in treatment eligibility at programme level (by 65% at enrollment) • Limited data in slow progressors and in children between 1-2 years of age
EID	• Virological testing allows early diagnosis of HIV infection. • DBS make EID possible in remote areas • New WHO algorithm increase sensitivity and specificity • Link with PMTCT improves retention	• EID services have still low coverage (23%) • EID implementation is affected by high lost to follow up rate, as a result only a third start ART • Cost and performance of virological tests are influenced by multiple factors such as HIV prevalence
First-line ART	• LPV/r-based regimen shows lower risk of virological failure in NVP-exposed and unexposed children less than 3LPV/r-based regimen • Induction-maintenance NNRTI-based strategy is promising (ARROW results expected in 2012) • PI-sparing strategies such as the NEVEREST strategy are safe but require additional LPV/r-based regimen are problematic for TB co-treatment	• LPV/r-based regimen are currently unfeasible in most countries • LPV/r acceptability likely to be low due to poor palatability and storage requirements • Treatment sequencing following a LPV/r-based 1st-line regimen is challenging in most countries Surveillance on transmitted drug resistance is needed • Lack of harmonization with adult regimens may negatively affect decentralization and procurement and supply chain

In conclusion, early diagnosis and early treatment in infants will have to be globally prioritised to achieve a rapid reduction of HIV-related mortality and optimize future treatment outcomes. The debate around the superiority of LPV/r-based regimen as first-line therapy is ongoing due to challenges in acceptability and feasibility. Impact studies are needed to formally assess the cost-implications of changes in policy and allow countries to plan resource allocation.

Once optimal treatment standards have been defined the international community should strive to guarantee that universal access to the best possible quality of care is achieved as soon as possible for every HIV-infected infant.

8.2 Research needed

Further evidence is urgently required to better inform policy on first-line treatment recommendations in young children. Data from non-randomised, observational studies might usefully be combined to inform an individual patient meta-analysis to further address the question of what regimen to start in children under 2 years of age. More robust data addressing non-virological outcomes are also needed. Concomitantly, HIV drug resistance surveillance studies perform in different countries to investigate the frequency of NNRTI resistance at a population level will have the potential of informing decision taken at the national level so that strategies can be tailored according setting-specific data.

Exploring in details the cost-implications of introducing a LPV/r-based regimen for HIV-infected infants as a first-line therapy will also be extremely important; the use of strong assumptions developed around data from LIC/MIC and the performance of a sensitivity analysis to allow for differences and uncertainty around assumptions and parameters will be of absolute importance in the next future. A strong economic evaluation would inform and extremely facilitate decisions around the adoption of international standards at a national level. The sustainability of providing LPV/r to all HIV-infected infants should also be investigated at a global level considering the significant changes which are expected in paediatric treatment financing, drug price and effective PMTCT scaling up.

More definite answers on the effectiveness of switching to nevirapine after virological suppression with LPV/r-based regimens are needed, particularly regarding how best to identify those children who may benefit from such a strategy and the mid- and long-term impact on treatment sequencing and virological control.
Data are also awaited from trials that are exploring the possibility of interrupting treatment beyond the critical period of rapid disease progression and neurological development. However, it will be necessary to undertake adequately powered trials that compare mortality and disease progression between infants undergoing early initiation combined with treatment interruption vs the current standard of care.

8.3 Conclusions

The issues concerning diagnosis and treatment of HIV-infected infants and young children were systematically explored with a wide methodological approach, which included essential elements of evidence evaluation, analysis of clinical observational data, operational research and evidence-based policy development.

The formal teaching in epidemiology, statistical methods for epidemiology, health economics, principal of social research and public health received over the past three years has led to adopt a more comprehensive approach to research questions. The technical knowledge acquired includes: conducting systematic literature reviews; applying standards for quality of evidence evaluation; carrying out comprehensive risks-benefits evaluations to inform policy development; developing study protocols; developing basic models for economic evaluation; developing epidemic curves and conduct outbreaks investigations; plan qualitative studies using qualitative and mixed methods; planning analysis of an observational study; analyzing data using statistical packages, such as Stata 11; collaborating with other researchers in discussing and interpreting the results obtained; and finally preparing deliverables for research dissemination (manuscripts, abstracts, posters or oral presentations).

The aim of future work will be to apply the newly acquired skills to facilitate and promote research to guide the development of international policies for life-saving public health actions in the field of infectious diseases in developing countries.

9. Publications

9.1 Articles in peer-review journals

1. Penazzato M, Prendergast A, Cotton M, Gibb D. Effectiveness of antiretroviral therapy in HIV-infected children under 2 years of age. Cochrane Database of Systematic Reviews 2010, Issue 10. Art. No.: CD004772. DOI: 10.1002/14651858.CD004772.pub2. Cochrane Library 2012(in press)

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9.2 Abstracts

1. Petrara R, Penazzato M, Massavon W, Nabachwa SM, Nannyonga M, Mazza A, Zanchetta M, Giaquinto C, De Rossi A. Dynamics of Epstein-Barr virus in HIV-1-infected children in Uganda. VI IAS Conference, Rome 2010: Abstract MOPE258

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10. Penazzato M, Crowley S. *What is available for early infant diagnosis?: Results from WHO survey 2008.* V IAS Conference, Cape Town 2009: Abstract WEPEB269.

11. Nannyonga M, Massavon W, Franceschetto G, Penazzato M, Morelli E, Nabachwa S, Sekavuga DB, Mazza A, Giaquinto C. *Switching from 1st line to 2nd line antiretroviral therapy (ART) regimen: experience of a cohort of Ugandan HIV-1 infected children.* V IAS Conference, Cape Town 2009: Abstract MOPEB091

12. Massavon W, Nannyonga M, Penazzato M, Nabachwa S, Sekavuga DB, Morelli E, Rinaldi E, Aiello E, Farina F, Mazza A, Giaquinto C. *Impact of home visits and psycho-social support on retention in care: experience from a cohort of Ugandan HIV-1 infected children.* V IAS Conference, Cape Town 2009:

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14. Mazza A, Morelli E, Penazzato M, Wool P.S, Massavon W, Franceschetto G, Nannyonga M, Giaquinto C. *Clinical presentation, diagnostic criteria and outcome of Tuberculosis in Ugandan HIV-infected children.* 1st International Workshop on HIV Pediatrics, Cape Town 2009. Abstract: P_82

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