
Kulkanya Chokephaibulkit, MD¹
Nirun Vanprapar, MD¹
Sanay Chearskul, MD¹
Ruengpung Sutthent²
Wanatpreeya Phongsamart, MD¹

¹Department of Pediatrics, ²Department of Microbiology
Faculty of Medicine Siriraj Hospital
Mahidol University, Bangkok, Thailand

Correspondence: Kulkanya Chokephaibulkit, MD
Department of Pediatrics
Faculty of Medicine Siriraj Hospital
2 Prannok Rd., Bangkok-noi, Bangkok 10700, Thailand
Tel: 661-6110371 Fax: 662-4180544
E-mail: sikch@mahidol.ac.th

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Abstract

**Background:** HIV-infected infants deserve antiretroviral therapy regardless of their staging. Unfortunately an appropriate treatment strategy for HIV-infected infants in resource-limited settings, where HAART is generally not feasible or affordable, is still unclear. Initiation with dual nucleoside reverse transcriptase inhibitors (NRTI) may be an alternative rationale for infected infants with mild to moderate disease. As of this time, no study has been performed to test feasibility, duration of efficacy, or outcome of dual NRTI therapy in infants living in developing countries.

**Methods:** A multicenter prospective open-labeled operational study was conducted. Eighty-eight antiretroviral naïve HIV-infected infants were enrolled in seven hospitals in Thailand. The infants were assigned a number in order when they were accepted into the study. Those with odd numbers received zidovudine (AZT) plus lamivudine (3TC), and those with even numbers received AZT plus didanosine (ddI) for 24 months. Infants who were in stage “C” and CD4 cell percentage < 15 were excluded from the study.

**Results:** Of the 88 infants who were in the study for at least 12 weeks, the mean starting age of treatment was 6.8 months, the mean initial CD4 cell counts and percentages were 1538 cells/mm³ and 21.4%. While the medication for seven infants was changed from ddI to 3TC due to vomiting or diarrhea, none of the infants in 3TC arms found to have drug intolerance. The z-scores for weight and height increased after 4-8 months of treatment, and
by the 24 months, were +0.89 and +0.69 higher than at enrollment. The CD4 cell percentage peak increased at 8 months of treatment, by a mean increment of 4.19%, but gradually decreased to the level of 1.08 % above baseline at 24 month of treatment. Three (3.4%) infants died, all within 6 months of treatment, 11 (12%) had disease progression, 7 (8%) had poor compliance and prematurely discontinued the study, and 37 (42%) were lost to follow-up. At the end of 24 months, 30 (34%) children were in stable condition with the chance of clinical and immunological stable of 68% by on-treatment analysis. In comparison with the historically controlled cohort, the infants in this study had a lower mortality rate and lesser chance of disease progression ($P<0.01$ for clinical and $P=0.02$ for immunological category progression).

**Conclusions:** Treatment initiation with dual NRTI in infants with mild to moderate disease stages a resource-limited setting may not be feasible due to high rate of lost to follow-up. However, in those who can adhere to the treatment, the clinical and immunological benefits were seen up to at least 24 months of treatment. This study underscored the need for careful case selection before initiating the treatment in infants in developing countries.
Introduction

Antiretroviral therapy (ART) is an important part of management of HIV-infection. Effective ART prolonged healthy life, prevent opportunistic infection and improve survival\(^1\).\(^2\). Initiation of ART soon after acquiring infection advantageous in preserving immune function, effectively suppress viral replication which may result in a better prognosis\(^3\)-\(^5\). With these potential benefits, infants acquiring perinatal HIV deserve ART once the infection is confirmed. Earlier studies confirmed that early initiation of ART in vertically acquired infants can produce long-term viral suppression and preserved immune function\(^6\)-\(^8\). Perinatal infection is generally more severe than infection in adult\(^9\). The HIV RNA pattern in perinatally infected infants is usually persistently high for prolonged periods and slowly decline after the first year of life due to immature immune system\(^9\)-\(^11\), therefore benefit of ART would be more pronounce. A recent study has shown a 70% reduction of mortality in HIV-infected infants with triple combination antiretroviral therapy\(^12\). Because of the theoretical benefit and the less predictive value of the available virological and immunological parameters\(^10\),\(^11\), the US treatment guideline suggested ART in HIV-infected infants regardless of stage\(^13\) although the clinical trial data on the benefit of this approach is not currently available.

Highly active antiretroviral therapy (HAART) has been recommended as the initial ART regimen in children\(^13\),\(^14\). The HAART regimens for infants generally include two nucleoside reverse transcriptase inhibitors (NRTI) plus one protease inhibitor (PI) or nevirapine. In developing countries with limited resource, protease inhibitor and NVP in pediatric formulation are mostly
unaffordable. Moreover, it is not easy to feed an infant many drugs everyday. To improve adherence and make it feasible and plausible to the family, dual NRTI regimens may be more appropriate in infants in particular among those who are not in advance HIV stage.

Dual NRTI has been known to be less effective in suppression of viral replication\textsuperscript{15}. However, many children who were on dual NRTI were clinically and immunologically stable despite presence of plasma HIV RNA\textsuperscript{1,2}. Moreover, suboptimal viral suppression is not uncommon in children even with HAART\textsuperscript{16}, and clinical and immunological benefit may be observed even with incomplete viral suppression\textsuperscript{17,18}. There is a great concern of suboptimal viral suppression which may later result in selective viral resistance and treatment failure. This may eliminate the drug options in the future. However, if dual NRTI benefit long enough, the infant will grow up into childhood and able to take more drug options. Initiation with dual NRTI in infants in less advanced disease stages may prolong healthy stages and delay the need for the more expensive and complex regimens. This may be an appropriate strategy in resource-limited settings.

We therefore conducted a prospective study to evaluate the feasibility of, as well as the magnitude and duration of efficacy of dual NRTI regimen initiating in infants in mild to moderate stage of HIV disease whose family cannot afford HAART. The result of the study will help considering the appropriate guidelines for initial treatment of HIV infection in infants in developing countries.
MATERIALS AND METHODS

A multicenter prospective open-labeled operational study was conducted in 7 hospitals in Thailand. The participating hospitals were Siriraj, Pramokutklao, Queen Sirikit National Institute of Child Health, Charoenkrung-Parchoruk, Vachira, Chulalongkorn and Hat-Yai Hospitals. The study protocol was approved by Ethical Review Board of the Ministry of Public Health and the Faculty of Medicine Siriraj Hospital. Infants younger than 12 months of age who were diagnosed of HIV infection, whose family cannot afford triple antiretroviral regimen and committed to return for long term follow-up in one of the participating hospitals were eligible. HIV-infection was defined as positive HIV PCR in at least 2 separate blood samples, with or without symptoms. Once they reached the age of 18 months, they must be confirmed of HIV infection by the presence of anti-HIV antibody. The enrollment required an informed consent by the parent or authorized caregiver.

The exclusion criteria at enrollment were age older than 12 months, in advance state of HIV disease i.e. in clinical stage “C” and immunological stage “3” (CD4 cell percentage of < 15%) by CDC classification system. The enrolled infants in odd numbers were assign to receive zidovudine (AZT) 100-120 mg/M²/dose every 8 hours plus didanosine (ddI) 100 mg/M²/dose every 12 hours, and those in even numbers were assigned to receive AZT as above dosage plus lamivudine (3TC) 4 mg/kg/dose every 12 hours. The infants who were unable to tolerate 3TC or ddI that was assigned will be switched to the other one. The infants who were unable to tolerate both 3TC and ddI; or AZT or did not take the medicine regularly by any reasons
were excluded from the study. The antiretroviral drugs were sponsored by the Ministry of Public Health of Thailand.

All the studied children were followed-up every 1-2 months, for physical examination and general check up. The disease staging was determined by using CDC classification system\(^\text{19}\). Complete blood count and CD4 level were measured every 4 months. Other laboratory investigations were performed upon clinical indication. Verification of adherence was by self report and leftover pill counts. The adherence was evaluated at each visit by the participating physicians who saw the patients. Repeated counseling on adherence was done periodically by the assistant nurses. Children with obvious poor adherence or frequently missed the pills even after counseling as judged by the study physicians were prematurely discontinued the study.

Primary end points of the studies were: i) disease progression with either clinical or immunological category change, or decline of CD4 count more than 30 percent of baseline, after at least 3 month of therapy, ii) death. The study was planned for 24 months of treatment. The children who were clinically and immunologically stable up to 24 months were offered to continue the same dual NRTI or to change to HAART.

Other treatments such as vaccination and opportunistic infection prophylaxis were offered according to standard of care in Thailand. Formula was offered free of charge from birth to at least 12 month-old. The study was integrated into the routine HIV care without other incentives except for the antiretroviral drugs, vaccines and formula.
Analysis of data

The tolerance of the treatment regimen was descriptively analyzed. The outcomes were measured in terms of CD4 cell percentage gain, weight and height z-score gain every 4 months. The data of those who lost to follow-up before 12 weeks of treatment were excluded from the analysis. Due to the natural decline of CD4 cell count with age in young children, CD4 cell count may not gain even with clinical improvement. Therefore CD4 cell count was not used for in the analysis. This study did not intend to compare the treatment outcome of the two regimens which will need a much larger sample size. The Kaplan-Meier survival analysis for the proportion of patients who were in stable condition without disease progression or death after treatment was performed by intention-to-treat method, in which all the patients that were lost to follow-up or premature terminated were counted as failure, and on-treatment method, in which the patients who were lost to follow-up or premature terminated were not counted. We also analyze the characteristics that might correlate with disease progression or death including the age at enrollment, baseline CD4 and disease stage, baseline weight and height z-score, and CD4 gain after 4 and 8 months of treatment.

Because the clinical and immunological status of HIV-infected infants will variously progress over time without treatment, the benefit of the treatment strategy was measured by comparison of the disease progression with the group of infants in the similar age and staging who did not receive treatment. A subset of children in this study were age-and disease stage-matched at the initiation of treatment at the ratio of 1:1 with the HIV-infected children in the previous long term cohort in our center. This control cohort
includes children who were born before 1990 and were generally not given antiretroviral therapy in infancy unless they were severely symptomatic, but were offered in later childhood if indicated. Each child was matched only once. There were 37 children with complete record from the previous cohort were available for matching. The age of the patient matched was less than 1 month difference, and must be in the same disease stage at the beginning. The comparative analysis was performed to evaluate the difference of time from the beginning of treatment initiation, with the equal age in the matched control, to the time of clinical staging change (to the poorer category), or immunological staging change (to the poorer category), or death by Kaplan-Meier survival analysis. The number of days and episodes of hospitalization during the comparison period were analyzed.

Results

The totals of 107 infants were enrolled into this study. Of these, 15 infants lost to follow-up within 12 weeks of treatment (7 received AZT+3TC, and 8 received AZT+ddI) these children were excluded from the analyses. Additionally, there were 4 children voluntary withdraw soon after enrollment due to: hepatitis and hemolysis of unknown cause (receiving AZT+ddI), persistent vomiting probably from the treatment (receiving AZT+3TC), and thrombocytopenia of unknown cause (receiving AZT+3TC), and the family decided that they were unable to adhere the treatment after a few weeks of treatment (receiving AZT+ddI). There were 88 children remained in the study for at least 12 weeks of treatment with at least one follow-up blood drawn after treatment (Fig 1).
Of the 88 infants; 40 girls and 48 boys, 45 were assigned to received AZT+ddI and 43 were assigned to AZT+3TC. Seven children who were assigned to AZT+ddI were unable to tolerate ddI because of vomiting or diarrhea, and were switched to AZT+3TC without problem. There was none of those who received 3TC needed to switch to ddI. There was no serious adverse event in these 88 children.

At enrollment, the mean age was 6.81 (SD = 3.68) months and the clinical stages were: 20 (23%) in clinical category N, 38 (43%) in category A, 30 (34%) in category B, and 4 (5%) in category C. The mean CD4 cell count at enrollment was 1,538 (SD = 985) cells/mm$^3$ and a mean CD4 cell percentage was 21.4% (SD = 7.8). Fifteen (18%) children had baseline CD4 cell percentage <15%. During the study period 3 (3.4%) children died from pneumonia (2), and sepsis (1), with the duration of treatment before death of 4, 6, and 6 months, and the disease stage at enrollment of A2, B2 and B2.

There were 37 (42%) infants (23 received AZT+3TC, 14 received AZT+ddI) lost to follow-up before reaching end-point and before finishing 24 months of the study (Table 1). Seven (8%) infants were found out during the study, mostly within the first 4 months of treatment, to miss many doses of the drugs and the family decided to prematurely discontinue the study after counseling. All of them received AZT+ddI. Eleven (12%) children, (5 received AZT+3TC, 6 received AZT+ddI) had disease progression before finishing 24 months of treatment (Table 1). At 24 months of treatment 30 (34%) children (21 received AZT+3TC and 9 received AZT+ddI) remained in the stable condition with the same or improved status compared to at enrollment. The group of children who remained stable at 24 months was significantly older
than those who had disease progression or death (7.53 vs 4.5 months, $P=0.005$), and tended to gain more CD4 cell percentage at 4 months of treatment (mean gain = 4.67% vs 0.98%, $P=0.29$). The proportion of patients in the study without disease progression or death over the treatment period by intention-to-treat (OTT) analysis and on-treatment (OT) analysis were in Fig 2, with the probability of 34% by ITT and 68% by OT at 24 months of treatment. Due to the high rate of lost to follow-up, we did the comparison analyses between the 37 children who lost and the 30 children who were stable through the 24 months of treatment. There was no difference in age, disease staging, and weight and height z-score at enrollment between the children who lost to follow-up and those who remained stable in the study.

The effect of treatment on weight and height demonstrated in Fig 3. The increased of z-score of weight and height was clearly seen after 4 months of treatment. At 24 months of treatment, the mean change from baseline of z-score of weight and height were + 0.89 and + 0.69. The CD4 cell percentage was found to increase from baseline from month 4 of treatment and reached its peak of mean increment of 4.19% at month 8 of treatment before slowly decreased thereafter. At 24 months of treatment, the CD4 cell percentage was 1.08% above the level at enrollment. The CD4 cell count was stable up to 12 months of treatment and gradually dropped thereafter (Fig 4).

In the matched-pair analysis, the duration of comparative analysis was 777 days. Of the 37 children in the previous cohort, 3 had antiretroviral initiated before 12 months of age and 9 initiated at 13-24 months of age. The antiretroviral used was either AZT or ddI monotherapy. The median age at the start of matched-pair analysis was 6 months, with 21 pairs started before 6
months of age. The mean CD4 cell count at start was not difference (1,939 cells/mm$^3$ in this cohort vs 2074 cells/mm$^3$ in the previous cohort, $P=0.55$).

There were 2 deaths in this cohort compared with 6 in the previous cohort ($P=0.19$). There were more children in the previous cohort developed clinical progression (26 vs 3, $P<0.01$) and immunological progression (26 vs 6, $P=0.02$) than in this cohort (Fig 5). There were more episodes, number of days, and rate of hospitalization in the previous cohort compared to this cohort (37 vs 9 episodes, 379 vs 80 hospitalization days, and 7.661 vs 4.286 hospitalization days per 1000 child-days).

**Discussion**

Perinatal HIV infection resulted in very high viral load during infancy and naturally progressed more rapid than infection in adults or in transfusion-acquired hemophiliac children$^{20, 21}$. Antiretroviral treatment during this period would protect the developing immune system of the infants. It is expected that the outcome of early initiation of ARV in infants would result in benefit similar to the treatment in adults with primary infection in which the studies have confirmed the clinical, immunological, and virological benefit and reduction of opportunistic infections and progression to AIDS$^{22, 23}$. Limited studies also supported the benefit of antiretroviral treatment in infants$^{6, 16, 24}$. The earlier age at initiation of treatment conversely correlate with the magnitude of recovery of naïve CD4 cell$^{25}$. Long-term medication in infants is, however, not simple. Adherence to treatment is totally dependent on caregivers, and could be very problematic in chaotic HIV-infected families. Incomplete adherence could promote development of resistance and eliminate future treatment.
options. Long-term therapy may also bring more chance of adverse effects such as metabolic problems, osteopenia, mitochondrial dysfunctions. And finally, the cost of treatment could be a significant barrier. Therefore, treatment in infants, especially those who are asymptomatic or mildly symptomatic must weight the benefit against all the potential problems. The US guidelines suggested initiation of antiretroviral therapy in all infants regardless of symptoms or CD4 level\(^{13}\). The European guideline, however, recommend the treatment in infants only when CD4<20% and consider initiation of treatment in infants with CD4>20\(^{14}\). WHO also recommend initiation of ART in infants with CD4<20% in developing countries\(^{26}\).

HAART including 2 NRTI plus one PI or nevirapine (NVP) is generally recommended as the initial regiment in infants. However, the available PI for infants, ritonavir (RTV), lopinavir/ritonavir (LPV/r), or nelfinavir (NFV), are mostly unaffordable in limited-resource setting. They are not palatable and frequently cause gastrointestinal side effects. Nevirapine is less expensive and may be locally produced but may cause significant adverse events of hepatitis and rashes in 15-20%. Dual NRTI may be the option for those who are unable to take PI or NNRTI when the benefit outweighed the risk of suboptimal viral suppression. In infants, the transmitted virus is mostly homogeneous NSI and rarely resist to AZT even with inutero AZT exposure\(^{27}\). In developing countries, the antiretroviral treatment during pregnancy is quite limited to AZT and NVP, therefore, the risk of resistant to the initial regimens of dual NRTI is low. In Thailand, NRTI’s are locally produced at low cost while PI is generally unaffordable by most families. NVP is the least expensive drug to compose a HAART regimen, but affordability is still limited. In most cases
in developing countries, dual NRTIs are the only available and affordable regimens for infants. Moreover, dual NRTI are easier to take and could help the patient to stay in good adherence.

Dual NRTI regimens are less potent than triple regimens and may result in failure in shorter duration\textsuperscript{15, 28-30}. In infants with advanced disease stages, dual NRTI regimens are not appropriate. However, clinical and immunological benefit of dual NRTI regimens were clearly demonstrated in many pediatric studies\textsuperscript{1, 2, 31, 32}. The relative magnitude of benefit if the treatment is started in infancy with less advance disease stages has not been well determined. A population-based study in Italy has found limited benefit of dual NRTI in reduction of mortality in treated children\textsuperscript{12}, however, about half of the children in that study were in advance stage of HIV and the median age of treatment initiation was 2.1 years. There was a concern of limited options and the less efficacy of salvage regimens after dual NRTI treatment failure. However, a study found that the delayed in initiation of PI in stable children who were on dual NRTI did not adversely affect the response\textsuperscript{29}. The caveat is not to wait for too late to start HAART.

It is still unclear that in the settings where the availability of HAART is limited, one should hold off the treatment in infected infants if only dual NRTI regimens are available. We hypothesize that initiation of ARV in the early stage of HIV-infection in infants, even with dual NRTI, will prolong healthy period and delayed the need for more expensive and difficult to take triple regimens. Early dual NRTI therapy may be better than not to initiate treatment and wait until the disease progress as it has been shown that disease in infants run fast with high mortality and morbidity\textsuperscript{9, 20, 21, 33, 34}. The drug options
other than NRTIs will be more available when the child grows older and remains in good health.

This study has demonstrated that implementation of ARV in infants in a developing country is a real challenge. Because it is an operational research, the patients were treated in the routine service without other incentives more than the ARV, vaccines and formula. This is more likely to simulate the real situation than in usual clinical trials. About half of the infants lost to follow-up, 15 children lost in the very first few visits, and another 37 children gradually lost along the period of 24 months. This drop-out rate is markedly higher than our routine service in older children, in which the rate has been less than 10% lost per year. From the patient interview, most of the lost to follow-up cases occurred because the infants were relocated to be taken cared of by the grandparents in up-country. The parents wanted to go back to work when they found the infants grew up normally without pressure for them to return for follow-up. In our hospital, most of our HIV-infected parents’ home-towns were outside Bangkok and the infants enrolled into this study were relatively healthy. We were unable to identify the factor associated with the risk of lost to follow-up. Another problem in this study is the evaluation of adherence. Of the seven cases that were prematurely terminate the study from poor adherence; many were found out after many visits of apparently good history of taking medicine and some with appropriate left-over pill count. Most cases of poor adherence were identified within the first 4 months of study. A previous European study has shown that full adherence to treatment was more likely in symptomatic children and older than 10 years of age\textsuperscript{35}. This
finding is probably applicable in our study. A more careful case selection before enrollment might be able to reduce these problems.

This study has confirmed the clinical and immunological benefit of the dual NRTI regimens. Due to limited resource, we did not routinely perform HIV RNA load for treatment monitoring. We found that if the treatment is well taken, 68% of children remained clinically and immunologically stable at 24 months of treatment (on-treatment analysis). In the comparative analysis with the previous cohort, in which only monotherapy was given in very few infants, dual NRTI treatment in this study reduced disease progression and hospitalization up to 24 months. The mortality in this study is also less but could not reach the statistical significance probably due to small sample size. Previous studies without treatment found the mortality rate of 17% in Florida and 9% in Italy in the first year of life, and 45% at 2 years of age in Rwanda. The rate of 3 (3.4%) deaths during 2 years of treatment in this study is obviously less. All of the death occurred in 6 months of treatment. These infants may need more potent regimens. They might have had a higher viral load that we did not evaluate. We were unable to identify any factor that would associate with the risk of death infancy.

In ACTG 152, 25% of the children received AZT+ddI, started at the mean age of 0.8 years, reached primary end points of death or disease progression at 28 months. In the ACTG 300 study, 7.6% of children who received AZT+3TC, started at the age of 2.7 years, had disease progression or death at 60 weeks. This study found somewhat higher rate of disease progression or death; 15% (10/66) and 32% (14/44) at 12 and 24 months of treatment respectively by on-treatment analysis. The PENTA-4 trial indicated
that weight and height gain can be good surrogate markers of response to treatment\textsuperscript{31}. This study has found a notable improvement in weight and height throughout the 24 months of treatment. The weight and height z-score gain in this study at 12 months was in the magnitude of 0.2-1.0, very similar to the ACTG 300 and PENTA-4. These data suggested that clinical benefit on growth of dual NRTI in this study is not difference from that found in the cohorts of older children. In this study the CD4 cell percentage gained from baseline was peaked at the level of 4.19\% at 8 months of treatment, similar to the PENTA-4 trial\textsuperscript{31} that found the gain of 3\% at 24 months after adding 3TC into the stable regimens of mono or dual NRTI. However, the CD4 cell percentage gain was no longer persist at 24 months of treatment.

We did not intend to compare the efficacy of 3TC and ddI, however, there was a trend of less disease progression and death in 3TC group (data not shown). A previous study also suggested that AZT+3TC is more efficacious than AZT+ddI\textsuperscript{32}. We found that 3TC is better tolerated than ddI. One of the advantages of 3TC over ddI for infants is the availability of liquid formulation. There were 2 serious adverse events of which might be related to the treatment, acute hepatitis and hemolysis a child who received AZT+ddI, and thrombocytopenia in the other child who received AZT+3TC. Both cases occurred in the first month of treatment and the family decided to withdraw from the study without rechallenging.

In conclusion, treatment initiation in HIV-infected infants in less advance disease stage in developing country is usually not feasible due to high rate of lost to follow-up. In the cases that can adhere to treatment, dual NRTI regimens in infants induced the clinical and immunological benefit and
resulted in stable condition up to 24 months in about two-third. 3TC is better tolerated in infants than ddI. The findings from this study underscore the importance of case selection if one decided to treat an infant. The impact on viral resistance would be greater if HAART is implemented in infants with potentially high drop-out rate.

References

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