COMPARISON OF VIROLOGICAL RESPONSES OF CHILDREN COMMENCED ON AN ABACAVIR VERSUS STAVUDINE BASED ANTIRETROVIRAL REGIMEN AT KING EDWARD VIII HOSPITAL, DURBAN.

By

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As the candidate's supervisor I have approved this thesis for submission

Signed:_____ Name: Dr M Archary Date: July 2016

Date of submission: July 2016

Declaration

I Stephane Montgomery declare that:

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Overview of thesis

The Joint United Nations Programme on HIV/AIDS (UNAIDS) estimated that in 2014 just over 160 000 children in South Africa were receiving highly active antiretroviral treatment(HAART), accounting for 20% of the global HAART cohort. Finding the appropriate HAART regimen that is safe, well tolerated and efficacious is of extreme importance in ensuring continued and ongoing success of the Paediatric HAART program. In 2010 the World Health Organisation(WHO), due to concerns of short and long term stavudine(d4T) toxicity changed the recommendation regarding first-line HAART regimen from a stavudine based regimen . In South Africa, an Abacavir (ABC) based regimen was chosen as the preferred background regimen. However questions have been raised as to whether this change has replaced the safety concerns associated with stavudine with a less efficacious regimen. A retrospective chart review was conducted to evaluate the virological responses at 6 and 12 months post HAART initiation in an abacavir cohort at King Edward VIII hospital between January 2012 – December 2012. Data of 94 children under the age of 12 years who were initiated on an abacavir and lamivudine with either lopinavir/ritonavir or efavirenz regimen (abacavir cohort) were analysed using Fisher's exact test and logistical regression to evaluate virological suppression at 12 months. The data was compared to a prior retrospective chart review conducted between 2004 – 2010 at King Edward VIII Hospital during which stavudine and lamivudine with either lopinavir/ritonavir or efavirenz regimen (stavudine cohort) was the standard of care. The primary objectives were to describe the demographic characteristics (age, sex, WHO stage), baseline characteristics (CD_4^+ count, viral load, diagnosis of tuberculosis (TB), HAART regimen) and the virological responses at six months and 12 months in children in the abacavir cohort and to compare these to children in the stavudine cohort. It is hoped that this information can be used as an adjunct to other studies that have been done at other centres in South- Africa, to support a safe and efficacious HAART regimen for children.

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Glossary

ABC	Abacavir
AIDS	Acquired immunodeficiency syndrome
CD4	Cellular differentiation
D4T	Stavudine
EFV	Efavirenz
FTC	Emtracitibine
HAART	Highly active antiretroviral therapy
HAZ	Height-for-age z score
HIV	Human immunodeficiency virus
HLA	Human leucocyte antigen
LPV/r	Lopinavir/Ritonavir
NRTI	Nucleoside reverse transcriptase inhibitor
NNRTI	Non nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
PMTCT	Prevention of Mother-to-child transmission
PENTA	Paediatric European Network for the treatment of AIDS
PI	Protease inhibitor
RCT	Randomised controlled trial
RNA	Ribosomal nucleic acid
RTV	Ritonavir
TAM	Thymidine analogue mutation
TDF	Tenofovir
UNAIDS	Joint United Nations program for HIV/AIDS
VL	Viral load

WHO	World Health Organisation
WAZ	Weight-for-age z score
3TC	Lamivudine

Abstract:

Background: UNAIDS estimated that in 2014 just over 160 000 children in South Africa were receiving HAART, accounting for 20% of the global HAART cohort. Finding the appropriate HAART regimen that is safe, well tolerated and efficacious is of extreme importance in ensuring continued and ongoing success of the Paediatric HAART program.

In 2010 the World Health Organisation, due to concerns of short and long term stavudine toxicity changed the recommendation regarding first-line HAART regimen from a stavudine based regimen. In South Africa, an abacavir based regimen was chosen as the preferred background regimen. However questions have been raised as to whether this change has replaced the safety concerns associated with stavudine with a less efficacious regimen.

Method: A Retrospective chart review was conducted to evaluate the virological responses at 6 and 12 months in a cohort of children initiated on an abacavir based regimen at King Edward VIII hospital between January 2012 – December 2012. Data of 94 children under the age of 12 years who were initiated on abacavir and lamivudine with either lopinavir/ritonavir or efavirenz regimen (abacavir cohort) were analysed using Fisher's exact test and logistical regression to evaluate virological suppression at 12 months. The data was compared to a prior retrospective chart review conducted between 2004 – 2010 at King Edward VIII Hospital during which a stavudine and lamivudine with either Lopinavir/ritonavir or efavirenz (stavudine cohort) was the standard of care.

Results: In both the abacavir cohort and stavudine cohort there was no difference in gender distribution and the mean age of initiation was 6years. In the abacavir cohort, 62,8% were initiated on ABC/3TC/EFV and 37,2% on ABC/3TC/KAL. 88,4% were initiated on D4T/3TC/EFV and 11,6% were initiated on D4T/3TC/KAL in the stavudine cohort.

The virological suppression rate in the abacavir cohort was 80.7% compared to 85.2% in the stavudine cohort, which was not a significant difference (p= 0,38:). In the abacavir cohort there was no statistical significant difference in virological suppression between patients on efavirenz versus lopinavir/ritonavir (p= 0.427:).

Conclusion: This study demonstrates that children treated with an abacavir based regimen have a good probability of virological suppression, and there was no statistical difference between patients initiating an abacavir-based regimen versus a stavudine based regimen. These findings are in keeping with data from several clinical trials and support the WHO recommendation of an abacavir-based regimen for infants and children initiating antiretroviral treatment.

Part 1 Literature Review

Objective

The objective of this literature review was to assess virological responses in children on different HAART regimens in order to determine which HAART regimen is the most safe and effective regimen to use in children.

A literature review was performed to address the following specific questions:

1. Is HAART effective in children in reaching virological suppression?

2. Which NRTI regimen will be the most efficacious for the HAART regimen in children?

3. Which NNRTI will be the most efficacious for the HAART regimen in children?

Search Strategy

A broad search was conducted on a number of database platforms:

- Pubmed
- The Cochrane database

Search terms used included MeSh terms and free text:

- HIV, Child, paediatric, Virological responses, abacavir, stavudine, NRTI regimen, Efavirenz based regimen, Protease inhibitor regimens.

The bibliographies of relevant articles were also searched for additional papers. The search was initially done in October 2013 and repeated again in June 2016 prior to this submission.

Acceptable studies/inclusion criteria

Population: Adults and children

Intervention: Antiretroviral regimens

Comparator: Different HAART regimens

Outcome: Treatment outcome- Virological response

Results of search:

There were several clinical trials that have been conducted to determine optimal ART regimens with careful attention being given to evaluating the efficacy of protease inhibitors (PI) versus non-nucleoside reverse transcriptase inhibitors (NNRTI) for paediatric first line regimens. Relatively little attention has been given to the nucleoside reverse transcriptase inhibitor(NRTI) "backbone" of the regimens.

There were 2 RCT studies, 3 cohort studies and one systematic review that compared an ABC based regimen to other NRTI regimens from paediatric data. There were one multicentre open labelled clinical trial and one meta-regression analysis found comparing ABC based regimen to other NRTI's from Adult data.

There were 3 clinical trials, one secondary analysis and one retrospective cohort study in the paediatric group comparing protease inhibitors vs. NNRTI's.

There were 4 retrospective chart reviews that looked at virological responses in the paediatric group on HAART.

Introduction

Improvement in the prevention of mother to child transmission (PMTCT) program and availability of treatment of human immunodeficiency virus(HIV) infected children has reduced the number of children acquiring HIV infection. In 2013, 200 000 (170 000–230 000) children were newly infected with HIV. This is 43% lower than in 2009. Twenty four per cent of children living with HIV received HAART, based on 2014 Joint United Nations Program for HIV/AIDS (UNAIDS) report.[1] In South Africa 158,539 children are on highly active antiretroviral treatment(HAART), of which 33,5% (53110) live in KZN.[2] In the absence of treatment, Newell et al suggested that without optimal therapy, 52,5% of HIV-infected children living in Africa died by 24 months of age.[3]

The South African National HAART program started in 2004, and the recommended first line paediatric antiretroviral regimen was stavudine, lamivudine with either lopinavir/ritonavir(LPV/r) for children under the age of three, or efavirenz for children over the age of three. In 2010, the South African National HAART guidelines replaced stavudine with abacavir (ABC) in the first line regimen due to concerns of stavudine toxicity, following World Health Organization (WHO) recommendations. The eligibility criteria also changed from based on the patients WHO clinical stage or CD4 count to a treat all strategy for children under five years of age in 2013.[4]

The ultimate aim of HAART is to reach virological suppression, which is defined as a viral load below 50copies/ml. Virological suppression is dependent on a variety of factors such as adherence and acquisition of mutations resulting in drug resistance.[5]

Stavudine has a comparatively higher genetic barrier to resistance than ABC, therefore theoretically a stavudine based regimen should be able to tolerate sub optimal compliance better than an ABC based regimen.[6] Exposure to sub-optimal HAART drug levels results in the virus acquiring mutations conferring resistance to the drug. The common drug resistance mutations to ABC are M184V/I, K65R,L74V. The combination of M184V/I and L74V reduces abacavir susceptibility more than fivefold, whereas the M184V mutation actually reduces the resistance to stavudine.[6] In comparison,

common drug resistance mutations to stavudine are thymidine analogue mutations (TAM) namelyM41L, L210W,D67N,K70R T215Yand K219Q/E. TAMs are involved in resistance to all nucleoside reverse transcriptase inhibitors(NRTIs), except lamivudine (3TC), but the degree of cross-resistance depends on the NRTI considered and the number of TAMs on the virus.

As mentioned earlier, adherence to HAART is essential to prevent drug resistance. Therefore the formulations of the different antiretroviral drugs are an important factor to ensure compliance. ABC is available as a paediatric syrup and tablet and can be stored at room temperature. However stavudine oral solution requires refrigeration after reconstitution. The storage of these drugs is an especially important factor to consider in the setting where most households access to electricity and refrigeration are limited.[7]

Coupled with the ease of administering and storage of treatment, the side effect profile is also of particular concern when considering the ideal regimen for children. Adverse events associated with stavudine include mitochondrial toxicity, peripheral neuropathy, lipoatrophy, pancreatitis, lactic acidosis/severe hepatomegaly, hyperlipidaemia and insulin resistance.Lipodystrophy syndrome refers to peripheral lipo-atrophy, central lipohypertrophy and dyslipidaemia associated with insulin resistance resulting in permanent disfigurement.

In a recent review article, Innes et al described that lipodystrophy syndrome in HIV infected children on HAART was common in patients on didanosine, stavudine or zidovudine. The authors concluded that paediatric dosing of stavudine need to be reduced urgently to minimize the risk of lipodystrophy.[8]

Abacavir is a NRTI with fewer side effects than stavudine. Its only potential dangerous side effect is a hypersensitivity reaction that is more common in the Caucasian population than African population due to the infrequency of the HLA B5701 haplotype in the African population. A study by Walter Hughes et al concluded that abacavir is safe and well tolerated in children and the single ABC related adverse event was the hypersensitivity reaction.[9]

Comparing ABC regimen to other NRTI regimens: Paediatric data

There were 2 randomized controlled trials , 3 cohort studies and one systematic review that compared an ABC based regimen to other NRTI regimens.

The Paediatric European Network for the Treatment of AIDS study (PENTA-5) trial published in the AIDS journal, 2007, main objective was to describe the long-term efficacy over 5 years of regimens including combinations of abacavir, lamivudine and/or zidovudine in previously untreated children. It was a 48-week randomised controlled trial that compared three dual nucleoside reverse transcriptase inhibitor (NRTI) combinations as part of HAART. 128 HAART-naïve children were randomised to zidovudine\lamivudine (n = 36), zidovudine\abacavir (45) or lamivudine\abacavir (47). Asymptomatic children (n = 55) were also randomised to nelfinavir or placebo; all other children received open-label nelfinavir. The median follow-up was 5.8 years. By 5 years, 17 (47%), 28 (64%) and 18 (39%) children had changed their randomised NRTIs in the zidovudine\lamivudine, zidovudine\abacavir and lamivudine\abacavir groups respectively, but 18%, 50% and 50% of these changes were either early single drug substitutions for toxicity or switches with viral suppression (HIV-1 RNA < 400 copies/ml; e.g. to simplify regimen delivery). At 5 years, 55%/32% zidovudine\lamivudine, 50%/25% zidovudine\abacavir and 79%/63% lamivudine\abacavir had HIV-1 RNA < 400/< 50 copies/ml respectively (p = 0.03/p = 0.003). The five year data demonstrated that lamivudine\abacavir is more effective in terms of HIV-1 RNA suppression and growth changes, with lower rates of switching with detectable HIV-1 RNA than zidovudine\lamivudine or zidovudine\abacavir, and they recommended that lamivudine/abacavir should be preferred as first-line NRTI backbone.[10]

Similarly, Musiime et al conducted an open labelled parallel-group, randomised controlled trial between Nov 8, 2010, and Dec 28, 2011,where they enrolled HIV- infected children from Uganda and Zambia. This was the first randomised controlled trial in African children, conducting a head-to-head comparison of the three most relevant NRTIs for paediatric treatment.480 Children were randomised: 156 to stavudine, 159 to zidovudine, and 165 to abacavir. After two were excluded due to

randomisation error, 156 children were analysed in the stavudine group, 158 in the zidovudine group, and 164 in the abacavir group, and followed for median 2·3 years (5% lost to follow-up). 365 (76%) were HAART naive .917 grade 2–4 clinical or grade 3/4.At 48 weeks, 98 (85%), 81 (80%) and 95 (81%) HAART-naive children in the stavudine, zidovudine, and abacavir groups, respectively, had viral load less than 400 copies per ml (p=0·58); most HAART-experienced children maintained suppression (p=1·00). Most HAART-naive children achieved viral load less than 400 copies per mL by 48 weeks, with no differences between randomised groups (p=0·58). Viral load less than 400 copies per ml was maintained at 48 weeks by more than 96% HAART-experienced children (p=1·0).[11] This study concluded that none of the NRTI's were superior and all the NRTI's were capable of producing virological suppression in children.

There were 3 local South African cohort studies conducted to evaluate virological responses in children on abacavir vs. stavudine based HAART regimen. A cohort analysis by Brennan et al had similar findings to Musiime et al. The study participants were initiated in one of 8 HIV clinics in Gauteng and Mpumalanga, South Africa.317 (56.9%) patients initiated stavudine and 240 (43.1%) abacavir. They defined virologic failure as the proportion of participants with a viral load of more than 400 copies/ml after 24 months of treatment. They detected no difference in virologic failure between abacavir regimen and stavudine regimen (RR 1.01; 95 % CI 0.73–1.39; n = 557).However the quality of the evidence for this outcome was considered low.[12]

The other two South African cohort studies showed contrasting evidence to the above clinical trials and cohort study.

Technau and Lazarus et al conducted a retrospective analysis of the virological outcomes among children receiving different starting regimens at Empilweni clinic at Rahima Moosa Mother and Child Hospital (RMMCH), a large paediatric HIV treatment centre in Johannesburg, South Africa. Among 2423 children who initiated HAART at RMMCH from April 2004 until 28 December2011, 2036 (84%) were included and had initiated d4T/3TC+LPV/r (n=672); ABC/3TC+LPV/r (n=192);

d4T/3TC+EFV (n=962) or ABC/3TC+EFV (n=210). The children excluded had initiated other regimens (n=387, including nevirapine, ritonavir, didanosine,zidovudine and 'super-boosted' LPV/r for concurrent rifampicin usage).At both 6 and 12 months, fewer children reached virological suppression and median VL logs were higher in children receiving ABC compared to d4T, in both the EFV and LPV/r treated children. In children treated with LPV/r-based regimens, 71% receivingd4T versus 40% receiving ABC had VL<400 copies/ml at 6 months (p<0.0001). Similarly, in those on EFV, 91% versus 67% had VL<400 copies/ml at 6 months when receiving d4TversusABC (p<0.0001). Time to viral suppression was significantly longer and time to viral rebound (>1000 copies/ml) after suppression shorter in the ABC-treated children for both LPV/r and EFV-based regimens. A stronger association was seen in the LPV/r-based regimens, where children on ABC had an almost 2-fold increased risk of failure to suppress (41% versus21%, log-rank p<0.0001) by 12 months. Children receiving EFV had an almost 2-fold higher risk of rebound by 12 months after first suppression (35% versus 18%, log-rank p=0.0001) if they were on ABC compared to d4T.These data demonstrate that children treated with ABC/3TC had a lower probability of viral suppression at 6 and 12 months and a higher probability of virological rebound than those treated with d4T/3TC in both LPV/r- and EFV-based regimens, even after adjustment for calendar time and other potential confounders.[13]

Similarly, a South African Multi-Cohort Analysis was then conducted by Technau and Schomaker et al evaluating the virological response in children treated with abacavir compared with stavudine-based antiretroviral treatment. Data for 9543 HAART-naïve children <16 years at treatment initiation started on either stavudine/lamivudine (d4T/3TC) or ABC/3TC with efavirenz (EFV) or ritonavir-boosted lopinavir(LPV/r) treated at six clinics in Johannesburg and Cape Town, South Africa, were analysed with Chi-square tests and logistic regression to evaluate viral suppression at six and twelve months. Prevalence of viral suppression at six months in 2174 children started on a d4T-based LPV/r regimen was greater (70%) than among 438 children started on an ABC-based LPV/r regimen (54%, p<0.0001). Among 3189 children started on a d4T-based EFV regimen a higher proportion (86%) achieved suppression at six months compared to 391 children started on ABC containing

EFV regimens (78%, p<0.0001). Relative benefit of d4T vs. ABC on six month suppression remained in multivariate analysis after adjustment for pre-treatment characteristics, cohort and year of program (LPV/r – OR 0.57 [CI: 0.46–0.72]; EFV – OR 0.46 [CI: 0.32–0.65]).They concluded that this expanded analysis is consistent with their previous report of worse virological outcomes after ABC was introduced as part of first-line ART in South Africa.[14]

Adetokunboh et al conducted a systematic review and meta-analysis to determine the efficacy and safety of abacavir-containing combination antiretroviral therapy as first-line treatment of HIV infected children and adolescents. They included two randomised controlled trials (RCTs) and two analytical cohort studies with a total of 10,595 participants. Among the RCTs they detected no difference in virologic suppression after a mean duration of 48 weeks between abacavir- and stavudine-containing regimens (2 trials; n = 326: RR 1.28; 95 % CI 0.67–2.42) with significant heterogeneity (P = 0.02; I2 = 81 %). They also found no significant differences between the two groups for adverse events and death. After five years of follow-up, virologic suppression improved with abacavir (1 trial; n = 69: RR 1.96; 95 % CI 1.11–3.44). For cohort studies, they detected that the virologic suppression activity of abacavir was less effective than stavudine in both the lopinavir/ritonavir (1 study, n = 2165: RR 0.79, 95 % CI 0.67–0.92) and efavirenz sub-groups (1 study, n = 3204: RR 0.79, 95 % CI 0.67–0.92) respectively. The quality of evidence from RCTs was moderate for virologic suppression but low for death and adverse events, while that of cohort studies was low for all three these outcomes. They concluded that available evidence showed little or no difference between abacavir-containing regimen and other NRTIs regarding efficacy and safety when given to children and adolescents as a first-line antiretroviral therapy.[15]

In order to extrapolate from the above results one has to take into account the nature of the studies. The two randomised controlled trials were done in an ideal study setting where patients are closely monitored and followed up, ensuring better compliance to treatment. Whereas the cohort studies are more reflective of the real world environment where compliance maybe suboptimal and drug stock-outs may result in interruptions in antiretroviral treatment. The low genetic barrier of ABC for the

development of drug resistance may therefore predispose patients on an ABC containing regimen with sub-optimal compliance to treatment failure.

Comparing ABC regimen to other NRTI regimens: Adult data

As with the paediatric data, there are also conflicting results in the literature with regards to virological performance of an abacavir based regimen.

DeJesus et al conducted a multicenter, randomized, double-blind non-inferiority clinical trial that compared the efficacy and safety of abacavir with that of zidovudine plus lamivudine and efavirenz in 649 HAART-naive HIV-infected patients. Their primary objective was a comparison of proportions of patients achieving plasma HIV-1 RNA levels<50 copies/ml through week 48 of the study. Their results concluded that 70% of patients in the abacavir group, compared with 69% in the zidovudine group, maintained confirmed plasma HIV-1 RNA levels of <50 copies/ml (in the intent-to-treat exposed population).Virologic failure was infrequent (6% in the abacavir group and 4% in the zidovudine group). There was a significant CD4+ cell response (209 cells/mm3 in the abacavir group and 155 cells/mm3 in the zidovudine group).Safety profiles were as expected. They concluded that abacavir provided an effective and durable antiretroviral response that was non-inferior to zidovudine, when combined with lamivudine and efavirenz.[16]

A meta-regression analysis of 12 clinical trials in 5168 patients was conducted by Hill et al. Their main objective was to determine the efficacy of Tenofovir/emtricitabine (TDF/FTC) vs. abacavir/lamivudine (ABC/3TC) with ritonavir (RTV)-boosted protease inhibitors (PIs).From the 12 clinical trials of 5168 HAART-naïve patients, 3399 patients on TDF/FTC and 1769 patients ABC/3TC was used with RTV-boosted PI. Across all the trials, HIV RNA suppression rates were significantly higher for those with baseline viral load below 100,000 copies/ml (77.2%) vs. above 100,000 copies/ml (70.9%) (P=0.0005). For the trials of lopinavir/ritonavir (LPV/r), atazanavir/ritonavir (ATV/r) and fosamprenavir/ritonavir (FAPV/r) using either TDF/FTC or ABC/3TC, the HIV RNA responses were significantly lower when ABC/3TC was used, relative to

TDF/FTC, for all patients (P=0.0015) and for patients with baseline viral load <100,000 copies/ml (70.1%vs. 80.6%, P=0.0161), and was borderline for those with viral load >100,000 copies/ml (67.5%vs. 71.5%, P=0.0523). They concluded that their analysis suggests higher efficacy for first-line use of a TDF/FTC NRTI backbone with boosted PIs, relative to use of ABC/3TC.[17]

It is difficult to apply adult studies to the paediatric population due to differences in pharmocokinetics and pharmacodynamics, however the meta-regression analysis findings is of concern regarding the efficacy of ABC and high VLs especially as paediatric patients often have higher VL at initiation than adult patients..

Comparing Protease inhibitors to NNRTI regimens: Paediatric data

Results from the previously 2 mentioned South African cohort studies on the comparison of abacavir vs. stavudine, actually found that patients on PI treatment had worse virological performances than patients on EFV.

The PENPACT- 1 trial was the first open label long term randomised trial to compare protease inhibitor(PI) and non-nucleoside reverse transcriptase inhibitor(NNRTI) first line HAART in HIV-infected children. The PENPACT-1 trial assessed the long-term effectiveness of HAART-naïve children from Europe and North/South America initiating 2NRTIs+PI vs. 2NRTIs+NNRTIin a randomised open-label factorial design. The primary outcome was VL change between baseline and 4 years. A total of 266 children (133 Europe, 77 North America, 56 South America) from 68 centres in 13 countries were randomised between September 2002 and September 2005.

266 children were randomised and 263 analysed. The median age was 6.5years; mean(SD), CD4 18%(11); VL 5.1(0.8)log10c/ml. Median follow-up was 5.0years; 188(71%) children were on first-line HAART at trial end. At 4 years, mean VL reductions were −3.16 vs. −3.31log10c/ml for PI vs. NNRTI(difference p=0.26).VL was <400c/ml in 82%PI vs82%NNRTI, p=0.91.There was no difference between initiating HAART with PI or NNRTI-based regimens; both achieved good long-term virological outcomes.[18]

Similar findings were found in the PROMOTE trial where they analysed virologic and immunologic outcomes of HIV-infected Ugandan children randomized to lopinavir-ritonavir or non-nucleoside reverse-transcriptase-inhibitor therapy. Of 185 children enrolled, 91 initiated LPV/r and 92 initiated NNRTI-based HAART. At baseline, the median age was 3.1 years (range: 0.4 to 5.9) and 131 (71%) were HAART-naive. The proportion of children with virologic suppression at 48 weeks was 80% (67/84) in the LPV/r-arm vs. 76% (59/78) in the NNRTI-arm, a difference of 4% (95%CI: −9% to +17%). Time to virologic failure, CD_4^+changes, were similar between arms. They concluded that LPV/r-based HAART was not associated with worse virologic efficacy, immunologic efficacy, or adverse event rates compared to NNRTI-based HAART.[19]

Local South African studies also confirmed that patients initiating a PI regimen showed satisfactory virological suppression rates. Teasdale et al conducted a secondary analysis of data collected during the pre-randomization phase of an HAART strategies trial conducted at a single site in Johannesburg, South Africa. The main objective of the study was to investigate if there is the association between adherence and viral suppression among infants and young children initiated on PI Therapy. By 24 weeks, 197/269 (73%) children achieved viral suppression. This study showed high proportions of viral suppression and medication adherence in this cohort of infants and young children initiating protease inhibitor based antiretroviral treatment in South Africa.[20]

Similarly Jaspan et al conducted retrospective cohort study to evaluate the clinical and laboratory outcomes of 391 children who received protease inhibitor (PI) or non-nucleoside reverse transcription inhibitor (nNRTI)-containing highly active antiretroviral regimens (HAART) from a Cape Town clinic. This cohort achieved a sustained doubling of median CD_4^+% from baseline, steady increase of median WAZ, and survival of 91%, despite only 49% virologic suppression at 24 months. However, when analysed according to regimen, PI-containing regimens had better virologic suppression at all time points. Their findings confirmed that PI regimens achieved greater virologic suppression than nNRTIs.[21]

The PROMOTE and PENPACT-1 trial showed no difference in virological outcomes in initiating with a ritonavir-boosted lopinavir (LPV/r)- vs. nevirapine-based therapy in prophylaxis-exposed children.

In contrast to these above mentioned studies the P1060 study which provided evidence of the superiority of ritonavir-boosted lopinavir–based regimens over nevirapine-based regimens in terms of both efficacy and safety.The P1060 trial was two parallel, randomized clinical trials comparing nevirapine with ritonavir-boosted lopinavir in nevirapine-exposed vs. nevirapine-unexposed, in addition to zidovudine and lamivudine, in HIV-infected, ART-eligible children between 2 and 36 months of age. The randomized trial was conducted in six African countries and India, The primary end point of both cohorts was virologic failure or discontinuation of treatment by study week 24.

The results of cohort 1(nevirapine exposed) showed a total of 164 children were enrolled. The median percentage of CD4+ lymphocytes was 19%; a total of 56% of the children had WHO stage 3 or 4 disease. More children in the nevirapine group than in the ritonavir-boosted lopinavir group reached a primary end point (39.6% vs. 21.7%; weighted difference, 18.6 percentage-points; 95% confidence interval, 3.7 to 33.6; nominal P=0.02). Baseline resistance to nevirapine was detected in 18 of 148 children (12%) and was predictive of treatment failure.

The results of cohort 2 showed a total of 288 children were enrolled; the median percentage of CD4+ T cells was 15%, and the median plasma HIV type 1 (HIV-1) RNA level was 5.7 $\log_{10}$ copies per milliliter. The percentage of children who reached the primary end point was significantly higher in the nevirapine group than in the ritonavir-boosted lopinavir group (40.8% vs. 19.3%; P<0.001). Among the nevirapine-treated children with virologic failure for whom data on resistance were available, more than half (19 of 32) had resistance at the time of virologic failure. In addition, the time to a protocol-defined toxicity end point was shorter in the nevirapine group (P=0.04), as was the time to death (P=0.06).The conclusion of both cohorts was that outcomes were superior with ritonavir-boosted lopinavir among young children. These data support ritonavir-boosted lopinavir as the basis for first-line ART in all children younger than 3 years of age, regardless of whether they have had prior NNRTI exposure.

Factors that may have contributed to the suboptimal results with nevirapine include elevated viral load

at baseline, selection for nevirapine resistance, background regimen of nucleoside reverse-transcriptase inhibitors, and the standard ramp-up dosing strategy. Since nevirapine is used for both treatment and perinatal prevention of HIV infection in resource-limited settings, alternative strategies for the prevention of HIV transmission from mother to child, as well as for the treatment of HIV infection, are urgently required.[22,23]

Virological outcomes of children on HAART: International studies

Several studies were also included to evaluate virological responses in children on HAART. These studies confirm that children had favourable virological responses to HAART.

A multi centre national cohort was conducted to assess the long term virological outcome in children on antiretroviral treatment in the UK and Ireland. Nine hundred and ninety-seven children started HAART at a median age of 7.7 years (inter-quartile range 2.9–11.7), 251 (25%) below 3 years: 411 (41%) with efavirenz and two nucleoside reverse transcriptase inhibitors (EFV+2NRTIs), 264(26%) with nevirapine and two NRTIs (NVP+2NRTIs), 119 (12%; 106 NVP, 13EFV) with non-nucleoside reverse transcriptase inhibitor and three NRTIs(NNRTI+3NRTIs), and 203 (20%) with boosted protease inhibitor-based regimens. Median follow-up after HAART initiation was 5.7 (3.0–8.8) years. Viral load was less than
400 copies/ml by 12 months in 92% [95% confidence interval (CI) 91–94%] of the children. Time to suppression was similar across regimens (P¼0.10), but faster over calendar time, with older age and lower baseline viral load. Their results showed that viral load suppression by 12 months was high with all regimens.NVP+3NRTIs regimens were particularly efficacious in the longer term and may be a good alternative to protease inhibitor-based ART in young children.[24]

Another study done in India , also assessed the immunological, virological and clinical responses to HAART in children. 175 children (boys: 74.9%) were included in the study, with a median follow up of 43 (IQR:17, 68) months. The median age at diagnosis was 119 (IQR: 75, 156) months. The median

CD_4^+ count at start of HAART was 340 cells/μL (IQR: 185,704), which increased to 924 cells/μL (IQR:591,1278) at 48 months after HAART and plateaued at 749 (IQR: 542,1056) cells/ μL after 90 months of therapy. Viral load was available in 76 children. After a median duration of 34.4 months (IQR: 10.4, 47) of HAART the median viral load documented was 400 (IQR:47, 958)RNA copies/mL; 49 (66%) children had undetectable viral load.

The weight for age (WAZ) and height for age (HAZ) z score both showed improvement with time after HAART initiation [baseline: WAZ −2.8 (IQR:-4,-1.6), HAZ −2.1 (IQR:-3.4,-0.69); at 42 months of therapy: WAZ −1.2 (IQR:-2.1, 0.01), HAZ −0.75(IQR:-1.6,-0.37)]. Adverse events were reported in 21 (12%)children. Non-adherence to therapy, treatment failure and death were noted in 35 (20%), 9 (5.1%) and 6 (3.4%) children respectively. This study did not specify the HAART regimens that the children were receiving. They concluded that HAART in HIV-infected children is effective, safe and is associated with good immunological and virological response as well as improvement in growth parameters.[25]

Similarly a study done in Central China concluded that HAART is an effective strategy for inhibiting HIV replication and reconstructing the immunological response in children with AIDS. Twenty-six HIV-1-infected children receiving HAART in Hubei province, China, were enrolled retrospectively in this study. The median duration of HAART was 41 months (18–72.3 months). In children showing clinical improvement, high viral suppression rate below log10 (2.7) copies/ml by the third months of HAART was observed. The median CD_4^+cell counts reached to 820.5/μl by 12 months and the median ratio of CD4/CD8 increased to 0.6 by 21 months. They concluded that HAART is an effective strategy for inhibiting HIV replication and reconstructing the immunological response in children with AIDS.[26]

A local study by Meyer et al also provided reassuring data as their findings demonstrated excellent virologic suppression rates and immunologic and somatic growth responses in children receiving HAART. Their results showed the cumulative probability of achieving a viral load < 400 copies/ml

was 59.4% (95% CI: 57.0%, 61.8%) 6 months after HAART initiation, 84.0% (95% CI: 82.6%, 86.2%) by 12 months, 96.2% (95% CI: 94.4%,96.7%) by 24 months. Children greater than 3 years at HAART initiation (correlating with those who started EFV-based regimens) were more likely to achieve virologic suppression early compared with children younger than 3 years (on LPV/r-containing regimens).[27]

Conclusion:

Abacavir seems to be an ideal NRTI considering its formulation and low side effect profile. However there are conflicting results from the literature regarding the efficacy of an abacavir based regimen in producing virological suppression.

There is currently no data available regarding the virological performance in children on an ABC based regimen in KwaZulu Natal.

The purpose of this retrospective chart review was to compare the virological responses of children commenced on an Abacavir versus a Stavudine based Antiretroviral regimen at King Edward VIII hospital, Durban.

Table 1: Summary of Literature

Study	Setting	Design	Study Population	Outcomes
Virological Outcomes: Comparing ABC regimen to other NRTI regimens: Paediatric data				
Musiime et al(2014)[11]	Uganda and Zambia	• Open labeled parallel • Randomised control trial • Comparing stavudine vs zidovudine vs abacavir as NRTI backbone in NNRTI-based first-line HAART	480 Children	• At 48 weeks, 98 (85%), 81 (80%) and 95 (81%) HAART-naive children in the stavudine, zidovudine, and abacavir groups, respectively, had viral load less than 400 copies per mL (p=0·58) • Most HAART-naive children achieved viral load less than 400 copies per mL by 48 weeks, with no differences between randomised groups (p=0·58). • Viral load less than 400 copies per mL was maintained at 48 weeks by more than 96% HAART-experienced children (p=1·0).
Penta 5 Trial	34 centres in	• 48-week	128	• At 5 years, 55%/32%

(2002)[10]	nine countries	randomised controlled trial • Comparing three dual nucleoside reverse transcriptase inhibitor (NRTI) combinations as part of first triple antiretroviral therapy .	Children	zidovudine\lamivudine, 50%/25% zidovudine\abacavir and 79%/63% lamivudine\abacavir had HIV-1 RNA < 400/< 50 copies/ml respectively (p = 0.03/p = 0.003). • The five year data demonstrate that lamivudine\abacavir is more effective in terms of HIV-1 RNA suppression
Technau and Lazarus et al(2013)[13]	Empilweni clinic at Rahima Moosa Mother and Child Hospital, Johannesburg, South Africa	• Retrospective analysis	2036 Children	• Children treated with LPV/r-based regimens, 71% receiving d4T versus 40% receiving ABC had VL<400 copies/ml at 6 months (p<0.0001). • Similarly, in those on EFV, 91% versus 67% had VL<400 copies/ml at 6 months when receiving d4T versus ABC (p<0.0001). • Time to viral suppression

				was significantly longer and time to viral rebound (>1000 copies/ml) after suppression shorter in the ABC-treated children for both LPV/r and EFV-based regimens • These data demonstrate that children treated with ABC/3TC had a lower probability of viral suppression at 6 and 12 months and a higher probability of virological rebound than those treated with d4T/3TC in both LPV/r- and EFV-based regimens
Technau and Schomaker et al(2014)[14]	Six clinics in Johannesburg and Cape Town, South Africa	• A South African Multi-Cohort Analysis • Retrospective analysis	9543 Children	• Prevalence of viral suppression at six months in 2174 children started on a d4T-based LPV/r regimen was greater (70%) than among 438 children started on an ABC-based LPV/r regimen (54%, p<0.0001). • Among 3189 children started on a d4T-based EFV

				regimen a higher proportion (86%) achieved suppression at six months compared to 391 children started on ABC containing EFV regimens (78%, p<0.0001). • Worse virological outcomes after ABC was introduced as part of first-line ART in South Africa
Brennan et al (2014)[12]	One of 8 HIV clinics in Gauteng and Mpumalanga, South Africa	• Prospective Cohort analysis	317 Children	• They detected no difference in virologic failure between abacavir regimen and stavudine regimen (RR 1.01; 95 % CI 0.73–1.39; n = 557)
Adetokunboh et al(2015)[15]		• Systematic review and meta-analysis	10,595 Children	• Among the RCTs they detected no difference in virologic suppression after a mean duration of 48 weeks between abacavir- and stavudine-containing regimens (2 trials; n = 326: RR 1.28; 95 % CI 0.67–2.42) • For cohort studies, they detected that the virologic

				suppression activity of abacavir was less effective than stavudine in both the lopinavir/ritonavir (1 study, n = 2165: RR 0.79, 95 % CI 0.67–0.92) and efavirenz sub-groups (1 study, n = 3204: RR 0.79, 95 % CI 0.67–0.92) respectively • Available evidence showed little or no difference between abacavir-containing regimen and other NRTIs
Virological outcomes: Comparing ABC regimen to other NRTI regimens: Adult data				
DeJesus et al(2004)[16]	78 sites in the United States (48 sites), Europe (17), South America (9), Central America (2), and Puerto Rico (2)	• Multicenter • Randomized, double-blind non-inferiority clinical trial	649 Adults	• 70% of patients in the abacavir group, compared with 69% in the zidovudine group, maintained confirmed plasma HIV-1 RNA levels of <50 copies/mL (in the intent-to-treat exposed population). • Virologic failure was infrequent (6% in the abacavir group and 4% in the zidovudine group). • They concluded that

				abacavir provided an effective and durable antiretroviral response that was non-inferior to zidovudine, when combined with lamivudine and efavirenz
Hill et al (2009)[17]		• Meta-regression analysis of 12 clinical trials	5168 Adults	• For the trials of lopinavir/ritonavir (LPV/r), atazanavir/ritonavir (ATV/r) and fosamprenavir/ritonavir (FAPV/r) using either TDF/FTC or ABC/3TC, the HIV RNA responses were significantly lower when ABC/3TC was used, relative to TDF/FTC, for all patients (P=0.0015) and for patients with baseline viral load <100,000 copies/mL (70.1%vs. 80.6%, P=0.0161), and was borderline for those with viral load >100,000 copies/mL (67.5%vs. 71.5%, P=0.0523). • They concluded that their

				analysis suggests higher efficacy for first-line use of a TDF/FTC NRTI backbone with boosted PIs, relative to use of ABC/3TC
Virological Outcomes: Comparing Protease inhibitors to NNRTI regimens: Paediatric data				
PENPACT 1 trial(2011)[18]	Europe and North/South America	• Open label long term randomised trial	266 Children	• At 4 years, mean VL reductions were −3.16 vs −3.31log10c/ml for PI vs NNRTI(difference p=0.26) • VL was <400c/ml in 82%PI vs 82%NNRTI, p=0.91 • There was no difference between initiating HAART with PI or NNRTI-based regimens; both achieved good long-term virological outcomes
PROMOTE trial(2014)[19]	Uganda	• Open-label randomized clinical trial	185 Children	• The proportion of children with virologic suppression at 48 weeks was 80% (67/84) in the LPV/r-arm vs. 76% (59/78) in the NNRTI-arm, a difference of 4% (95%CI: −9% to +17%) • LPV/r-based HAART was

				not associated with worse virologic efficacy, immunologic efficacy, or adverse event rates compared to NNRTI-based HAART
P1060 trial (2010,2012)[22, 23]	Six African countries and India	• Open labeled randomised control trial	Cohort 1 164 children Cohort 2 288 children	• Cohort 1:The medianpercentage of CD4+ lymphocytes was 19%; a total of 56% of the children had WHO stage 3 or 4 disease. More children in the nevirapine group than in the ritonavir-boosted lopinavir group reached a primary end point (39.6% vs. 21.7%; weighted difference, 18.6 percentage-points; 95% confidence interval, 3.7 to 33.6; nominal P=0.02). Baseline resistance to nevirapine was detected in 18 of 148 children (12%) and was

				predictive of treatment failure. • The results of cohort 2 showed a total of 288 children were enrolled; the median percentage of CD4+ T cells was 15%, and the median plasma HIV type 1 (HIV-1) RNA level was 5.7 $\log_{10}$ copies per milliliter. The percentage of children who reached the primary end point was significantly higher in the nevirapine group than in the ritonavir-boosted lopinavir group (40.8% vs. 19.3%; P<0.001). • Among the nevirapine-treated children with virologic failure for whom data on resistance were available, more than half (19 of 32) had

				resistance at the time of virologic failure • The conclusion of both cohorts was that outcomes were superior with ritonavir-boosted lopinavir among young children
Teasdale et al (2013)[20]	Johannesburg, South Africa	• Secondary analysis of data collected during the pre-randomization phase of an HAART conducted retrospective cohort study strategies trial	296 Children	• By 24 weeks, 197/269 (73%) children achieved viral suppression. • This study showed high proportions of viral suppression and medication adherence in this cohort of infants and young children initiating protease inhibitor based antiretroviral treatment in South Africa
Jaspan et al (2008)[21]	Cape Town Clinic, South Africa	• Retrospective cohort study	391 Children	• This cohort achieved a sustained doubling of median CD_4^+% from baseline, steady increase of median WAZ, and survival of 91%, despite only 49%

				virologic suppression at 24 months. • However, when analyzed according to regimen, PI-containing regimens had better virologic suppression at all time points. • Their findings confirmed that PI regimens achieved greater virologic suppression than nNRTIs.
Long term virological outcomes of children on HAART: International studies **Paediatric Data**				
Duong et al(2014)[24]	UK and Ireland	• Multi centre national cohort	997 children	• Viral load was less than400 copies/ml by 12 months in 92% [95% confidence interval (CI) 91–94%] of the children. Time to suppression was similar across regimens (P¼0.10) • Their results showed that viral load suppression by 12 months was high with all regimens. • NVP+3NRTIs regimens

				were particularly efficacious in the longer term and may be a good alternative to protease inhibitor-based HAART in young children.
Mukherjee et al(2014)[25]	India	• Retrospective chart review	175 Children	• Viral load was available in 76 children. • The median viral load documented was 400 (IQR:47, 958)RNA copies/mL; 49 (66%) children had undetectable viral load • HAART in HIV-infected children is effective, safe and is associated with good immunological and virological response as well as improvement in growth parameters.
Zheng et al (2014)[26]	Hubei province, China	• Retrospective chart review	26 Children	• In children showing clinical improvement, high viral suppression rate below log10 (2.7) copies/ml by the third months of HAART was

				observed • They concluded that HAART is an effective strategy for inhibiting HIV replication and reconstructing the immunological response in children with AIDS
Meyer et al(2011)[27]	Soweto, South Africa	• Retrospective chart review	2216 Children	• Their results showed the cumulative probability of achieving a viral load < 400 copies/ml was 59.4% (95% CI: 57.0%, 61.8%) 6 months after HAART initiation, 84.0% (95% CI: 82.6%, 86.2%) by 12 months, 96.2% (95% CI: 94.4%,96.7%) by 24 months

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PART 2:A submission ready manuscript.

Title page

Comparison of virological responses of children commenced on an Abacavir versus a Stavudine based Antiretroviral regimen at King Edward VIII hospital, Durban.

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Abstract:

Background: UNAIDS estimated that in 2014 just over 160 000 children in South Africa were receiving HAART. In 2010 the World Health Organisation, replaced stavudine with abacavir in the first line regimen due to concerns of stavudine toxicity. However questions have been raised as to whether this change has replaced the safety concerns associated with stavudine with a less efficacious regimen.

Method: A Retrospective chart review was conducted to evaluate the virological responses at 6 and 12 months in a cohort of children initiated on an abacavir based regimen at King Edward VIII hospital between January and December 2012. Data of 94 children under the age of 12 years who were initiated on abacavir and lamivudine with either lopinavir/ritonavir or efavirenz regimen (abacavir cohort) were analysed using Fisher's exact test and logistical regression to evaluate virological suppression at 12 months. The data was compared to a prior retrospective chart review conducted between 2004 and 2010 at King Edward VIII Hospital during which stavudine and lamivudine with either lopinavir/ritonavir or efavirenz (stavudine cohort) was the standard of care.

Results: In both the abacavir cohort and stavudine cohort there was no difference in gender distribution and the mean age of initiation was 6 years. In the abacavir cohort, 62, 8% were initiated on ABC/3TC/EFV and 37, 2% on ABC/3TC/KAL. 88, 4% were initiated on D4T/3TC/EFV and 11, 6% were initiated on D4T/3TC/KAL in the stavudine cohort.
The virological suppression rate in the abacavir cohort was 80.7% compared to 85.2% in the stavudine cohort, which was not significant (p= 0, 38).

Conclusion: This study demonstrated that children treated with an abacavir based regimen have a good probability of virological suppression. These findings are in keeping with data from several clinical trials and support the WHO recommendation of an abacavir-based regimen for infants and children initiating antiretroviral treatment.

Introduction

Improvement in the prevention of mother to child transmission (PMTCT) program and availability of treatment of human immunodeficiency virus(HIV) infected children has reduced the number of children acquiring HIV infection. In 2013, 200 000 (170 000–230 000) children were newly infected with HIV. This is 43% lower than in 2009. Twenty four per cent of children living with HIV received HAART, based on 2014 Joint United Nations Program for HIV/AIDS (UNAIDS) report.[1] In South Africa 158,539 children are on HAART, of which 33,5% (53110) live in KZN.[2] In the absence of treatment, it is estimated that 10-20% of infants can progress rapidly to acquired immunodeficiency syndrome (AIDS) in the first year of life and 80% of infected infants will demise in first 2 years of life.[3]

The South African National HAART program started in 2004, and the recommended first line paediatric antiretroviral regimen was stavudine, lamivudine with either lopinavir/ritonavir(LPV/r) for children under the age of three, or efavirenz for children over the age of three. In 2010, the South African National HAART guidelines replaced stavudine with ABC in the first line regimen due to concerns of stavudine toxicity, following World Health Organization (WHO) recommendations. The eligibility criteria also changed from based on the patients WHO clinical stage or CD4 count to a treat all strategy for children under five years of age in 2013.[4]

The ultimate aim of HAART is to reach virological suppression, which is defined as a viral load below 50copies/ml. Virological suppression is dependent on a variety of factors such as adherence and acquisition of mutations resulting in drug resistance.[5]

Stavudine has a comparatively higher genetic barrier to resistance than abacavir(ABC), therefore theoretically a stavudine based regimen should be able to tolerate sub optimal compliance better than an ABC based regimen.[6]Exposure to sub-optimal HAART drug levels results in the virus acquiring mutations conferring resistance to the drug. The common drug resistance mutations to ABC are M184V/I, K65R,L74V. The combination of M184V/I and L74V reduces abacavir susceptibility more

than fivefold, whereas the M184V mutation actually reduces the resistance to stavudine.[6]In comparison, common drug resistance mutations to stavudine are thymidine analogue mutations (TAM) namelyM41L, L210W,D67N,K70R T215Yand K219Q/E.TAMs are involved in resistance to all nucleoside reverse transcriptase inhibitors(NRTIs), except lamivudine (3TC), but the degree of cross-resistance depends on the NRTI considered and the number of TAMs on the virus.

As mentioned earlier, adherence to HAART is essential to prevent drug resistance. Therefore the formulations of the different antiretroviral drugs is an important factor to ensure compliance. ABC is available as a paediatric syrup and tablet format and can be stored at room temperature. However stavudine oral solution requires refrigeration. The storage of these drugs is an especially important factor to consider in the rural setting where most households don't have access to electricity.[7]

Coupled with the ease of administering and storage of treatment, the side effect profile is also of particular concern when considering the ideal regimen for children. Adverse events associated with stavudine include mitochondrial toxicity, peripheral neuropathy, lipoatrophy, pancreatitis, lactic acidosis/severe hepatomegaly, hyperlipidaemia and insulin resistance.Lipodystrophy syndrome refers to peripheral lipo-atrophy, central lipohypertrophy and dyslipidaemia associated with insulin resistance resulting in permanent disfigurement.

In a recent review article , Innes et al described that lipodystrophy syndrome in HIV infected children on HAART was common in patients on didanosine, stavudine or zidovudine. The authors concluded that paediatric dosing of stavudine need to be reduced urgently to minimize the risk of lipodystrophy.[8]

Abacavir is a NRTI with fewer side effects than stavudine. Its only potential dangerous side effect is a hypersensitivity reaction that is more common in the Caucasian population than African population due to the infrequency of the HLA B5701 haplotype in the African population. A study by Walter Hughes et al concluded that abacavir is safe and well tolerated in children and the single abacavir related adverse event was the hypersensitivity reaction.[9]

Abacavir seems to be an ideal NRTI considering its formulation and low side effect profile. However there are conflicting results from the literature regarding the efficacy of an abacavir based regimen in producing virological suppression.

The results from the Paediatric European Network for the Treatment of AIDS study (PENTA-5)showed that an abacavir-containing NRTI regimen is more effective than zidovudine/lamivudine regimen and recommended that an abacavir containing NRTI will provide a good NRTI backbone for use with protease inhibitors and non-nucleoside reverse transcriptase inhibitors.[10]
Similarly ,results from a randomised controlled trial of 480 African children that compared zidovudine, ABC and stavudine concluded that most HAART-naive children achieved viral load less than 400 copies per ml by 48 weeks, with no differences between randomised groups (p=0·58).[11]
In contrast to the two clinical trials, Technau and Lazarus et al conducted a retrospective analysis of the virological outcomes among children receiving different starting regimens and concluded that children on an abacavir based regimen had a lower probability of virological suppression at six months and 12 months than those on a stavudine based regimen.[12]

Similarly a South African Multi-Cohort Analysis was then conducted by Technau and Schomaker et al evaluating the virological response in children treated with abacavir compared with stavudine-based antiretroviral treatment. The study concluded that there was reduced virological suppression at six months and 12 months in those children who were commenced on ABC based regimen compared to a stavudine based regimen.[13]

There is currently no data available regarding the virological performance in children on an ABC based regimen in KwaZulu Natal.

This study is a retrospective chart review to compare the virological responses of children commenced on an Abacavir versus a Stavudine based Antiretroviral regimen at King Edward VIII hospital, Durban.

The primary objectives were to describe the demographic characteristics (age, sex, WHO stage), baseline characteristics (CD_4^+ count, viral load, diagnosis of tuberculosis (TB), HAART regimen) and the virological responses at 12 months in children in the Abacavir cohort and to compare these to children in the stavudine cohort.

Research design

Study Design

This was a retrospective chart review of the routine patient clinic files.

Study setting

King Edward VIII hospital ARV clinic serves a large paediatric population in KwaZulu Natal. In 2012, 220 children under the age of 12 yrs were diagnosed and initiated on HAART.

Antiretroviral Treatment

Patients were managed in compliance with the appropriate South African National HAART guidelines (2004-2013).

In 2004 the first line regimen for children 6 months to 3 yrs was stavudine, lamivudine, lopinovir/ritonavir and for children older than 3 years the treatment was stavudine, lamivudine and efavirenz.

The 2010 guideline changed the first line regimen for infants and children under the age of three to abacavir, lamivudine (3TC) and lopinavir/ritonavir and children older than three or more than 10kg,abacavir, lamivudine and efavirenz(EFV).

The initiation criteria for starting HAART in 2004 was ,2 or more hospital admissions for HIV related conditions in one year, or if the patient was classified as WHO stage 2 or 3, or if the patient was younger than eighteen months and CD_4^+ less than 20% , and if patient is older than eighteen months and CD_4^+ is less than 15%. The patient also had to meet specific psychosocial criteria.

The eligibility criteria for initiation of HAART in 2010 were (1) all children less than 1 year of age, (2)symptomatic infection (WHO stage 3 or 4) or CD4 <25% or <750cells/mm^3 if age 1-5 years, and (3) symptomatic infection (WHO stage 3 or 4) or CD4 <350 cells/mm^3 if age >5years. changed to all children under the age of five were eligible for HAART and children five to fifteen years of age qualified for HAART if they were WHO stage three or four or CD_4^+ <350cells/ul.

The 2010 guidelines required CD_4^+ counts and viral loads to be done at initiation of treatment. CD_4^+ counts were then monitored at 6 months, then at 12 months into HAART and then every 12 months. Viral loads were monitored at six months and then at 12 months, and then every 12 months.[4]

Study participants

All children under the age of 12 years ,who were commenced on an ABC based HAART regimen from 01 January 2012 to 31 December 2012 were included in the study and assigned to the ABC cohort group.

The children had to be on an ABC based HAART regimen for at least 6 months.

The exclusion criteria was children who were commenced on ABC based regimen for less than 6 months and children commenced on a Stavudine based regimen.

Our data was compared to data that was previously collected on all children that were commenced on a Stavudine based regimen at King Edward Hospital VIII between 2004 and 2009.[15] This data obtained from 2004 to 2009 were assigned to the stavudine cohort group.

Virological failure was defined as viral load less than 50copies/ml.

Data collection and analysis

In the abacavir cohort , the patient's demographics, clinical data, HAART regimen and laboratory results were obtained from file records and captured on a data capture sheet. Data was extracted on the 05 March 2015 from all children under the age of twelve years initiated on an ABC/3TC first-line regimen in combination with either EFV or LPV/r .

In the stavudine based cohort , the patient's demographics, clinical data, HAART regimen and laboratory results were obtained from file records and captured in a standardized questionnaire and entered into an access data base. This data was transferred onto a new Microsoft excel spreadsheet and analysed using Intercooled Stata version 13.

The data from the abacavir cohort group was then compared to the data from the stavudine cohort. The data from the abacavir cohort was captured in Microsoft Excel and analysed using Intercooled Stata version 13. Descriptive statistics such as frequencies and percentages were used to summarise

results. Fisher's exact test was used to test for association between virological suppression and other categorical variables. Logistic regression was used to test the effect of factors such as, age, sex, TB and WHO staging on viral suppression. To compare the median CD4 percentages at initiation between efavirenz and kaletra in the abacavir cohort, a two - sample Wilcoxon Rank Sum (Mann- Whitney) will be used. A p-value less than 0.05 was considered to be statistically significant.

Viral load and CD_4^+ testing

The laboratory tests are conducted by the central laboratory by the National Health Laboratory service. HIV diagnosis is based on two positive HIV antibody tests or one single positive HIV PCR in children > 18months or a single positive HIV PCR with a confirmatory viral detection assay in children <18months.Viral loads are measured with NucliSENSEasyQ HIV-1 version 1.2 from April 2004–Nov 2009 and then NucliSENSEasyQ HIV-1 version 2.0 till Sept 2010, thereafter COBAS AmpliPrep/COBAS Taqman HIV-1 test.

Ethics Approval

Permission for the collection and analysis of routine clinic data has been obtained from the

Human Research Ethics Committee of the University of Kwa– Zulu Natal. Brec Ref: BE025/15.

Results:

In the abacavir cohort there were 97 children who initiated highly active antiretroviral treatment at King Edward Hospital VIII from 01 January 2012 to 31 December 2012,of which 96% (94) were included in the study.The 3 files not included was due to missing files. Of the 94 children, 62, 8%(59) were initiated on ABC/3TC/EFV and 37.2%(35) were initiated on ABC/3TC/KAL.

In the stavudine cohort 305 files were reviewed of which 65%(198) were included in the study. Of the patient files not included, 47,7%(51/107) were due to missing data and 52,3% (56/107) were due to missing files. As the stavudine data were collected for a previous retrospective chart review, I cannot provide an explanation as to why there were missing data and missing files.

There was also a low rate ,52%(51/107) of viral load testing done among the stavudine group. It is unclear as to why there was such a low rate as the 2004 guidelines clearly state that viral load testing should be done at baseline and every 6 months. Of the 198 children, 88.4%(175) were initiated on D4T/3TC/EFV and 11.6% (23) were initiated on D4T/3TC/KAL. The demographic characteristics of children in the abacavir cohort and stavudine cohort initiated on HAART are tabulated in table 1.

In the abacavir cohort there were no difference in gender distribution and the median age of initiation was six years. Approximately forty eight percent of children were on TB treatment prior to initiation of HAART. The median CD_4^+ was 18% at time of initiation and the pre–treatment median viral load was 18915 copies/ml.

Children on the kaletra based regimen had higher viral load copies as compared to children on efavirenz (p=<0.001). There was no statistical significant difference between CD4 at initiation of treatment (p=0.745) or WHO staging prior to treatment (p=0.091) between kaletra and efavirenz p=0.75). The median CD4 at initiation of Efavirenz was 18,2% and the median CD4 of kaletra was 17,5%.Majority of the patients were classified as WHO stage 3 or 4.

In the stavudine cohort there were predominantly more males 55.6% that were initiated on HAART as compared to 44.4% females. The mean age of initiation was 6.5 years. The WHO staging and TB

status were not obtained from the stavudine cohort as this data was not available from the stavudine data sheet that was collected previously.

The virological suppression rates in the abacavir cohort at the 12 month follow up, were 83.3% of children commenced on ABC/3TC/EFV reached virological suppression and 76.5% of children commenced on ABC/3TC/KAL reached virological suppression. There was no statistical difference regarding virological suppression between Kaletra and Efavirenz group (p=0.43). These results are presented in table 3.

In the stavudine based cohort , 85.2% of children commenced on the D4T based regimen reached virological suppression at the 12 month follow up. Approximately 14.8% did not reach virological suppression at 12 months. There is no statistical difference regarding virological suppression between KAL and EFV group (p=0.78). The results are presented in table 3.

Logistic regression analysis was performed and CD_4^+, Age, TB, Sex, WHO staging were not associated with virological failures. See Table 2.

There is no statistical significant difference ,when comparing the stavudine cohort to the abacavir cohort, with regards to reaching virological suppression at 12 months with a p value of 0.39. See table 4.

Table 1:Demographic Characteristics:

	Total Number	**ABC regimen**	Total Number	**D4T regimen**
Age in years at initiation **Median**	86	6yrs (2-11yrs)	89	6.5yrs (4-8,8)
Gender	86	Male: 52.3% (45) Female: 47.7%(41)	196	Male:55.6%(109) Female:44.4%(87)
CD4 % (at initiation) **Median**	93	18%		
Viral load copies/ml at initiation **Median**	75	18915 copies/ml		
HIV regimen	94	ABC/3TC/EFV : 62.8% (59) ABC/3TC/KAL :37.2% (35)	198	D4T/3TC/EFV 88%(175) D4T/3TC/KAL: 12% (23)
TB (current TB at time of Diagnosis)	89	48.3%		
WHO Stage 1	94	8.5%		
WHO Stage 2		9.6%		
WHO Stage 3		49.0%		
WHO stage 4		32.9%		

Table 2 Logistic Regression analysis to determine if the variable , CD_4^+, Age, TB, Sex, WHO staging were associated with virological failures

	ABC		D4T	
	Odds Ratio	**95% Conf. Interval**	**Odds Ratio**	**95% CI**
WHO staging	0.6	0.3 - 1.3		
TB	1.1	0.4 - 3.3		
Age in years	1.1	0.9 - 1.2	1.1	0.8 - 1.5
Sex	1.5	0.5 – 4.6	0.8	0.3 - 2.3

Table 3 The proportion of virologically suppressed children in both the ABC- and D4T regimen groups after 12 months of HAART

			EFV	KAL	Total
ABC regimen	**Virological Suppression**	**ABC % Virologically Suppressed**	**83.3.3%**	**76.5%**	**80.7%**
		Number of Patients	**45**	**26**	**71**
	Not Virologically Suppressed	**ABC % Virologically Not Suppressed**	**16.7%**	**23.5%**	**19.3%**
		Number of Patients	**9**	**8**	**17**
D4T regimen	**Virologically suppressed**	**D4T % Virologically Suppressed**	**84.8%**	**87.5%**	**85.2%**
		Number of Patients	**95**	**14**	**109**
	Not Suppressed	**D4T % Virologically Not Suppressed**	**15.2%**	**12.5%**	**14.8%**
		Number of Patients	**17**	**2**	**19**

Table 4 Virological suppression at 12 months -Comparing ABC vs. D4T

	Virological suppression	**Not Virologically Suppressed**	**Total Number**
ABC	80.7%(71)	19.3% (17)	88
D4T	85.2% (109)	14.8% (19)	128
Total	87.6%(180)	16.7% (36)	216

Pr =0.38

Discussion

The primary objective of this study was to compare the virological responses between an abacavir cohort and a stavudine based cohort. The importance of this study was to identify if lower virological effectiveness were seen in an abacavir cohort in our setting, as two other studies reported poorer virological responses in an abacavir containing regimen.

In our study we found no difference in virological suppression rates between the abacavir cohort and stavudine cohort. (p value 0.38). Our study also provided evidence that there was good virological suppression (81.91%) in both the EFV/KAL group.

These results are in keeping with the Penta- 5 trial and Musiime trial, as these two randomised control trials showed no difference in virological suppression rates between an abacavir based regimen versus other NRTI regimens.[10,11]

This is in contrast to the Gauteng studies where their findings showed a poorer performance of ABC-containing regimen as compared to the stavudine based regimen. In the kaletra group , 76.5% on ABC versus 87.5% on D4T reached virological suppression, and similarly in the EFV group, 83.3% on ABC versus 84.8% on D4T reached virological suppression.[13]

Similarly in the retrospective analysis done by Technau et al they found differences in virological suppression rates with LPV/r- versus EFV-based regimens. In their study a stronger association was seen in the LPV/r-based regimens, where children on ABC had an almost 2-fold increased risk of failure to suppress (41% versus21%, log-rank p<0.0001) by 12 months. Children receiving EFV had an almost 2-foldhigher risk of rebound by 12 months after first suppression (35% versus 18%, log-rankp=0.0001) if they were on ABC compared to d4T.[14]

Our study provides reassuring data that children in our setting have a good probability of virological suppression on an abacavir regimen.

We also aimed to describe the demographic and baseline characteristics of children on HAART in the abacavir cohort as the baseline data for the stavudine cohort was not available . In the abacavir cohort, children on the kaletra based regimen had higher viral load copies as compared to children on efavirenz (p=<0.001). There was no statistical significant difference between CD4 at initiation of

treatment (p=0.745) or WHO staging prior to treatment (p=0.091) between kaletra and efavirenz. The higher viral load at initiation of treatment in children on a kaletra based regimen can be explained by the fact that children on kaletra are younger compared to children on efavirenz. A possible explanation for the high viral load could be that the level of viral replication is higher in infants and infants have a higher concentration of circulating CD4 T lymphocytes, which are the major target cells for HIV-1 replication. Another possibility for the high viral load can be secondary to a less robust early immune response to infection in infants. This is in keeping with a study done by Richardson et al that found that the average peak plasma HIV-1 viral loads are 1 $\log_{10}$ higher in infants than in adults.[16]

The limitation of this study is that it's a descriptive retrospective chart review with a small sample size and findings are from a single centre. Another limitation to the study was missing data and several patients were lost to follow up especially in the stavudine cohort. An important limitation to this study was the limited number of variables used to explore determinants of virological suppression e.g no information was obtained on adherence and therefore the inability to undertake a comprehensive evaluation of the determinants of virological suppression. An important concerning finding, was also the low rate of VL testing in all treatment groups at the six months and twelve months follow up.

Conclusion

This study is a retrospective chart review that compared virological responses between an abacavir cohort and a stavudine based cohort at King Edward Hospital Durban. This study demonstrates that children treated with an abacavir based regimen have a good probability of virological suppression, and there was no statistical difference between patients initiating an abacavir-based regimen versus a stavudine based regimen. These findings are in keeping with data from several clinical trials and support the WHO recommendation of an abacavir-based regimen for infants and children initiating antiretroviral treatment.

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Appendices

Appendix 1: The final Study Protocol

Research Proposal

Research topic: Comparison of virological responses of children commenced on an Abacavir versus a Stavudine based Antiretroviral regimen at King Edward VIII hospital, Durban.

Nature of study:

Quantative Study

Type of study

Retrospective chart review

Objective

Primary Objective:

1. To describe the demograpic characteristics (age, sex, WHO stage) of children commenced on an Abacavir(ABC) based HAART regimen at King Edward VIII Hospital from January 2012 to December 2012.
2. To describe the baseline characteristics (CD4 count, Viral load, Diagnosis of TB, ART regimen) of children commenced on an Abacavir(ABC) based HAART regimen at King Edward VIII Hospital from January 2012 till December 2012.
3. To describe the virological responses (VL < 1000 = virological response/ VL > 1000 = virological failure) at 12 months in children commenced on an Abacavir(ABC) based

HAART regimen at King Edward VIII Hospital from January 2012 till December 2012 and to compare these to children in the stavudine cohort

Summary of the proposed research

Improvement in the PMTCT program and availability of treatment of HIV infected
Children have reduced the number of children acquiring HIV infection. Following the World Health Organization recommendations, the South African Antiretroviral treatment guideline in 2010,replaced stavudine with abacavir in the first line HAART regimen, due to the concerns of stavudine toxicity.

The proposed research aim to evaluate the virological performance in all children started on the ABC based HAART regimen at King Edward Hospital VIII Hospital from January 2012 till December 2012.This is a retrospective chart review that will review selected patient records from the hospital database.

Viral load will be used to assess the virological performance. Ethical permission will be sought from BREC and EThekwini Municipality. Data will be analysed using a statistical software package.

Keywords

HIV

HAART regimen

Side effects of treatment

Abacavir

Stavudine

Epidemiology

Viral load

CD4 count

Literature review.

Improvement in the PMTCT program and availability of treatment of HIV infected children has reduced the number of children acquiring HIV infection. In 2011, 330 000 children acquired HIV infection. This represents a 43% decline since 2003.More than 90% of these children acquiring HIV infection live in sub-Saharan Africa.[1]

The route of infection in children is mainly through vertical transmission(95%). Of which approximately 10% is acquired transplacentally,60% in the peripartum period and 30% through breastfeeding (30%). Children can also acquire the virus through sexual abuse and blood product transfusion. In resource poor communities in the absence of treatment, it is estimated that 10-20% of infants can progress rapidly to AIDS in the first year of life and 80% of infected infants will demise in first 2 years of life.[2]

The plasma HIV RNA (viral load) provides an accurate means of quantifying the response to treatment. Effective HAART regimens taken with high levels of adherence result in a decrease of viral load by at least 1 log per month and suppression to a viral load below 50copies/ml will generally be achieved in 16-24 weeks. The use of $CD4^+$ cell counts and viral load provide a better estimate of the risk of disease progression.[3]

The initial treatment included in the first line regimen was stavudine, lamivudine with either Ritonavir- lopinavir for children under the age of 3,or efavirenz for children over the age of 3.

In 2010, the South African Antiretroviral treatment guidelines replaced stavudine with abacavir in the first line regimen due to the concerns of stavudine toxicity, following the World Health Organization recommendations. In 2013 the eligibility criteria also changed in that all children under the age of 5 must start HAART irrespective of CD4 count.[4]

The adverse events of stavudine include mitochondrial toxicity, peripheral neuropathy, lipoatrophy, pancreatitis, lactic acidosis/severe hepatomegaly, hyperlipidaemia and insulin resistance.
A study done by Steve Innes et al, published in August 2010 looked at lipodystrophy syndrome in HIV infected children on HAART which is common in those taking didanosine, stavudine or zidovudine. Lipodystrophy syndrome refers to peripheral lipo-atrophy, central lipohypertrophy and dyslipidaemia associated with insulin resistance. They concluded that paediatric dosing of stavudine need to be reduced urgently to minimize the risk of lipodystrophy.[5]
Linda Barlow-Mosha et al published an article in the Journal of the International AIDS society in April 2013 on metabolic complications and treatment of perinatally HIV- infected children and adolescents and concluded that nucleoside reverse transcriptase inhibitors, particularly Stavudine, zidovudine and didanosine are linked to development of lipodystrophy and lactic acidosis.[6]

Abacavir is a nucleoside reverse transcriptase inhibitor with fewer side effects than stavudine. Its only potential dangerous side effect is a hypersensitivity reaction that is more common in the Caucasian population than African population. A study done by Walter Hughes et al in 1998 on safety and single dose pharmacokinetics of abacavir in human immunodeficiency virus type 1- infected children concluded that abacavir is safe and well tolerated in children and the single abacavir related adverse event was the hypersensitivity reaction.[7]
Another study by Chaponda et al published in August 2010 looked at hypersensitivity reactions to HIV therapy and concluded that the Abacavir hypersensitivity reaction occurs in 2.3-9% of adults and children with some differences in ethnicity.[8]

A study done at the Empilweni clinic at Rahima Moosa Mother and Child hospital in Johannesburg published in 2013, compared the virological performance of the abacavir based first line regimen with the stavudine based regimen. The study concluded that the Abacavir based regimen showed a significantly poorer virological performance as compared with the Stavudine based regimen.[9]

The Paediatric European Network for the treatment of AIDS (PENTA-5) trial from 1998-1999 demonstrated that the combination of lamivudine/abacavir has virological superiority over AZT/Lamivudine and AZT/ABC. This trial also concluded that Abacavir has the least effect on mitochondrial DNA and its only associated serious side effect is hypersensitivity reaction that is more prevalent in Caucasians than the African population.[10]

Another study that was done from April 2004 to March 2008, looked at Antiretroviral therapy responses among children attending a large public clinic in Soweto. This study concluded that children that were started on the lamivudine/stavudine/LPV/r or lamivudine/stavudine/ EFV regimen achieved an 84% and 96% virological suppression at 12months and 24 months respectively. Virological suppression was defined as achieving a viral load less than 400 copies/ml. Children younger than 3yrs and with higher viral loads suppressed their viral loads more slowly than older children. The mean CD4 percentage doubled within 12months of initiation, rising from 12.7% to 25.1%. Their findings demonstrated excellent virological and immunological suppression rates.[11]

The PenPact- 1 trial from 2002-2005 conducted in children from Europe and South America, compared first line antiretroviral therapy with a protease inhibitor versus a non- nucleoside reverse transcriptase inhibitor and demonstrated no significant differences in virological, immunological and clinical outcomes.[12]

This study aims to evaluate the virological performance of the abacavir based HAART regimen.

Research design

Study Design

This was a retrospective chart review of the routine patient clinic files.

Study setting

King Edward VIII hospital ARV clinic serves a large paediatric population in KwaZulu Natal. In 2012, 220 children under the age of 12 yrs were diagnosed and initiated on HAART.

Antiretroviral Treatment

Patients were managed in compliance with the appropriate South African National HAART guidelines (2004-2013).

In 2004 the first line regimen for children 6 months to 3 yrs was stavudine, lamivudine, lopinovir/ritonavir and for children older than 3 years the treatment was stavudine, lamivudine and efavirenz.

The 2010 guideline changed the first line regimen for infants and children under the age of three to abacavir, lamivudine (3TC) and lopinavir/ritonavir and children older than three or more than 10kg,abacavir, lamivudine and efavirenz(EFV).

The initiation criteria for starting HAART in 2004 was ,2 or more hospital admissions for HIV related conditions in one year, or if the patient was classified as WHO stage 2 or 3, or if the patient was younger than eighteen months and CD_4^+ less than 20% , and if patient is older than eighteen months and CD_4^+ is less than 15%. The patient also had to meet specific psychosocial criteria.

The eligibility criteria for initiation of HAART in 2010 were (1) all children less than 1 year of age, (2)symptomatic infection (WHO stage 3 or 4) or CD4 <25% or <750cells/mm^3 if age 1-5 years, and (3) symptomatic infection (WHO stage 3 or 4) or CD4 <350 cells/mm^3 if age >5years. changed to all children under the age of five were eligible for HAART and children five to fifteen years of age qualified for HAART if they were WHO stage three or four or CD_4^+ <350cells/ul.

The 2010 guidelines required CD_4^+ counts and viral loads to be done at initiation of treatment. CD_4^+ counts were then monitored at 6 months, then at 12 months into HAART and then every 12 months. Viral loads were monitored at six months and then at 12 months, and then every 12 months.[4]

Study participants

All children under the age of 12 years ,who were commenced on an ABC based HAART regimen from 01 January 2012 to 31 December 2012 were included in the study and assigned to the ABC cohort group.

The children had to be on an ABC based HAART regimen for at least 6 months.

The exclusion criteria was children who were commenced on ABC based regimen for less than 6 months and children commenced on a Stavudine based regimen.

Our data was compared to data that was previously collected on all children that were commenced on a Stavudine based regimen at King Edward Hospital VIII between 2004 and 2009.[15] This data obtained from 2004 to 2009 were assigned to the stavudine cohort group.

Virological failure was defined as viral load less than 50copies/ml.

Data collection and analysis

In the abacavir cohort , the patient's demographics, clinical data, HAART regimen and laboratory results were obtained from file records and captured on a data capture sheet. Data was extracted on the 05 March 2015 from all children under the age of twelve years initiated on an ABC/3TC first-line regimen in combination with either EFV or LPV/r .

In the stavudine based cohort , the patient's demographics, clinical data, HAART regimen and laboratory results were obtained from file records and captured in a standardized questionnaire and entered into an access data base. This data was transferred onto a new Microsoft excel spreadsheet and analysed using Intercooled Stata version 13.

The data from the abacavir cohort group was then compared to the data from the stavudine cohort. The data from the abacavir cohort was captured in Microsoft Excel and analysed using Intercooled Stata version 13. Descriptive statistics such as frequencies and percentages were used to summarise

results. Fisher's exact test was used to test for association between virological suppression and other categorical variables. Logistic regression was used to test the effect of factors such as, age, sex, TB and WHO staging on viral suppression. To compare the median CD4 percentages at initiation between efavirenz and kaletra in the abacavir cohort, a two - sample Wilcoxon Rank Sum (Mann- Whitney) will be used. A p-value less than 0.05 was considered to be statistically significant.

Viral load and CD_4^+ testing

The laboratory tests are conducted by the central laboratory by the National Health Laboratory service. HIV diagnosis is based on two positive HIV antibody tests or one single positive HIV PCR in children > 18months or a single positive HIV PCR with a confirmatory viral detection assay in children <18months.Viral loads are measured with NucliSENSEasyQ HIV-1 version 1.2 from April 2004–Nov 2009 and then NucliSENSEasyQ HIV-1 version 2.0 till Sept 2010, thereafter COBAS AmpliPrep/COBAS Taqman HIV-1 test.

Ethics Approval

Permission for the collection and analysis of routine clinic data has been obtained from the

Human Research Ethics Committee of the University of Kwa– Zulu Natal. Brec Ref: BE025/15.

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Appendix 2

DATA SHEET

Number	**Age**	**Sex**	**WHO Stage**	**HAART regimen**	**TB**	**CD4 count**	**Viral Load before initiation**	**Viral load at 6 months**	**Viral load at 1 year**

UNIVERSITY OF
KWAZULU-NATAL

STUDY IDENTIFICATION NUMBER (SID) ☐☐-☐☐☐☐

A retrospective review of outcomes of HIV positive children on Antiretroviral Therapy in King Edward VIII Hospital, Durban, South Africa

A. ARV Chart – Philani Clinic Card

Department of Paediatrics and Child Health
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KwaZulu-Natal
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PRIMARY CAREGIVER'S DEMOGRAPHICS

1. **What is the relationship of primary care giver to patient?**

   (a) Biological mother ☐

   (b) Biological father ☐

   (c) Grandmother ☐

   (d) Foster parent ☐

   (e) Sibling ☐ (g) Unknown ☐

   (f) Other ☐ State relationship _____

2. **Highest level of education of primary caregiver**

   (a) No Education ☐ (b) Primary/Secondary Education ☐

   Grade (enter grade e.g. 8, 12) ☐

   (c) Tertiary Education ☐ (d) Unknown ☐

3. Age of Primary Caregiver _____

Biological Mother's Clinical Data

4. Age: ☐

5. PMTCT for mother:

   (a) NIL ☐ (b) NVP only ☐

   (c) NVP and AZT ☐ (d) HAART ☐

   (e) Other ☐ (d) Unknown ☐

6. If you answered **NO** to question 6 above: Reason for No PMTCT

   (a) Unknown HIV status ☐ (b) Negative HIV status ☐

   (c) Not offered VCT ☐ (d) Not offered PMTCT ☐

   (e) declined testing ☐ (d) declined /defaulted PMTCT ☐

7. CD4 count

(a) <200 ☐ (b) 200 to 350 ☐

(c) >350 ☐ (d) Unknown ☐

8. HAART Y ☐ N ☐ Unknown ☐

9. Is the Mother dead ☐ or alive ☐

Patient's Clinical Data

10. Date of START of ART Treatment ☐☐☐☐☐☐

11. What was/is the patient's WHO Stage at:

BASELINE Clinical Staging: _____ ☐☐☐☐☐☐

12. Patients Program Status (at date of last visit when completing the Chart Review)

(a) Currently on ART ☐ (b) Deceased ☐ (c) Lost to follow up ☐

(e) Transferred to another HIV/Care ART ☐ (f) other ☐

IF PATIENT IS DECEASED:

13. Date of death ☐☐☐☐☐☐ Unknown ☐

14. Are their medical records relating to their death? Y ☐ N ☐

15. Suspected cause of death: (as noted in patient file)

(a) HIV-related death _____ (insert cause of death) ☐

(b) ART-related ☐ (c) Other e.g. gun shot, MVA or other illnesses ☐

(d) Unknown ☐

PATIENT'S ART Regiment

16. **FIRST ART REGIMEN AT THIS CLINIC**

(a)	D4T	☐	(f)	DDI	☐
(b)	3TC	☐	(g)	Nevirapine	☐
(c)	KALETRA	☐	(h)	Abavavir	☐
(d)	EFV	☐	(i)	Other _____	☐
(e)	AZT	☐	(j)	Other_____	☐

17. Was there a change in regimen during the follow up period? Y ☐ N ☐

Unknown ☐

IF YES

18. Reason for change in ART regimen:

side effects / intolerance ☐ treatment failure ☐ TB treatment started ☐

non-adherance ☐

Other ☐ _____ (specify)

19. **SECOND ART REGIMEN AT THIS CLINIC:**

20. Date of starting regimen 2: ☐☐☐☐☐☐

(a)	D4T	☐	(f)	DDI	☐
(b)	3TC	☐	(g)	Nevirapine	☐
(c)	KALETRA	☐	(h)	Abavavir	☐
(d)	EFV	☐	(i)	Other _____	☐
(e)	AZT	☐	(j)	Other_____	☐

27.1 Suspected resistance Y ☐ N ☐ Unknown ☐

27.2 Resistance testing done Y ☐ N ☐ Unknown ☐

Patient's Clinical Follow up

28. **ARV scheduled visits:**

Visit Choose date closest to each time period	Weight (kg)	Height (cm)	Other clinical problems	Adherence check (U) = Unknown (P) = Problems noted (N) = Normal (O) = Other – state
Initiation				
First 6 months				
12 months				
18 months				

Patient's Clinical Follow up

Tuberculosis Summary Page 1

29. Has the patient been treated for TB in the past 5 years?

Y ☐ N ☐ Unknown ☐

30. How many episodes?

episode Date 1 ☐☐☐☐☐☐ episode Date 2 ☐☐☐☐☐☐

episode Date 3 ☐☐☐☐☐☐

Summary Sheet: Test Results 1

10.1	CD4	DATES	Results	VIRAL LOAD	DATES	Results
	BASELINE			BASELINE		
	6 months			6 months		
	12 months			12 months		
	18 months			18 months		
	24 months			24 months		

10.2	FBC	DATES	H	WCC	P	FBC	DATES	H	WCC	P

FBC = Full Blood Count

11	LFT	DATES	AP	ALT	AST	D_B	T_B	GGT	T_P	ALB
	BASELINE									

Appendix 3: The Guidelines for Authorship for the Journal selected for submission of the manuscript

Instructions to authors: SAHIVJ: www.sajhivmed.org.za

Structure and style of your original research article

The page provides an overview of the structure and style of your original research article to be submitted to the *Southern African Journal of HIV Medicine*. The original article provides an overview of innovative research in a particular field within or related to the focus and scope of the journal presented according to a clear and well-structured format (between 3500 and 5500 words with a maximum of 60 references).

Please use British English, that is, according to the Oxford English Dictionary. Avoid Americanisms (e.g. use 's' and not 'z'). Consult the Oxford English Dictionary when in doubt and remember to set your version of Microsoft Word to UK English.

- **Language:** Manuscripts must be written in British English.
- **Font:**
  - **Font type:** Palatino
  - **Symbols font type:** Times New Roman
  - **General font size:** 12pt
- **Line spacing:** 1.5
- **Headings:** Ensure that formatting for headings is consistent in the manuscript.
  - First headings: normal case, bold and 14pt
  - Second headings: normal case, underlined and 14pt
  - Third headings: normal case, bold and 12pt
  - Fourth headings: normal case, bold, running-in text and separated by a colon.

Our publication system supports a limited range of formats for text and graphics. Text files can be submitted in the following formats only:

- Microsoft Word (.doc): We cannot accept Word 2007 DOCX files. If you have created your manuscript using Word 2007, you must save the document as a Word 2003 file before submission.
- Rich Text Format (RTF) documents uploaded during Step 2 of the submission process. Users of other word processing packages should save or convert their files to RTF before uploading. Many free tools are available that will make this process easier.

 For full details on how to ensure your manuscript adheres to the house style, **click here**.

The structure and style of your original article

Page 1

The format of the **compulsory cover letter** forms part of your submission and is on the first page of your manuscript and should always be presented in English. You should provide all of the following elements:

- **Article title:** Provide a short title of 50 characters or less.

- **Significance of work:** Briefly state the significance of the work being reported on.
- **Full author details:** Provide title(s), full name(s), position(s), affiliation(s) and contact details (postal address, email, telephone and cellular number) of each author.
- **Corresponding author:** Identify to whom all correspondence should be addressed to.
- **Authors' contributions:** Briefly summarise the nature of the contribution made by each of the authors listed.
- **Summary:** Lastly, a list containing the number of words, pages, tables, figures and/or other supplementary material should accompany the submission.

Page 2 and onwards

Title: The article's full title should contain a maximum of 95 characters (including spaces).

Abstract: The abstract, written in English, should be no longer than 250 words and must be written in the past tense. The abstract should give a succinct account of the objectives, methods, results and significance of the matter. The structured abstract for an original research article should consist of five paragraphs that are labelled. These labelled paragraphs should deal with the background, objectives, method, results and conclusion.

- **Background:** *Why do we care about the problem?* State the context and purpose of the study. (What practical, scientific or theoretical gap is your research filling?)
- **Objectives:** *What problem are you trying to solve*? What is the scope of your work (e.g. is it a generalised approach or for a specific situation)? Be careful not to use too much jargon.
- **Method:** *How did you go about solving or making progress on the problem*? State how the study was performed and which statistical tests were used. (What did you actually do to get the results?) Clearly express the basic design of the study; name or briefly describe the basic methodology used without going into excessive detail. Be sure to indicate the key techniques used.
- **Results**: *What is the answer*? Present the main findings (that is, as a result of completing the procedure or study, state what you have learnt, invented or created). Identify trends, relative change or differences on answers to questions.
- **Conclusion**: *What are the implications of your answer*? Briefly summarise any potential implications. (What are the larger implications of your findings, especially for the problem or gap identified in your motivation?)

Do not cite references in the abstract and do not use abbreviations excessively in the abstract.

The following headings serve as a guide for presenting your research in a well-structure format. As an author you should include all first level headings but subsequent headings (second and third level headings) can be changed.

Introduction (first-level heading)
The introduction contains two subsections, namely the background section and the literature review. The introduction section should be written from the standpoint of readers that is without specialist knowledge in that area and must clearly state the introduction to the research and its aims in the context of previous work bearing directly on the subject. The introduction section to the article normally contains the following five elements:

- **Key focus (third-level heading):** A thought-provoking introductory statement on the broad theme or topic of the research.

- **Background (third-level heading:** Providing the background or the context to the study (explaining the role of other relevant key variables in this study).
- **Trends (third-level heading):** Cite the most important published studies previously conducted on this topic or that has any relevance to this study (provide a high-level synopsis of the research literature on this topic).
- **Objectives (third-level heading):** Indicate the most important controversies, gaps and inconsistencies in the literature that will be addressed by this study. In view of the above trends, state the core research problem and specific research objectives that will be addressed in this study and provide the reader with an outline of what to expect in the rest of the article.
- **Contribution to field (third-level heading):** Explanation of the study's academic (theoretical and methodological) or practical merit and/or importance (provide the value-add and/or rationale for the study).

Research design (first-level heading)

- **Research appraoch (second-level heading)**
- **Research method (second-level heading)**
  - **Materials (third-level heading):** Describe the type of organism(s) or material(s) involved in the study.
  - **Setting (third-level heading):** Describe the site and setting where your field study was conducted.
  - **Design (third-level heading):** Describe your experimental design clearly, including a power calculation if appropriate. Note: Additional details can be placed in the online supplementary location.
  - **Procedure (third-level heading):** Describe the protocol for your study in sufficient detail (clear description of all interventions and comparisons) that other scientists could repeat your work to verify your findings.
  - **Statistical analysing (third-level heading):** Describe how the data were summarised and analysed, additional details can be placed in the online supplementary information.
  - **Reliability (third-level heading):** Reliability is the extent to which an experiment, test, or any measuring procedure yields the same result on repeated trials. Without the agreement of independent observers able to replicate research procedures, or the ability to use research tools and procedures that yield consistent measurements, researchers would be unable to satisfactorily draw conclusions, formulate theories, or make claims about the generalisability of their research.
  - **Validity (third-level heading):** Validity refers to the degree to which a study accurately reflects or assesses the specific concept that the researcher is attempting to measure. While reliability is concerned with the accuracy of the actual measuring instrument or procedure, validity is concerned with the study's success at measuring what the researchers set out to measure. Researchers should be concerned with both external and internal validity. External validity refers to the extent to which the results of a study are generalisable or transferable. Internal validity refers to (1) the rigor with which the study was conducted (e.g. the study's design, the care taken to conduct measurements, and decisions concerning what was and wasn't measured) and (2) the extent to which the designers of a study have taken into account alternative explanations for any causal relationships they explore. In studies that do not explore causal relationships, only the first of these definitions should be considered when assessing internal validity.
  - **Ethical considerations (third-level heading)**:Articles based on the involvement of people must have been conducted in accordance with relevant national and international guidelines. Approval must have been obtained for all protocols from the author's institutional or other

relevant ethics committee and the institution name and permit numbers provided at submission.

- **Potential benefits and hazards (fourth-level heading):** What risks to the subject are entailed in involvement in the research? Are there any potential physical, psychological or disclosure dangers that can be anticipated? What is the possible benefit or harm to the subject or society from their participation or from the project as a whole? What procedures have been established for the care and protection of subjects (e.g. insurance, medical cover) and the control of any information gained from them or about them?
- **Recruitment procedures (fourth-level heading):** Was there any sense in which subjects might be 'obliged' to participate – as in the case of students, prisoners, learners or patients – or were volunteers being recruited? If participation was compulsory, the potential consequences of non-compliance must be indicated to subjects; if voluntary, entitlement to withdraw consent must be indicated and when that entitlement lapses
- **Informed consent (fourth-level heading):** Authors must include how informed consent was handled in the study.
- **Data protection (fourth-level heading:** Authors must include in detail the way in which data protection was handled.

Results (first-level heading)

This section provides a synthesis of the obtained literature grouped or categorised according to some organising or analysis principle.

Tables may be used and models may be drafted to indicate key components of the results of the study.

- Organise the results based on the sequence of Tables and Figures you will include in the manuscript.
- The body of the Results section is a text presentation of the key findings which includes references to each of the Tables and Figures.
- Statistical test summaries (test name, p-value) are usually reported parenthetically in conjunction with the biological results they support.
- Present the results of your experiment(s)/research data in a sequence that will logically support (or provide evidence against) the hypothesis, or answer the question, stated in the Introduction.

All units should conform to the **SI convention** and should be abbreviated accordingly. Metric units and their international symbols are used throughout, as is the decimal point (not the decimal comma).

Discussion (first-level heading)

This section normally contains the following elements (it is strongly suggested that sub-headings are used in this section):

- **Outline of the results (second-level heading):** Restate the main objective of the study and reaffirm the importance of the study by restating its main contributions; Summarise the results in relation to each stated research objective or research hypothesis; link the findings back to the literature and to the results reported by other researchers; provide explanations for unexpected results.
- **Practical implications (second-level heading):** Reaffirm the importance of the study by restating its main contributions and provide the implications for the practical implementation your research.

- **Limitations of the study (second-level heading):** Point out the possible limitations of the study and provide suggestions for future research.
- **Recommendations (second-level heading):** Provide the recommendations emerging out of the current research.

Conclusion (first-level heading)
This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance, with a recommendation for future research (implications for practice). Provide a brief conclusion that restates the objectives, the research design, the results and their meaning.

Acknowledgements (first-level heading)
If, through your study, you received any significant help in conceiving, designing, or carrying out the work, or received materials from someone who did you a favour by supplying them, you must acknowledge their assistance and the service or material provided. **Authors should always acknowledge outside reviewers of their drafts and any sources of funding that supported the research.**

- **Competing interests (second-level heading):** A competing interest exists when your interpretation of data or presentation of information may be influenced by your personal or financial relationship with other people or organisations that can potentially prevent you from executing and publishing unbiased research. Authors should disclose any financial competing interests but also any non-financial competing interests that may cause them embarrassment were they to become public after the publication of the manuscript. **Where an author gives no competing interests, the listing will read 'The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this article.'**
- **Authors' contributions (second-level heading)*:** This section is necessary to give appropriate credit to each author, and to the authors' applicable institution. The individual contributions of authors should be specified with their affiliation at the time of the study and completion of the work. An 'author' is generally considered to be someone who has made substantive intellectual contributions to a published study. Contributions made by each of the authors listed, along the lines of the following (please note the use of author initials):

J.K. (University of Pretoria) was the project leader, L.M.N. (University of KwaZulu-Natal) and A.B. (University of Stellenbosch) were responsible for experimental and project design. L.M.N. performed most of the experiments. P.R. made conceptual contributions and S.T. (University of Cape Town), U.V. (University of Cape Town) and C.D. (University of Cape Town) performed some of the experiments. S.M. (Cape Peninsula University of Technology) and V.C. (Cape Peninsula University of Technology) prepared the samples and calculations were performed by C.S., J.K. (Cape Peninsula University of Technology) and U.V. wrote the manuscript.

References (first-level heading)
Begin the reference list on a separate page with no more than 60 references. *Southern African Journal of HIV Medicine* uses the **Vancouver referencing style**, details of which can be downloaded from the journal website. **Note: No other style will be permitted.**

Appendix 4:Ethical approvals

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

05 May 2015

Dr Stephane Montgomery
Unit 3, 280 Montpelier Road
Morningside
Montgomery.stephane@gmail.com

Dear Dr Montgomery

PROTOCOL: Evaluation of the virological performance of children commenced on an Abacavir based antiretroviral regimen at King Edward VIII Hospital from January 2012 to December 2012. School of Clinical Medicine: MMed 213571438. BREC REF: BE025/15

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 14 January 2015.

The study was provisionally approved pending appropriate responses to queries raised. Your responses received on 22 April 2015 to queries raised on 25 March 2014 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval.

This approval is valid for one year from **05 May 2015.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with **UKZN** BREC ethics requirements as contained in the **UKZN** BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its meeting taking place on **09 June 2015.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor J Tsoka-Gwegweni
Chair: Biomedical Research Ethics Committee

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

RESEARCH OFFICE
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Email: [redacted]
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

23 May 2016

Dr Stephane Montgomery
Unit 3, 280 Montpelier Road
Morningside
Montgomery.stephane@gmail.com

Dear Dr Montgomery

PROTOCOL: Evaluation of the virological performance of children commenced on an Abacavir based antiretroviral regimen at King Edward VIII Hospital from January 2012 to December 2012. School of Clinical Medicine: MMed 213571438. BREC REF: BE025/15

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved:	05 May 2016
Expiration of Ethical Approval:	04 May 2017

I wish to advise you that your application for Recertification dated 19 April 2016 for the above protocol has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

This approval will be ratified at the next full BREC meeting to be held on 14 June 2016.

Yours sincerely

Mrs A Marimuthu
Senior Administrator: Biomedical Research Ethics

Department:
Health
PROVINCE OF KWAZULU-NATAL

OFFICE OF THE HOSPITAL CEO
KING EDWARD VIII REGIONAL HOSPITAL
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www. kznhealth.gov. za

Ref.: KE 2/7/1/ (09/2015)
Eng.: Mrs. R. Sibiya
Research Programming

18 February 2015

Dr. Montgomery
Unit 3, 280 Montpelier Road
MORNINGSIDE
4053

Dear Dr. Montgomery

Protocol: Evaluation of the virological performance of children commenced on an abacavir based antiretroviral regimen at King Edward VIII Hospital from January 2012 to December 2012. School of Clinical Medicine: MMed 213571438. BREC REF: BE025/15

Permission to conduct research at King Edward VIII Hospital is provisionally granted, pending approval by the Provincial Health Research Committee, KZN Department of Health.

Kindly note the following: -

- The research will only commence once confirmation from the Provincial Health Research Committee in the KZN Department of Health has been received.
- Signing of an indemnity form at Room 8, CEO Complex before commencement with your study.
- King Edward VIII Hospital received full acknowledgement in the study on all Publications and reports and also kindly present a copy of the publication or report on completion.

The management of King EdwardD III—hospital reserves the right to terminate the permission for the study should circumstances so dictate.

Yours faithfully

SUPPORTED/ ~~NOT SUPPORTED~~

19/02/2015

DR. OSB BALOYI
ACTING CHIEF EXECUTIVE OFFICER

DATE

PERMISSION TO CONDUCT A RESEARCH STUDY/TRIAL

This must be completed and submitted to the /Medical Superintendent/s / Hospital Manager/s for signature.

For King Edward VIII Hospital (KEH) and lnkosi Albert Luthuli Central Hospital (IALCH) studies please submit the document together with the following:

1. Research proposal and protocol.
2. Letter giving provisional ethical approval.
3. Details of other research presently being performed by yourself if in the employment of KEH (individually or as a collaborator).
4. Details of any financial or human resource implications to KEH, including all laboratory tests, EEGs, X-rays, use of nurses, etc. (See Addendum 1)
5. Declaration of all funding applications / grants, please supply substantiating documentation.

Once the document has been signed it should be returned to the Biomedical Research Ethics Administration, Room N003, Govan Mbeki Building, Westville Campus, University of KwaZulu-Natal.

To: Chief Medical Superintendent / Hospital Manager

Permission is requested to conduct the above research study at the hospital/s indicated below:

Site 1 address:
King Edward VIII Hosp.
_____
_____

Investigator/s:
Principal: S Montgomery
Co-investigator: _____
Co-Investigator: _____

Signature of Chief Medical Superintendent/Hospital Manager:
_____

Date: 19/02/2015

Site 2 address:
_____
_____
_____

Investigator/s
Principal: _____
Co-investigator: _____
Co-investigator: _____

Signature of Chief Medical Superintendent / Hospital Manager:
_____

Date: _____

HB: vertical Superintendent/s / Hospital Manager/s to send a copy of this document to Natalia

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

330 Langalibalele street,
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Email: hrkm@kznhealth.gov.za
www.kznhealth.gov.za

DIRECTORATE:
Health Research & Knowledge Management (HKRM)

Reference: **HRKM214/16**
KZ_2016RP25_555

19 July 2016

Dear Dr S Montgomery

Subject: Approval of a Research Proposal

1. The research proposal titled **'Evaluation of the virological response of children commenced on an Abacavir (ABC) based ART regimen at King Edward VIII Hospital from January 2012 to December 2012'** was reviewed by the KwaZulu-Natal Department of Health (KZN-DoH).

   The proposal is hereby **approved** for research to be undertaken at King Edward VIII Hospital.

2. You are requested to take note of the following:
   a. Make the necessary arrangement with the identified facility before commencing with your research project.
   b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.

3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Ms G Khumalo on 033-395 3189.

Yours Sincerely

Dr E Lutge
Chairperson, Health Research Committee
Date: 19/07/16