Title: Treatment options for protease inhibitor-exposed children

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Abstract
We propose an unblinded randomized clinical trial to evaluate a simplification, protease-inhibitor (PI)-sparing treatment strategy among nevirapine (NVP)-exposed HIV-infected children treated initially with lopinavir/ritonavir (LPV/r). HIV-infected children aged 3-5 years, who have a history of exposure to NVP as part of prevention of mother-to-child HIV transmission (PMTCT), initiated LPV/r-based therapy in the first 36 months of life or who were enrolled on the control arm of Neverest 2 and who are virally suppressed with a viral load < 50 copies/ml will be included. These children will be randomized to either substitute efavirenz (EFV) for LPV/r or to continue on their LPV/r-based regimen. Eight weeks prior to the primary randomization, eligible children will also be randomized to either remain on stavudine (D4T) or switch to abacavir (ABC). Children will be followed with regular viral load and other clinical tests for 48 weeks after the primary randomization. Children in the experimental arm who have breakthrough viremia (-defined as two subsequent viral loads > 1000 copies/ml) on the EFV-based regimen will reinitiate the LPV/r regimen. The primary objective is to test whether the durability of viral suppression (< 50 HIV RNA copies/ml) is equivalent when children are switched to EFV-based therapy. The primary study endpoint is failure to have HIV RNA < 50 copies/ml at 24 weeks (6 months). Secondary aims include comparison of immune preservation, toxicities, selection of resistance mutations, and adherence across the two arms. Antiretroviral drug concentrations and adherence will be investigated as possible explanations for the success and/or failure of this simplification regimen. The overall goal of the study is to contribute to the evidence base to allow expansion of treatment options for HIV-infected children in low resource settings.

Background

Despite significant advances in worldwide prevention of mother-to-child HIV transmission (PMTCT) efforts, the pediatric HIV epidemic rages unabated, particularly in Sub-Saharan Africa. At the end of 2007, it was estimated that 2 million children were living with HIV, 370,000 children were newly-infected with HIV and 270,000 pediatric deaths worldwide were attributed to HIV/AIDS.[1] While there have been noteworthy successes in global efforts to increase access to antiretroviral treatment (ART) for HIV-infected individuals in low resource settings, the expansion of programs for PMTCT has been more limited.[2] Less than 33% of HIV-infected pregnant women received any form of intervention to reduce transmission.[2] Furthermore, most PMTCT programs rely on single-dose nevirapine (NVP), a regimen of modest efficacy for reducing transmission compared to more complex, multi-drug combinations.[3-6] Hundreds of thousands of children will continue to acquire HIV infection during the years ahead.

Infants and young children with HIV infection are particularly vulnerable to rapid HIV disease progression and early death.[1] In a cohort of infants who became infected despite PMTCT with single dose NVP, 85% had significant clinical and immunologic progression and met ART eligibility criteria within the first 6 months of life. Without treatment, at least 50% of HIV-infected children can be expected to die by 2 years of age.[7]

The availability of potent ART regimens for treatment of pediatric HIV infection is slowly transforming the dire landscape created by this epidemic. About 200,000 children were receiving ART globally in 2007, a 1.7 fold increase from 2006.[2] More than 32,000 children were reported to be receiving ART in South Africa, one of the highest burden countries in Sub Saharan Africa (280,000 children are estimated to be living with HIV in South Africa). Despite significant challenges in the treatment of children with HIV in resource-constrained settings, ART outcomes appear to be comparable to those in better-resourced countries.[8-10] Studies from a variety of settings in Africa and Asia demonstrate marked improvements in clinical and immune parameters after ART initiation as well as high rates of viral suppression (where it can be measured), comparable to what has been seen in the US and Europe.[8;11-21]

There are many challenges facing clinicians prescribing ART for HIV-infected children in resource-poor settings, among them; scarcity of appropriate pediatric formulations, cold-chain requirements for some liquid formulations, poor palatability of many liquid formulations, and unknown long-term side-effects of treatment during critical growth and development phases.[22-28] The evidence base for pediatric ART options lags behind that available for HIV-infected adults. This is lamentable because there are many complexities in treating HIV-infected children that could be better informed by focused clinical trials. New antiretroviral drugs are usually first investigated in adults resulting in lengthy delays in registration of the same drugs for pediatric use. Furthermore, formulations for children are often not developed or tested until efficacy is determined in adults. For example, raltegravir, darunavir, and etravarine are now available for adults but are only just entering clinical phase I/II trials in children and are not available in pediatric or liquid formulations. While it is imperative to continually and urgently investigate new agents for children, it is equally important to investigate treatment strategies which will rationalize the use of available pediatric formulations in order to maximize efficacy, minimize toxicity and prolong treatment durability.[22-24]

Standard virologic, immunologic and clinical criteria to establish ART eligibility perform poorly in infants < 12 months of age making it difficult to predict which children will progress rapidly and which will have a slower course and can safely delay treatment.[29-31] Therefore until recently this critical strategy question when best to initiate ART was unanswered[32] and it has been a

particularly complex issue in infants and young children where the rate of disease progression can be very rapid and neither CD4 nor viral load accurately predict the risk for progression to AIDS or death.[29;30] Studies of children initiating ART show high mortality during the first months of therapy,[8;13;14;16;33] similar to adults and attributable to ART initiation at advanced stages of disease. For children, low CD4 percent, low weight-for-age, advanced WHO stage, tuberculosis (TB) and young age have all been associated with an increased risk of death within the first months of ART.[8;13;14;16;18] CHER, a randomized clinical trial of early ART in infants has provided valuable data to inform the question of when to initiate ART in young children and infants.[34;35] HIV-infected infants <12 weeks of age without evidence of immune suppression were randomized to initiate ART immediately or to defer treatment until meeting established immunologic or clinical criteria. The study, which was stopped by the DSMB, demonstrated a clear survival advantage with early treatment initiation.[35] A second part of CHER evaluating treatment interruption is ongoing. In April 2008, after review of the CHER findings, WHO updated pediatric ART guidelines to recommend that all HIV-infected infants <12 months of age rapidly initiate treatment.[36] There are broad implications of these recommendations. It is anticipated that HIV-related mortality will be significantly diminished with early ART. At the same time, without a rapid scale-up of the depth and breadth of PMTCT programs, as many as 1000 babies worldwide will need to initiate treatment every day.[2] Thus there is a clear need to consider strategies for long-term management and treatment of children initiating ART during the first year of life.

The choice of starting regimen for children <12 months is constrained by possible selection of mutations conferring resistance to non-nucleoside reverse transcriptase inhibitors (NNRTI) following NVP used in PMTCT as “single-dose” or in combination with other drugs, most commonly zidovudine (ZDV).[37] Single dose NVP alone or in combination with ZDV is widely used for PMTCT in low resource settings.[2] While the efficacy of Single dose NVP has been repeatedly demonstrated, particularly for lowering the risk of peripartum and early postnatal transmission, prophylactic use of NVP results in selection of viral mutations resistant to this agent and to EFV the other NNRTI commonly used for therapeutic treatment.[38-44] In a trial in Thailand where NVP was added to a short-course ZDV PMTCT regimen, when women later initiated NNRTI-based therapy, those who had been randomized to the NVP arm were less likely to achieve viral suppression than those whose PMTCT regimen did not include it.[45] In a subsequent trial in Botswana with a similar design, the effect of past exposure to NVP was found to be confined to those women who initiated therapy within 6 months of delivery.[46] Recent results from OCTANE, in which women exposed to single dose NVP within 24 months were randomized to initiate therapy either with NNRTI- or with Lopinavir/ritonavir (LPV/r)-based therapy, demonstrated superiority of the LPV/r-based regimen.[47]

There are fewer data on the implications of PMTCT NVP exposure for pediatric treatment efficacy. Lockman et al. reported on treatment outcomes for 30 children, (15 NVP-exposed, 15 placebo-exposed) who started ART at median of 8+ months after exposure in a PMTCT trial.[46] There was a significantly increased risk of virologic failure in NVP-exposed children, 76.9% in the exposed vs. 9.1% in the unexposed cohort (P<0.001), as well as lower CD4 percents and lower CD4 increases after 6 months of NVP-based ART.[46] Data on somewhat older children in Uganda, mean age 1.7 years for single dose NVP-exposed children, found no differences in immunologic improvement or viral suppression, suggesting that the impact of NVP exposure may diminish with duration of time from exposure.[48] The IMPAACT clinical trials network is currently conducting a large randomized clinical trial, P1060, to compare the efficacy of PI vs. NNRTI-based ART for children, 6-36 months of age, exposed and unexposed to NVP for PMTCT (http://clinicaltrials.gov). The study is being conducted in multiple African countries and

has enrolled 286 of the 573 subjects. The recent interim data and safety review has found the LPV/r-based regimen to be more effective than the NVP-based regimen in HIV-infected children who received a single dose of NVP at birth. By 24 weeks after treatment initiation among all NVP-exposed children, 40% in the NVP arm had failed to virally suppress or stopped taking their treatment regimen compared to only 22% in the lopinavir arm. In addition, when considering viral suppression alone, only 7% among the NVP exposed children in the lopinavir arm had failed to reach viral suppression compared to 24% among the NVP-exposed children in the NVP arm. Consequently, the study team has stopped enrolling children who received single-dose NVP at birth.[49] These findings support the revised WHO pediatric treatment guidelines which recommend that HIV-infected infants previously exposed to NVP for PMTCT initiate ART with a protease-inhibitor (PI) in combination with two nucleoside reverse transcriptase inhibitors (NRTI)[36] and nowadays LPV/r - Kaletra™ is the recommend PI for treatment of infants.

In Johannesburg, South Africa, PMTCT programs that use NVP have been widely implemented since 2001. Pediatric ART programs have also been successfully implemented at the major referral hospitals since 2004 when the government-sponsored ART program began. Infant diagnosis has been made available in the public sector since 2004 but programs have low coverage and most HIV-infected infants and children still only reach clinical attention when they are symptomatic. Clinical and CD4 criteria have been used to decide when to initiate therapy. The National Department of Health adopted guidelines which were based on consensus among clinicians nationally,[50] recommending LPV/r-based ART in combination with two NRTIs (mostly 3TC and D4T) for children < 3 years of age and EFV-based therapy in combination with two NRTIs for children > 3 years as the first-line regimen.[50] Therefore, there has been considerable experience in South Africa with the approach recommended by WHO.

At the same time, a number of concerns have emerged regarding the long-term use of LPV/r as first-line, and in effect life-long, therapy for children including adherence challenges due to poor palatability for children too young to swallow tablets, inability to use with standard TB treatment, long-term toxicities, limited options for second-line treatment and high cost. While studies are examining the safety of treatment interruption in children, at this point initiation of ART implies lifelong continuation.[34;51] Thus ART will be continued throughout the period of rapid childhood development and into adolescence, spanning critical developmental periods for exacerbation of long-term drug toxicities and leading to new challenges for adherence. Guidelines offer no specific advice about whether LPV/r-based therapy should be continued life-long for all HIV-infected children starting therapy at a young age. There are several risks associated with indefinite, long-term use of LPV/r-based therapy including its poor palatability (raising adherence challenges in toddlers and older children),[52;53] interactions with rifampicin used for co-treatment for TB,[54] the lack of any suitable available second-line regimens, and uncertainty about its long-term metabolic toxicities when used in developing children.

Both the liquid and solid formulations of LPV/r present specific adherence challenges for children. The liquid is poorly palatable with a taste difficult to mask with sweeteners or other foods. While most infants can learn to take the medication, many parents report new adherence difficulties as their children age.[55] Infants who reluctantly swallow their medications become toddlers who run from their mothers as the hour for treatment approaches.[53] Abbott recently launched a heat tolerant pediatric tablet formulation of LPV/r, (Aluvia™) but it is only appropriate for children able to swallow tablets. These tablets cannot be crushed, broken or cut so that children, particularly those aged 3-6 years who cannot swallow tablets, must take the liquid formulation. Hence, toddlers and young children encounter significant adherence challenges

with LPV/r. Poor adherence is the most important threat to virologic suppression with ART. [56-58]. A recent study from Cape Town, South Africa, demonstrated that adherence was significantly worse for children receiving PI-based regimens compared to children receiving NNRTI-based regimens.[52]

Another unanswered question is related to effects of a life long therapy. Little is known about the long-term toxicities associated with LPV/r for children initiating therapy early in life. The experience with LPV/r in more resourced settings has been primarily in older children requiring salvage therapy.[59] Nonetheless, PI-based treatment has been associated with a variety of toxicities in children including fat maldistribution, dyslipidemias, insulin resistance and increased risk for cardiovascular morbidity.[60-72] In adults, the preference is to initiate therapy with NNRTI-based regimens secondary to the lower burden of long-term toxicities as well as adherence advantages. There is ongoing concern that early PI-based therapy may lead to significant long-term morbidities as children grow and develop. PI-associated toxicities may be less common in young children, particularly prior to puberty. This issue will require further study as large numbers of young children initiate lifelong therapy.

Other problems arise from drug-drug interactions. LPV/r is extensively metabolized in the liver via the CYP450 enzyme systems.[73] Therefore clinically-significant drug interactions may occur when LPV/r is administered concomitantly with other drugs metabolized by the CYP450 system which can result in increased or decreased concentrations.[54] Higher concentrations can in turn result in toxicities or, alternatively, sub-therapeutic drugs levels may lead to increased risk of drug resistance and/or treatment failure. These drug interactions are particularly problematic in TB endemic regions where standard short-course chemotherapy includes rifampicin as the standard of care TB treatment. Co-administration of rifampicin with LPV/r resulted in a 90% to 99% reduction in trough concentrations of lopinavir in 2 studies in healthy adult volunteers.[74;75] A number of strategies have been used in countries using LPV/r for treatment of HIV-infected children requiring co-treatment for TB including ritonavir alone, boosting LPV/r with additional doses of ritonavir and doubling the dose of LPV/r.[76] Preliminary data from the prerandomization period of our soon to be completed trial demonstrate that children co-treated for TB while receiving a ritonavir-based regimen were less likely to achieve viral suppression. A recent study conducted in Cape Town in addition revealed inadequate LPV/r levels when the double-dose LPV/r is used with rifampicin. [77] Data on TB co-treatment among adults suggest that there are no differences in viral suppression among co-treated vs. not co-treated adults when EFV-based ART treatment regimens are used[78] and a recent pediatric study found that co-administration of rifampicin based Tb therapy did not significantly reduce the EFV concentration in 15 children aged 3-15 years[79]. However, at least one study has reported lower viral suppression rates for NVP-based therapy.[78] A recent study conducted in the Western Cape estimated 23.4 cases of active TB per 100 HIV infected children per year[80] and TB is the most common HIV-associated infection.[81] As TB is a common co-infection among HIV-infected children it is essential that treatment regimens address the special challenges of TB co-treatment.

Another complexity of LPV/r-based therapy is that there are currently limited second-line options. As NNRTIs are part of the pMTCT intervention the current recommendations are to start children on a LPV/r based regimen, which in fact is a second line regimen. In case of viral failure there are no good alternatives for a so called third line regimen. While new drugs for treatment of drug resistant virus are in development and some are being studied in children (Raltegravir, TMC 125, Maravaroc, Vicriviroc), it can be anticipated that it will be many years before these medications are widely available in low resource settings for pediatric use. For example, tenofovir has been routinely prescribed for adult treatment for a number of years but a

pediatric formulation is still in development and dosing and toxicity of the existing formulation is still being evaluated in older children and adolescents. Given the limited formulary of pediatric antiretroviral medications, it is important to examine different strategies to maximize use of existing medication. Therefore, we propose to evaluate whether it is safe for NVP-exposed, HIV-infected children initiated on a LPV/r-based regimen to switch to a simpler, PI-sparing, NNRTI-based regimen.

The public health approach promoted by WHO relies on immunologic and clinical monitoring to determine treatment failure in lieu of viral load monitoring routinely used in the US and Europe. Therefore, treatment failure is often determined well after viral escape and in the context of ongoing viral replication and selection of drug resistant mutations.[82] It is not unlikely that children failing first line LPV/r treatment will develop extensive NRTI mutations and PI mutations. We present new data below demonstrating selection of NRTI- and PI-associated mutations among a large percentage of children who fail their initial LPV/r-based regimen consistent with other studies.[59;73;83] While it may be reasonable to consider NNRTI-based second-line therapy in children failing PI, background NRTI mutations will likely compromise the efficacy of such regimens, independent of the potential impact of archived NNRTI resistance in children with perinatal NVP exposure.[84] In any case, options for second line regimens are limited and clinicians are struggling to determine optimal treatment approaches for children who fail first-line LPV/r treatment. Switching children from their LPV/r regimen to a NNRTI containing regimen once they are virologically suppressed, introduces the chance to save the PI regimen as a treatment option in case of viral failure.

Data from adult studies suggest it is better to avoid initiation of NNRTI-based therapy among NVP-exposed women. Data from P1060 in children recently reported similar results in children. WHO guidelines encourage the use of LPV/r-based therapy as the first-line regimen for infants. These trials and recommendations consider only which regimen to initiate among NVP-exposed adults and children. Neither current scientific nor WHO recommendations address whether children need to continue PI-based therapy indefinitely (i.e. is safe to switch back to NNRTI treatment?) or how best to treat children who fail first-line PI treatment. There are several disadvantages of continuing LPV/r-based therapy indefinitely among HIV-infected children, such as adherence challenges with the poorly palatable regimen that can results in virologic failure, adverse interactions with rifampicin that may result in virologic failure, and high cost. Given these risks, it is necessary to evaluate whether other options, such as switching PI to NNRTI-based therapy, may provide better and safer options for long-term treatment. In addition switching to NNRTI can preserve LPV/r for use as a second-line agent, thus extending the duration of effective treatment that can be accomplished with currently available agents.

Preliminary data from our soon to be completed trial suggests that >80% of NVP-exposed children can be successfully suppressed on a NVP-based regimen after a period of induction with LPV/r if adherence is maintained and viral load is <50 copies/ml at the time of switch. We have also demonstrated that children who have breakthrough viremia on the experimental regimen can be promptly identified, returned to their prior regimen before any clinical consequences accrue and can re-suppress on their original regimen. We propose to recruit a cohort of HIV-infected, NVP-exposed children aged 3 to 5 years who have been receiving LPV/r-based therapy and have a viral load <50 copies/ml and to randomize them either to remain on LPV/r-based therapy or to switch to EFV-based therapy. PIs and especially Kaletra™ are more costly than NNRTI's and because of the complexity of the molecules used to produce this class of drugs, are unlikely to be available as cheaply even if generic drugs are produced. (The technology (Maltrex) required to co-formulate lopinavir and ritonavir in a single

pill is particularly complex.) EFV is a desirable agent to use as it is highly effective, is available in a once daily, easily palatable formulation, has minimal toxicities and can be used simultaneously with TB co-treatment.[85;86] For children < 3 years, appropriate EFV dosing has not yet been determined. Since EFV is also significantly less expensive than LPV/r it is also desirable to switch to a PI-sparing strategy from a cost perspective. From a public health perspective in countries where the burden of disease is high, economic consideration must be given to regimen choices for children and adults. Several countries in Africa are actively considering whether or not to implement the new WHO recommendations because of the additional costs.

Simplification studies are an accepted study design for evaluation of HIV treatment options and have been widely used in HIV-infected adults.[87-90] These studies include studies evaluating the safety and efficacy of a) moving from three drug regimens to PI monotherapy; b) changes from PI-based to NNRTI-based therapy; and c) changes from PI-based to a three NRTIs containing regimen; and d) changes in the NRTI backbone.[91-120] Simplification trials are designed with the goal of reducing toxicities, facilitating easier adherence and improving quality of life. Six reviews and one meta-analysis including over 25 PI to NNRTI switch protocols in adults have been published.[87-89;121-124] The overall conclusions from these studies in adults is that switching PI to an NNRTI (NVP or EFV) can be accomplished safely, while maintaining virological suppression, improving adherence and reducing some toxicities.[87;94;108;114;115;124;125] Simplification studies have also been conducted in children. The reason why only well-controlled patients are enrolled is so as not to select new resistance mutations if the new regimen is not fully suppressive. There are two reports of children switched from a PI regimen to an NNRTI regimen.[102;126] In the most detailed study, 15 heavily pretreated children were switched from a PI to EFV, all achieved viral suppression during a median follow-up of 40 weeks.[126] In addition, triglyceride and cholesterol levels decreased and adherence improved. In the second report, 7 children were switched. All but one maintained viral suppression and lipid profiles improved.[102] Data from these studies suggest that simplification of PI to NNRTI-based highly active ART is safe and feasible, even in children.

Treatment of pediatric HIV disease poses a formidable challenge in countries in sub-Saharan Africa with major HIV/AIDS epidemics. Despite acknowledgement of the importance of including children in clinical trials, studies on how to best optimize the agents available for pediatric ART treatment are remarkably few. An important reason to evaluate a switch strategy is to preserve a second-line option for children. Using LPV/r as a first-line regimen is problematic since the drug is intended to be used as part of a second-line regimen, and for children, there are as yet no other drugs that could be easily recommended for second-line use. Switching to an NNRTI-based regimen only once failure of the LPV/r-based regimen has occurred is suboptimal as the accumulation of resistance mutations as a result of long periods of non-suppressive ART is likely to render the second-line regimen ineffective. Switching to NNRTI-based therapy when viral suppression is achieved is likely to be more effective than waiting for viral failure and will allow re-use of the LPV/r with NNRTI-based failure. We propose to investigate a PI-sparing treatment strategy, which introduces the possibility to spare LPV/r to be used at a later point following the eventual failure of the NNRTI-based regimen, designed based on the results on our existing pediatric clinical trial that we describe in more detail below.

Specific Aims:

The primary specific aim of the trial is to investigate whether it is necessary to continue LPV/r-based antiretroviral therapy among NVP-exposed children indefinitely or until treatment failure. 400 HIV-infected children exposed to NVP as part of PMTCT who initiated LPV/r-based treatment in the first 36 months of life and who are suppressed (viral load <50 copies/ml) will be included. All children currently enrolled in Neverest II regardless of age, and children between 3 and 5 years of age if recruited from other sources, will be eligible. Children will be randomized to one of two treatment arms: (1) the experimental arm – substitute EFV for LPV/r vs. (2) the standard-of-care arm – remain on the LPV/r containing regimen.

Primary outcome

- maintenance of viral suppression < 50 copies/ml
- confirmed viral rebound >1000 copies/ml

Secondary outcome

- immunologic and clinical preservation
- drug-related toxicities, including fat distribution and metabolic parameters
- drug-related toxicities when co-treated for tuberculosis
- adherence

Study design

This study is designed as an unblinded, randomized clinical trial among 400 HIV-infected children exposed to NVP as part of PMTCT who initiated LPV/r-based treatment in the first 36 months of life and who are suppressed with at least one HIV RNA viral load <50 copies/ml at time of enrollment into the study. The two randomized treatment arms are: (1) the experimental arm – substitute EFV for LPV/r vs. (2) the standard-of-care arm – remain on the LPV/r antiretroviral regimen. Children will be followed with regular blood tests and clinical examinations until 48 weeks post-randomization.

Study site and team

We propose to undertake the trial at the clinical research site that we have established at Rahima Moosa Mother and Child Hospital Hospital (RMMCH) in Johannesburg, South Africa. The site is set up as a research clinic with consulting rooms, a separate research pharmacy and administrative offices and is currently staffed by two medical officers, two nurses, a pharmacist, pharmacy assistant, two counselors, data capturer, project coordinator and administrator. RMMCH, a teaching affiliate of the University of Witswatersrand Medical School, is a tertiary-level hospital providing in-patient and out-patient pediatric care as well as antenatal and maternity care. It has the second largest pediatric HIV treatment clinic in Johannesburg. We have successfully enrolled more than 300 HIV-infected children into the previous clinical trial at this site. There are existing collaborative relationships in place to recruit and retain eligible patients who live within a reasonable travel distance to the site from other pediatric clinics in the area including from the Harriet Shezi Clinic at Chris Hani Baragwanath Hospital (the largest pediatric HIV clinic in the region), Johannesburg Hospital and community primary health care clinics.

The team located at Columbia University, including two of the prinicipal investigators, will supervise the study as part of the weekly phone calls between the American and the South African site.

South Africa, and Johannesburg in particular, is an ideal place to undertake this research as it is a setting where the question is highly relevant - with one of the highest HIV seroprevalence rates among pregnant women in the world (estimated to be 30.3% according to the annual South African Department of Health Antenatal Survey) [127]and large numbers of HIV-infected infants and children in need of and receiving treatment. This huge clinical need exists alongside the capacity (including expertise and infrastructure) to undertake high quality clinical trials relatively easily. There has also been reasonably good implementation of PMTCT services over the past 5 years: through to the end of 2007 using almost exclusively SDNVP and beginning in 2008 using short-course ZDV with NVP. Resources exist to undertake infant diagnosis using PCR and there exists the laboratory infrastructure to monitor therapy including performing viral load and drug resistance testing, CD4 determination, and toxicity monitoring. It is also a setting where large numbers of HIV-infected children have been started on LPV/r-based therapy as this regimen has been recommended as part of local guidelines since 2004 for all children < 3 years of age who met clinical and CD4-based criteria for ART initiation**.**

Study population
All children currently enrolled in the control arm of Neverest II will be screened as potential subjects for the new study. In addition, other children will be recruited from other sites if they meet the following criteria: NVP-exposed, HIV-infected children aged 3 to 5 years at the time of study screening, who were originally initiated on LPV/r-based ART at <36 months of age. Children will need to have been on LPV/r therapy for at least 12 months and to be virologically-suppressed (viral load <50 copies/ml) to be eligible. Only those with available and complete medical records for HIV treatment will be eligible for enrollment. Records of exposure to NVP will be sought but the lack of ability to confirm NVP exposure will not be an exclusion criteria if a reliable history can be provided. We also anticipate that perhaps a third of the participants may be recruited from our existing clinical trial NEVEREST II.

Children will be recruited from several sites but all will be followed up at the RMMCH clinical research site. Children will be recruited preferentially from the control arm of the existing clinical trial NEVEREST II (anticipate about 80 eligible children), from the pediatric HIV treatment program at RMMCH (anticipate about 150 eligible children) and from the Harriet Shezi clinic at Chris Hani Baragwanath Hospital (anticipate about 400 eligible children). These three sources will provide access to children initiating LPV/r-based therapy <36 months of age and followed at standard intervals with CD4 and viral load measurements every 6 months. Detailed paper records are maintained on all these patients and key laboratory and clinical data have been maintained in computer databases. The specific inclusion/exclusion criteria we propose are described below:

Inclusion criteria:
- HIV-infected child 3 to 5 years of age at time of screening for this trial if enrolled from outside the current trial. Any age if enrolled from control arm of Neverest II. The rationale for the different criteria is that for the Neverest II subjects we already have the most detailed possible data on these children from the start of treatment. Setting an upper limit on age for the other children mostly pertains to the feasibility of finding medical records pertaining to their initiation of therapy.
- Reliable history or documented exposure to NVP used as part of PMTCT

- Initiated antiretroviral therapy with LPV/r at age < 36 months
- Receiving LPV/r-based ART for at least 12 months
- At least one viral load measurement < 50 copies/ml conducted as part of screening for the study
- ALT measurement grade I or less (DAIDS Toxicity Tables 2004) (Appendix A). These may be repeated until ALTs normalize if necessary.

Reliable histories for confirmation of NVP-exposure status will be accepted and will be taken in a standardized way with attention to implausible and inconsistent answers. Only caretakers who provide histories that are consistent and clear are eligible for enrollment.

Exclusion criteria:

- Prior treatment with any NNRTI drug as part of a therapeutic regimen
- Co-treatment for tuberculosis will be an exclusion criterion for enrollment into the study and randomizations will not occur during such times.
- Substitution of other NRTI drugs (instead of 3TC & D4T which are the standard first line regimen) will be allowed.

Screening and Recruitment

Potential participants will be located and screened for eligibility for enrollment after signing informed consent (Consent form 1). As part of this screening, blood samples will be collected for viral load measurement to establish whether viral load is <50 copies/ml and other inclusion criteria are met. Screening will also involve establishing the availability of the necessary records to confirm start of treatment and exposures to PMTCT. Record confirmation of NVP exposure is not required but will be sought. A questionnaire will be used to establish a reliable history. Since some participants may be referred to the research site, screening will also establish whether it is feasible for the participants based on their current residence to attend services at the research site. Adherence will be evaluated during screening and those who have a clear commitment to long-term treatment will be enrolled. Social criteria will not be used to exclude participants. In our experience almost all of the potential participants have complex and sub-optimal social circumstances and it precisely to this high risk group of children to whom we wish to generalize the results.

Informed consent: The study staff (nurses and doctors) will obtain informed consent. The consent will meet the requirements of the Institutional Review Boards of Columbia University and the University of the Witwatersrand. The study subject will be asked to read the consent form and study staff will describe the study and requirements of participation. If the study subject is unable to read, the consent form will be read out loud. The study subject will sign the consent form, or if she cannot write, she will place her thumbprint on the form. Mothers will provide informed consent for the child. If the child is no longer being cared for by the mother, the current primary care giver will sign the informed consent form.

Screening of Neverest II participants:

All current Neverest II participants will be screened as potentially eligible for the new trial. As part of this screening, blood will be drawn (arranged to be drawn after an at least 8 hour period of fasting) for viral load measurement, CD4 counts, ALT and metabolic parameters, clinical examinations, including anthropometric and fat distribution measurements, will be done and questionnaires will be administered to the caregiver. Children who were randomized to the control group in Neverest II (remain on LPV/r) will be eligible to be enrolled in the new trial if they meet the criteria above and they are interested in participating. Children who were

randomized to the intervention group in Neverest II (switch to NVP) will not be eligible for the new trial, regardless of whether or not they were switched back to LPV/r. However, these children will be screened following the same procedures to ensure orderly exit from Neverest II and supervised and monitored transition back into the regular clinic population.

Intervention group children follow-up: Children in the switch group who are currently receiving NVP and who have a viral load <1000 copies/ml at screening will be transitioned onto an EFV-based regimen. Children in the switch group who are no longer receiving NVP-based regimen or who have a viral load >1000 copies/ml will be followed on the same schedule. Those still on the NVP-based regimen will be switched to LPV/r-based regimen. The NRTI backbone currently in use (in most cases 3TC and D4T) will be preserved unless there are specific indicators to suggest that any of the drugs are contraindicated. Children will be followed at 2, 4 and 6 months post-EFV switch with clinical examinations and questionnaires. Viral load monitoring will be done at 6 months post-switch to EFV. CD4 and ALT measurements will be done at 6 months. Children will then be referred to the routine clinical services most convenient for the participant. Children who have a viral load >50 copies/ml at this point will be advised to switch back to LPV/r-based therapy. The motivation to transition the children to EFV is two-fold. First this drug is considered one of the safest and most effective agents for children and is the recommended drug for all children initiating therapy older than age 3 in the routine program. Thus it is the drug most likely to be consistently available and prescribed in routine clinical care programs when these children are no longer seen as part of a clinical trial. Since these children are suppressed at the time of switch they have also already demonstrated that underlying NNRTI-associated resistance is not of immediate clinical concern. Secondly, continuing on an NNRTI-based regimen allows LPV/r to be preserved for second-line therapy if this regimen fails later on. There are currently 77 children still in follow-up who were randomized to switch to NVP. We estimate that about 50 of these children are still receiving NVP-based regimens and have not been switched back to LPV/r. Children on D4T will remain on this drug unless clinical parameters suggest otherwise.

Control group screening and follow-up: Children in the control group will undergo the same screening procedures to identify who will be eligible to enroll in the new trial. Those who are found to meet the above criteria will continue to enrollment. Those who are screened and found not to meet the inclusion criteria will be referred to the most convenient services with the recommendation that they remain on a LPV/r-based regimen and will not continue in follow-up as part of the study. If participants are known or suspected not to meet the inclusion criteria, the screening tests should still be conducted to ensure complete data. Currently there are 87 participants still in follow-up in the control group and we anticipate that 70 are likely to meet criteria.

Screening of new participants: Participants who are known or thought to meet the study criteria will be recruited from pediatric services around Johannesburg. They will be asked to attend a screening visit at the study site at which time blood will be drawn, examinations will be done and questionnaires will be administered to determine whether the children meet criteria for enrollment. Those who do not meet criteria will be referred back to routine services for follow-up care. Combined with the expected numbers from Neverest II, we anticipate having to screen an additional 400 children to yield the 300 required for this study, However, we will continue to screen potential participants until the study sample size is achieved.

Enrollment
At the second visit the results of the blood test taken at the initial screening visit will be discussed. Once it is established that the participant is eligible for the study, they will be re-consented for the randomized study (Consent form 2). If blood results are abnormal at first screening they may be repeated until abnormalities resolve. Caregivers of the participants will need to agree that they will follow their assigned treatment arm.
At enrollment, the child will be reviewed to determine whether they are eligible for the NRTI backbone randomization (randomization A). Criteria for randomization include all the criteria for enrollment in the trial described above. In addition, in order to eligible for randomization A, the child also needs to be currently prescribed D4T and not have any signs of lipodystrophy severe enough to warrant change to ABC. If the child is eligible, general adherence counseling will be conducted and the child randomized or a visit will be scheduled for randomization A. Children who are not eligible for this randomization will proceed straight to the visit for randomization M.

Randomization A: Stavudine (D4T) vs. Abacavir (ABC): Through December 2009, D4T was used as part of the NRTI backbone for treating children in South Africa. Almost all the Neverest II participants are receiving treatment with this agent and it is likely that the participants that we recruit from routine services will also be receiving treatment with D4T. It is now recommended that treatment programs move away from treatment with D4T. The current South African guidelines recommend that no new children be started on D4T but that children who are already receiving the drug remain on it unless clinical signs of lypodystrophy/atrophy are present in which case abacavir (ABC) should be used instead. While this is a reasonable recommendation from a programmatic point of view, the adverse effects of D4T on lipodystrophy/atrophy are well described in adults and are known to occur in children as well although the precise frequency is not well established. In an audit of over 3000 children treated at the Harriet Shezi clinic, <5% were found to have clinical signs sufficient to prompt a change in regimen. Some programs are reluctant to end use of D4T given its low cost and generally good tolerability and concerns about ABC hypersensitivity. Our trial offers a unique opportunity to examine issues related to D4T in more depth.

We propose to introduce a separate randomization among the participants who are enrolled in the trial. All participants who are currently being treated with D4T and who do not have clinical signs that would make them eligible to switch to ABC will be randomized to either remain on D4T or to switch to ABC. The current protocol did not make any specific recommendation regarding the NRTI backbone and any combinations were considered accepted. Thus this randomization does not affect the primary question being addressed by this trial. This design deviates from “standard of care” by providing what is considered better quality treatment by some and considered not necessary but more expensive by others. The randomization will allow us to determine whether there are measurable benefits of switching away from D4T more rapidly than the current South African guidelines advise.

Randomization A will be done 8 weeks before the main randomization for the study. Random assignment lists will be generated separately to the main trial in case it is necessary to terminate this randomization during the trial if recommendations regarding D4T and ABC change over the next few years. Random assignment A will be prepared and placed in opaque envelopes to be opened at the time of randomization. Either at the enrollment visit or at a specially scheduled randomization A visit, the envelopes will be opened and the child assigned to either remain on D4T (Group D) or switch to ABC (Group A). Children will remain on their assigned backbone throughout the trial unless it is necessary to switch one or more of the drugs for toxicity reasons. In this case, the switch will be made but the child will continue to be

followed for the duration of the protocol. No specific data will be collected for this randomization and the outcomes already being collected as part of the trial, including viral load, CD4 count, anthropometric, fat distribution and metabolic parameters will be used as the outcomes. If children randomized to remain on D4T develop symptoms they will be changed to ABC. Only children who enter the study receiving D4T as part of their regimen will be eligible for this randomization. If children are receiving ABC or AZT or another NRTI they will remain on the drugs they are already receiving unless there are clinical grounds for a change.

Week 4 post-randomization A (W4PRA)

A visit 4 weeks after randomization A will be scheduled. At this visit, adherence will be reviewed and a clinical exam will be done. If children are thought to have hypersensitivity reactions, they will immediately and permanently discontinue ABC. Once appropriate, a suitable alternative drug will be considered. If these children still meet study criteria, they may be randomized in the primary study.

Week 8 post-randomization A (W8PRA)

A visit 8 weeks after randomization A will be scheduled. At this visit, adherence will be reviewed and a clinical exam will be done. Bloods will be drawn. Body composition measurements will be taken. This visit may also correspond with the randomization M visit.

Randomization M: LPV/r (group 1) vs. EFV (group 2)

At the randomization visit an opaque envelope will be opened to establish random assignment related to whether to switch to EFV (group 2) or to remain on LPV/r (group 1). It is not possible to blind the participants to their randomized arm since the medications are quite distinct in look and taste. Those assigned to the intervention arm i.e. changing to EFV will have counseling with the pharmacist about dosing and administration of the new drug. Those assigned to the control arm i.e. continuing on LPV/r will also have counseling reminding them about the dosing and administration of LPV/r to ensure comparability. The study allows enrollment of children who started ART before 3 years of age. However, children who started ART <12 months of age are of greatest interest. We will review enrollment after the first 150 participants have been enrolled to determine the proportion who started treatment before 12 months of age. Recruitment strategies may need to be revised thereafter to target those who started therapy at younger ages.

Eligibility criteria for randomization

In summary, the following criteria are required before the enrolled children may be randomized:
Viral suppression (<50 copies/ml).
ALT measurement grade I or less (Appendix A: DAIDS Toxicity Tables 2004)

In most cases, if randomization M is done at the same time as W8PRA, then no second viral load result will yet be available after the original screening one that established eligibility. If , however, the W8PRA viral load is >50 copies/ml and the child has not yet been randomized, randomization should be delayed. Possible causes of the elevated viral load should be investigated, adherence counseling should be done and a follow-up viral load done 4 weeks later. If this viral load is still > 50 copies/ml, the child should no longer be considered eligible for the trial and should be referred into the routine services. If the viral load is now <50 copie/ml the child may continue and be randomized. If the child has already been randomized and the viral load is elevated, then the viral load should be repeated within 4 weeks, adherence counseling done and the child followed as per protocol for elevated viral loads.

Efavirenz (EFV) initiation
At the time of initiation of the post-randomization M treatment regimen, the pharmacy will dispense sufficient EFV, 3TC and D4T or ABC for the first four weeks. The patient will be contacted by phone at one week and will be asked standard questions about the child's clinical status and understanding of the new treatment regimen. If any problems are identified on this phone call, the child will be scheduled for a visit as soon as possible. Blood collection and follow-up visits will take place as described in the Table below.

Drug treatment post-randomization:
South African guidelines previously recommend the NRTIs 3TC and D4T as part of the first line regimen.[50] Given the recent concerns about D4T we propose the randomization to either remain on D4T or switch to ABC that we describe above. If other drugs have already been substituted for these, then the child will remain on that regimen and will still be eligible for the study. If toxicities or viral failure develops that requires changing one of these drugs, then the drug will be changed according to the local guidelines[50] and the child will remain in the study. Other than the substitution of EFV for LPV/r in the experimental arm, clinical management of the children will follow standard local guidelines.[50] Dosing for the ARVs will follow standard guidelines based on body surface area calculations or on body weight depending on drug, and will be in accordance with the South African treatment guidelines and where necessary be updated in line with WHO recommendations. [50] Should a child require treatment for TB whilst on the study, the child will remain in the study and if in the intervention (EFV) arm will continue with study drugs. If in the control group, doses of RTV will be increased to ensure "boosted LPV/r" as per local guidelines. [50] Measurements of ALT should be done before TB treatment and ARV regimen change or if not possible, as close to the start of TB treatment as possible. Measurements of ALT should be repeated after 2, 4, and 6 months after TB treatment initiation.

Children randomized to switching to EFV, will be maintained on the EFV-containing regimen up to 48 weeks post-randomization unless toxicity to EFV develops (described in more detail below) or unless there is evidence of virologic failure. These treatment safety criteria will be put in place to ensure that children do not remain in the EFV switch arm if there is any evidence of treatment failure (unrelated to adherence). In this way, the close monitoring provided by the study ensures the safety of this group. Children in both groups will have viral load measured on the schedule described below. The usual turnaround time of viral loads from the laboratory is 1 week. For any viral load received >1000 copies/ml the child will be called in to the site by phone or through home visit if unreachable by phone. An extra blood draw for viral load will be conducted at this unscheduled visit to rule out laboratory error. At this visit, the study team will investigate with the mother possible lapses in adherence and re-emphasize the importance of adherence. A visit 4 weeks from the "special visit" will be scheduled at which time the viral load will be repeated. If a laboratory error was ruled out for the first measurement and the viral load has not substantially declined and the staff feel confident that the care-giver is adherent, then the child will be switched back to the original LPV/r-based regimen. For children where adherence problems and treatment interruptions are clearly identified as the explanation for the elevated viral load, close monitoring with at least 4 weekly viral load measurements will be done and the child returned to the LPV/r-based regimen after no longer than 3 months has elapsed if viral load does not decline below 50 copies/ml. HIV-infected children in the EFV -switch arm will be considered as candidates to change therapy back to a LPV/r containing regimen if they meet the following criteria:

Two or more viral load measures greater than 1,000 copies/ml on sequential protocol-scheduled blood draws at least 4 weeks apart, and
Viral load does not respond to adherence counseling during a follow up time of no longer than 3 months after the first viral load > 1,000 copies/ml
HIV-infected children in the LPV/r arm will be candidates to change therapy to another regimen if criteria for a switch according to the local guidelines are met.[50]

Toxicities / other clinical management issues: The study protocol will follow the DAIDS (2004) Toxicity Tables (Appendix A) to grade adverse reactions as well as toxicity markers in terms of abnormal lab results. This will be used in conjunction with a toxicity standard operating procedure (SOP) that would guide and inform decisions relating to when to start, when to stop or substitute drugs. Children will be assessed at each clinical visit for any visible signs of lipodystrophy or lipoatrophy, complications that may arise from the use of PIs or D4T. All cases of toxicity presumed or confirmed will be discussed as team on the weekly conference call, so as to ensure appropriate management of these cases. Likewise all difficult or complicated clinical scenarios or conditions will discussed as a team.

Adherence counseling: As we will be enrolling participants who have been on treatment and suppressed for some time, we expect that knowledge and adherence will be excellent. However, since most participants will have been part of other programs, all caregivers will undergo mandatory adherence sessions conducted by the pharmacy team. This will involve one or more sessions during which the caregivers' prior knowledge and attitudes towards treatment are discussed as well as expectations. These sessions include a discussion on the caregivers' routine, meal times, work, child's school times, the family's use of traditional medicines, nutritional supplements and immune-boosters. Adherence counseling will also be done by the study doctors and nurses.

At the time of randomization, for both groups, but specifically for those changing their medications, special counseling explaining the changes will be put in place. The pharmacy team will explain the changes to the care-giver and the study coordinator will independently confirm that the caregiver understands the new directions before leaving the clinic. A week after the change, the counselor will call the caregiver to enquire about how the child is doing and to confirm that the new regimen is being given correctly.

As with NEVEREST II, we apply an open door policy whereby the caregiver can call in at any time to report on any concerns that they have or adverse reactions that they or their child is experiencing so that they may be offered prompt assistance. A 24 hour emergency line is available and one of the study staff members is always on call. Throughout the duration of the study, all caregivers will have counseling by the pharmacy staff whenever the need arises as warranted by either a poor drug reconciliation indicating poor adherence or where there is failure to suppress virus. These ad hoc adherence counseling sessions will be conducted by the pharmacy team or by any other member of the study team that the PI has delegated such a responsibility. These cases of poor adherence would be the subject of study team discussion at the weekly team meetings.

Follow-up schedule
The study will follow children to 48 weeks after randomization M. We select this period of follow-up to address the primary specific aims relating to the durability of viral suppression. If underlying drug NNRTI resistance or difference between the regimens in pharmacokinetics will compromise the durability of viral suppression we anticipate that it will be evident within the first

24 weeks post-switch. Later breakthrough viraemia is more likely to be due to social circumstances interfering with adherence. We extend the follow-up to 48 weeks consistent with other protocols and in case our assumption about the rapidity of viral rebound is incorrect.

	Screen	Follow-up ABC random	4 w	8 w EFV random	4w	8w	16w	24w	32w	40w	48w
Viral load	X			X	X	X	X	X			X
CD4 %/count		X						X			X
FBC/diff		X							X		
ALT	X								X		
Metabolic*	X			X						X	
Storage**	X	X		X	X	X	X	X	X	X	X
Drug levels	X	X		X	X	X	X	X	X	X	X
Drug resistance					All Failure i.e. VL >1000 copies/ml						

* HDL, LDL, TG, glucose/insulin, CRP (must be done fasting for at least 6 hours, preferably overnight – glucose will be done in the clinic) Other parameters may be done later on stored samples if funds permit

** Bloods may be more frequent if viral load is elevated and confirmatory tests have to be done. All bloods collected should be sent for storage in addition to the required tests. Both plasma and buffy coat will be stored. These samples will be sent for drug resistance and drug levels if funds permit. If funds permit, other tests to understand immunologic, virologic or genetic factors involved in HIV disease and its treatment may be conducted on these samples at a later date.

Shaded are visits that may occur on the same day i.e. be the same visit

Laboratory testing and management of specimens

We will contract with a clinical or research laboratory to process and store the samples on the schedule described above.

Specimen collection and processing: 7-10ml of venous blood will be collected into appropriate blood collection tubes specific to each visit contained in the pre-packaged study blood sampling kit. The date and time of blood collection will be recorded. The date and time that the last dose of antiretrovirals were consumed will be recorded. For the visits involving fasting bloods, the date and time of last consumption of any foods will be recorded. Blood collected at every visit will be separated into plasma and a buffy coat for storage at -80 degrees Celsius. CD4 counts, Alanine transaminase (ALT), full blood counts with differential (FBC/diff) and drug levels will be done at the visits indicated above. In case of remaining blood after conducting all the planned tests the remainder will be stored additionally to the primarily planned storage volume. Instructions will accompany each specimen concerning what tests and procedures are needed. For the screening and W8PRA and 40 week visit, the blood be collected after at least a 8 hour fast. Caregivers will be explained the need for fasting blood and will be asked to either bring their child in the morning without breakfast (i.e. overnight fast) or if that is not possible then to give a small breakfast as early as possible and blood will only be drawn 8 hours after the time the caregiver reports the child last had something to eat. Ideally, no food or caloric drinks should be consumed 8 hours before the blood draw. Visits will be scheduled as early in the morning as possible and care-givers will be encouraged to bring the child after an over-night fast. If, however, it is known that the child has consumed a meal before the blood draw, the clinical staff will at their discretion either wait for at least 4 hours to pass before taking the blood, will re-schedule the blood draw or will not undertake the glucose and insulin measurements.

Lab transport system and timing**:** Specimens will be collected at the study site and within 4 hours of blood collection samples will be transported by road to the laboratory. This service will be specifically scheduled for this study. The last courier will collect samples from Coronation hospital no later than 15h30 to ensure enough time for specimen processing. A specimen transport log will be completed at the study site and the courier will check and sign the log to

ensure all specimens are received. The specimens will be processed according to the specimen labeling forms that will contain specific instructions for each visit. The study site will be notified immediately of any problems with specimens (e.g. clotted blood, spillage, laboratory accidents, etc) in order to maximize the chances of re-bleeding the patient and keeping to the study protocol.

Should problems be experienced with assays (e.g. faulty instruments, quality assurance problems, etc) that necessitate additional blood samples, this will be communicated to the clinical site as soon as possible. Additional plasma will only be retrieved from storage for viral load assay testing once cleared with the clinical site (each problem will be reviewed to decide based on the circumstances, the patient history and stage of the study protocol whether additional blood needs to be drawn and/or whether stored sample can be used for testing).

Specimen labeling and forms to go to the lab**:** Prepackaged specimen packs tailored to each study visit and containing carbonized request forms and blood collection tubes, all of which will be pre-labeled with bar-coded labels, will be supplied. A copy of the request slip will remain on site as a record and for tracking purposes.

Reporting of results from laboratory to study site: Urgent results and serious adverse events will be reported telephonically to the project coordinator within an hour. Hard copies of routine results will ordinarily be available within 3 days of receiving the specimen and will be hand delivered to the study site. These results will be reviewed by the responsible doctor, placed into the clinical file and will also be entered into the study database.

Storage and shipping logistics: Plasma and buffy coat samples will be stored over the full duration of the study and beyond or until needed using the LDMS system in freezers that have back up energy supplies and are linked to a security company in the event of a power failure. When stored specimens are needed for testing, the study coordinator will generate a list of which specimens are needed (based on STUDYID, type of specimen (e.g. plasma or buffy coat) and which visit the specimen was collected at, the date and laboratory specimen number) and they will be located and sent to the relevant laboratory including Dr. Lynn Morris' laboratory at NICD for resistance testing or Dr. Maartens laboratory at UCT for drug level measurements.

Viral load**:** HIV-1 RNA quantity in plasma will be measured at the visits as outlined above. Patterns of virologic response to treatment will be analyzed in detail and will be the primary study end-points. We have been notified that that the Roche Amplicor VL test kits version 1.5 for use with sub-type C infections will be discontinued by 2010. We plan to utilize the primary platform that will be used for viral determination after 2010 as soon as the study begins to strive to have all viral load results conducted using the same assay. Research at the University of the Witwatersrand is comparing various high through-put assays.[128]

CD4 T-cells: Panleucogating, done at CLS will be used to enumerate CD4+ T cells using flow cytometry. For interpretability, both the CD4 count and the CD4 percent will be reported out.

Metabolic measurements: We will measure a variety of metabolic parameters as funds become available such as total cholesterol, LDL, HDL, Triglycerides, apo-lipoprotein A, insulin and glucose and C-reactive Protein (CRP) at the times indicated above. Glucose will be measured in the clinic using the bed-side machine currently available.

Other clinical and covariate information: During screening, a detailed history will be taken from the care-giver and a study-specific questionnaire will be administered to collect clinical and social data. The child's medical records will be sought and abstracted onto study forms including information about pre-treatment viral loads, CD4 counts and exposures to and response to ART. At each clinical encounter, the child's weight (Kg) and height (cm) will be measured and a routine measurement of body temperature (^{0}C via tympanic membrane scan). The child and caregiver will be seen by one of the study doctors. Each consultation will involve an enquiry on the child's general health and specific problems if any since the preceding visit. The history taken during this session will include concomitant medications, specific complaints, adverse reactions, other health care visits, consultations, or hospital admissions and use of any alternative therapies e.g. herbal or health tonics, traditional medication or "immune boosters." The child will be examined thoroughly and results will be recorded on standardized study forms as well as in detailed notes in the source documentation. Prior to the care-giver leaving the clinic, the study coordinator will review all the forms to ensure that they are complete.

At targeted intervals, we will include a set of questions about possible EFV side effects.

Fat distribution measurements: At screening and at W8PRA and W40PRM, a detailed body composition examination will be done. This will include measurements of mid arm circumference, triceps skin fold, sub-scapular skin fold, waist circumference, supra iliac skin fold, mid thigh circumference and mid thigh skin folds. We will also conduct a bioelectrical body composition analysis using a small BIA device. This is a hand-held machine that poses no risk to the patient and can be read on-site. These measurements will done in a systematic and standardized way, repeated for each measurement and if found to be discrepant, repeated again and an average taken. A special work sheet will be developed for this purpose.

Maternal/care-giver health: At screening, detailed information about maternal or care-giver health will be collected. This will include whether on antiretroviral therapy, CD4 counts, body mass index, mid-upper arm circumference.

Adherence

Our study will be enrolling a cohort of children who have "undetectable" viral loads implying adequate adherence prior to enrollment. However, difficulties with treatment fluctuate over time as serious social events interfere with domestic routines of the family. From NEVEREST II it seems that 'catastrophes' e.g. care-giver deaths, death of extended family members, relocation, loss of job, pregnancy, domestic violence can upset otherwise strictly adherent patients. In the new study we propose to take a broader view of adherence and have included Dr. Claude Mellins, a social scientist with considerable experience in measurement of these issues, as part of the study team. We will continue as we have done in the past with reconciliations of medicines dispensed and brought back at each study visit. This is complicated in children as it requires weighing of syrups. The pharmacist is experienced with this method of reconciliation, but it relies on the care-givers returning with all medicine bottles. We have found that when problems arise care-givers start forgetting to bring their remaining bottles. The age of the children enrolled in this study means that we will be including children at the age during which they transition from syrups to tablets. We will work with caregivers to make this transition when developmentally-appropriate. Pharmacy reconcilations will involve simple pill counts once the transition is made. We will record on the data forms which formulation of the medications the child is receiving. We will also continue asking standardized questions about adherence. We have adapted the questionnaire used in the IMPAACT pediatric clinical trials but we find that patients seldom report problems with adherence unless confronted with elevated viral load

results. We therefore propose to add in the new study a more general focus on changing social and domestic circumstances, particularly who is responsible for giving the child medication, living arrangements, travel and family deaths, care-giver illness and pregnancy, employment and financial status. These quantitative instruments will be used at randomization and at each study visit. We will also ask about barriers to adherence. For children who experience an elevated viral load and are recalled for repeat testing and additional counseling will have additional behavioral and social data recorded.

Antiretroviral drug concentration measurements

We plan to measure ARV concentrations at scheduled visits from week 0 to 24 for 2 primary reasons: 3TC concentrations will be used as a proxy measure of adherence; EFV and LPV concentrations (in the intervention and control arms respectively) will be evaluated as determinants of the maintenance of viral suppression. Taken together with the behavioral and virologic data we plan to utilize the measurements of drug concentrations to investigate possible explanations for the success or failure of the simplification strategy.

A 1 mL blood sample,which is part of the 7-10ml sample that drawn, will be collected at routine monthly clinic visits from week 4 to 24. The exact time of taking the sample will be noted and care givers will be asked when the last dose was administered. Concomitant medication will be recorded at each PK sampling. After centrifugation, the plasma will be stored at -80° C before transfer in batches to the Division of Clinical Pharmacology at the University of Cape Town for analysis. Validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) methods will be used to determine the ARV concentrations. The laboratory participates in the International Inter-laboratory Control Programme of Stichting Kwaliteitsbewaking Klinische Geneesmiddelanalyse en Toxicologie (KKGT; Netherlands) on an ongoing basis.

3TC concentrations will be evaluated against time-adjusted reference concentrations (based on a population model of 3TC concentrations from prior PK studies in > 100 South African children after observed treatment doses). Children with a 3TC concentrations $<5^{th}$ percentile will have a low probability of adherence to the previous dose. This pharmacokinetic measure of adherence will be compared to the measures of adherence based on sub-therapeutic concentrations of LPV or EFV, caretaker report and pharmacy reconciliations to determine the best predictor of virologic rebound. The proportion of children with low probability of adherence to the previous dose will be compared between the 2 study arms. Lastly, 3TC concentrations will be used to interpret EFV and LPV concentrations: children with time-adjusted 3TC concentrations $<5^{th}$ percentile of the model values will be assumed to have compromised treatment adherence.

Population PK models (using as a basis existing data from PK studies in > 70 children) will be used to predict individual estimates LPV exposure over 12 h area under the curve (AUC) and trough LPV concentrations. LPV AUC and trough concentrations, as well as EFV mid-dose interval concentrations in each treatment arm respectively, will be compared between children who remain virologically suppressed and those with viral rebound. The proportion of children (all children, and those who adhered to the last treatment dose based on 3TC concentration, respectively) with LPV or EFV concentrations lower than recommended therapeutic ranges will be described. We will conduct these studies if funds permit.

Drug resistance testing

Participants will be recruited into the study when they are already suppressed on treatment. Due to the low viral load levels pre-treatment samples will therefore not be available for resistance testing. For those children who are eligible for this study who were also in

NEVEREST II, drug resistance tests pre-treatment will already be completed. There are as yet no assays to investigate whether there might be archived resistance in children who are otherwise suppressed. However, we plan to store pre-randomization samples to be tested if these methodologies become available. We will test post-randomization viral rebound samples for drug resistance i.e. those >1000 copies/ml on at least 2 consecutive occasions. We focus on these viral rebound samples as viral amplification is generally too inconsistent to be used for research purposes if VL is <1000 copies/ml. We will select the first available rebound sample for testing. We have observed in our adult study (NEVEREST I) that viral drug resistance mutations in "failing" samples differ depending on whether or not women were exposed to SDNVP. We will use standard genotyping methods (i.e. population sequencing) to detect viral mutations in reverse transcriptase (RT) and protease (PR) and the Stanford HIV database and the IAS mutation list for interpretation. These assays detect viral mutations if present in >20% of the viral population and are generally adequate for post-treatment failing samples where minority variants are not the focus on interest. These assays are routinely performed in Dr. Lynn Morris' laboratory in South Africa, a laboratory that is also a reference laboratory on drug resistance for the region and which participates in the NIH quality assurance programs including those accrediting drug resistance assays. These assays perform well on subtype C-infected samples (the most common subtype in South Africa).

Viral RNA will be isolated from plasma using a MagNa Pure LC Total Nucleic Acid Isolation kit on the MagNa Pure Automated System (Roche Diagnostics, Indianapolis, IN). Nested PCR products spanning the protease gene and 309aa of the reverse transcriptase gene will be generated using in-house methodology. These PCR products will be sequenced using BigDye Terminators v3.1 on an ABI3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Genotypic resistance is defined as the presence of resistance mutations associated with impaired drug susceptibility or virologic response, using the Stanford Genotypic Resistance Interpretation Algorithm (http://hivdb.stanford.edu/pages/algs/HIVdb.html). Consensus sequences are aligned and manually edited using the Sequencher v4.5 software (GeneCodes, Ann Arbor, MI), and phylogenetic analysis of nucleic sequences performed using PHYLIP. Drug resistance testing will not necessarily be conducted in real time and if viral failure, described as above occurs, the switch of the regimen if necessary is independent on drug resistance testing. Drug resistance studies will only be done if funds permit.

Study endpoint: This study has two primary endpoints: HIV RNA >50 copies and two or more sequential viral loads >1000 copies. 24 weeks is selected as the first time point to examine these endpoints and the study continues through 48 weeks to determine the persistence of any effects.

Post Trial

On completion of the study, patients will be referred into routine care provided by the site the child was originally referred from or by one of the provincial ARV rollout sites. Study personnel will review the participants' circumstances and consider the best regimen to advise the routine services to continue. Children who have a viral load >50 copies/ml at week 48 will be recalled at week 52 to confirm the elevation but will not be followed further thereafter.

Data storage and management

We have in place a system of data checking and cleaning prior to data entry by the project coordinator. All clinical, laboratory and other data will be recorded on study forms. A full time data clerk enters the data on-site usually within one or two days of the visit into a user-friendly ACCESS database with front-end checking for illogical and out of range values. Once data are

entered, the database is emailed in a password protected form weekly to the team in New York where regular checking for completeness and internal consistency is done. Queries are returned to the data clerk who reviews the original forms with the project coordinator and makes necessary corrections.

Sample size calculation: We calculate the required sample size of the trial to address the two primary specific aims of the trial: (1) to examine whether the switch strategy has equivalent durability of virologic suppression <50 copies/ml; and (2) whether the switch strategy has no increased risk of viral rebound (two concurrent viral load measurements >1000 copies/ml). Based on the results of NEVEREST II, we anticipate that the first outcome will be very common. In NEVEREST II, we have observed that 55% of children in the two groups combined (since there were no significant differences between the groups if not stratified by age) have low level viraemia (>50 copies/ml) within the first 48 weeks post-randomization. The second outcome is less common with 4% of children in the control arm displaying this outcome. For calculation purposes, we elect to use sample size calculation methods based on simple comparison of proportions.[129] As we describe below, the primary analyses will mostly make use of Kaplan-Meier life-table methods. However, the comparison-of-proportion methods are simpler and allow for more straight-forward assumptions for sample size calculations. The results are more conservative (i.e. demand a slightly larger sample size). We assume one-tailed tests (as this is an equivalence study and we do not expect the intervention to be better than the standard of care for these outcomes), set significance level at 5% and power at 80% by convention. For the first specific aim, we consider what percentage of children with viral loads >50 copies/ml we would consider "equivalent" to that in the control group (i.e. the difference between the proportion in the intervention group (p1) and the proportion in the control group (p2) = p1-p2). With a sample size that exceeds 135 in each group, for aim 1 (p1-p2)=0.15 or, in other words, we consider a percentage of 70% or lower as equivalent to 55%. For aim 2, with a sample size that exceeds 135 in each group, (p1-p2)=0.07; essentially we consider a percentage of 11% or less in the intervention group to be equivalent to 4% in the controls. Assuming that we obtain 90% follow-up, the sample size required is 150 per group with roughly the same number in each group n=300. We assume we will need to enroll and screen 400 HIV-infected children to yield the required sample size. If the proportion who are eligible for the study is less than anticipated then we will continue screening until the required sample size of 300 is reached.

Analysis plan for primary & secondary endpoints: The analysis plan for the primary specific aim will follow "intent-to-treat" practices comparing children by the group to which they were assigned regardless of whether or not they actually changed their regimen and remain on it. The study endpoint for the first specific aim is the first plasma viral load >50 copies/ml post-randomization. The analysis will examine time to non-suppression (>50 copies/ml) up to 48 weeks post-randomization using Kaplan-Meier life-table methods and comparing across the two groups using log-rank tests.[130] For those meeting this endpoint, time will be taken as the first viral load test >50 copies/ml and for those not meeting this endpoint, time will be censored at the last available study visit or at 48 weeks if the participant completes the study. If endpoints are not reached and the study participant does not complete the study, the reason for failing to complete the study will be reviewed. Secondary sensitivity analyses will be done considering all these events as either censored or all as failure events to investigate their effect on the analysis. Other secondary analyses to address this specific aim, will examine viral loads >50 copies/ml at all the measured time points through 48 weeks, and compare the two groups in the proportions with non-suppressed virus using generalized estimated equations (GEE).[131] If there is evidence of imbalance between the two groups pre-randomization, then multivariable analyses using Cox Proportional Hazards models in the first approach or GEE models in the second will be done.

The study endpoint for the second specific aim is two sequential plasma viral load measurements >1000 copies/ml post-randomization. The analysis will examine time to viral rebound using Kaplan-Meier life-table methods and log-rank tests as described above. For those meeting this endpoint, time will be taken as the second viral load test >1000 copies/ml. Secondary analyses will investigate predictors of this endpoint and will also investigate whether there are subgroups among which this endpoint is more common, e.g. age at randomization, age at initiating antiretroviral therapy, CD4 count etc.

For the secondary specific aims, including immunologic preservation and clinical recovery, drug-related toxicities, and adherence we will compare the proportions with the outcomes of interest across the groups comparing categorical variables using chi-squared tests and continuous variables using Wilcoxon tests or t-tests as appropriate. All analyses will be done in SAS (Cary, NC) at Columbia University under the supervision of Dr. Wei-Yann Tsai.

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Minutes for NEVEREST 3 DSMB Meeting

Date: September 18, 2012

DSMB:
Avy Violari, Brian Eley, Mary Glenn Fowler, Carl Lombard, Peter Havens, Paul Palumbo; Regrets: Mark Cotton

NEVEREST:
Louise Kuhn, Ashraf Coovadia, Renate Strehlau, Leigh Martens, Francoise Pinillos, Wei-Yann Tsai, Stephanie Shiau, Elaine Abrams, Alison Zerbe

NICHD:
Lynne Mofenson, Yasaman Shirazi

Three main agenda issues: 1) regularly scheduled NEV3 DSMB meeting (n = 276 with 192 at 24 week follow-up); 2) evaluation of non-inferiority status; 3) address future strategy.

A brief update was provided by Dr. Kuhn and the team. At the time of the DSMB report there were 276 children enrolled with 248 reaching the primary randomization and 192 having completed a 24 week time point visit. An interim analysis has been performed and was reported by Dr. Kuhn which demonstrates statistically significant ($p<0.0001$) support for non-inferiority based on an equivalence criterion of 15%.

Summary of discussion and recommendations:

1) No safety concerns were identified.
2) Enrollment has been good during the last year – only approximately 20 more to reach n = 300.
3) This second interim analysis to evaluate non-inferiority supports such ($p < 0.001$) as well as the pre-defined "stopping rules".

Specific questions proposed by the study team were addressed:

1) How to proceed with participants who have not yet reached Randomization M? The DSMB reached a consensus and supports continuing enrolment to completion (n = 300) and following each individual to 48 weeks post-Random M (anticipated end of study with this plan is estimated to be end of calendar year 2013). Given that there are no perceived safety risks and the treatment strategies appear non-inferior, there does not appear to be participant risk to study continuation. There may well be strengthening of the non-inferiority findings which will be important for determination of and informing public health policy. The possible trend observed in the 'ever VL > 50 copies' K-M analysis may undergo clarification with larger participant numbers and longer follow-up. Additional long term follow-up will be accomplished by rolling over study participants into a recently funded study specifically for this follow-up.

2) Recommendations for treatment regimens for those who have and will complete the study? The DSMB recommends that the study team maintain their current approach, ie to refer children to routine local services advising that they remain on their current treatment regimen until clinical indications require a change. National Guidelines should be followed when feasible, eg switching d4T to abacavir when the latter drug is reliably available.

The DSMB also noted/discussed that the study team employed an equivalence criterion of 15% (10% when the event rates in the kaletra treatment group are <0.1). It may ultimately be useful to evaluate equivalence criterion of 10% or less.

The DSMB supports sharing the NEVEREST 3 Interim Findings with the WHO Guidelines group and other guidance groups in a confidential (non-public) fashion. The DSMB does not encourage or support public dissemination of interim findings and, rather, recommends waiting for final results before public presentation and sharing of the data.

Minor items:

- For Table on page 6, the probabilities for '> 1000 copies/ml twice' appear inverted; please consider adjusting
- For Table 2 [Events post- randomization M]: consider displaying a 'cumulative Grade III/IV event rate' and a summary measure across the 2 treatment groups.

The DSMB wishes to congratulate the study team on a remarkable accomplishment to date and the generation of findings of clinical import.