

The Conference on Retroviruses and Opportunistic Infections is held annually in the USA. This meeting, organised by the Foundation on Retroviruses and the Centers for Disease Control, has a mission to provide a forum for basic scientists and clinicians to present, discuss, and critique their investigations into the biology and epidemiology of human retroviruses and the diseases they produce with the ultimate goal of translating laboratory and clinical research into progress against the AIDS epidemic. The 14th conference was held in Los Angeles from 25 to 28 February 2007. This conference is relatively small with steep competition to get abstracts accepted for posters and presentations, yet South Africa has a growing representation at this prestigious and excellent meeting. The *Journal* invited a selection of South African authors whose abstracts were accepted and published to describe the background and relevance of their work and contextualise their findings for us in South Africa.

### SEVERE HYPERLACTATAEMIA COMPLICATING ANTIRETROVIRAL THERAPY WITH STAVUDINE FIRST- LINE THERAPY IN SOUTH AFRICA: INCIDENCE, RISK FACTORS AND OUTCOMES

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Symptomatic hyperlactataemia (SHL) and lactic acidosis (LA) result from mitochondrial toxicity caused by nucleoside reverse transcriptase inhibitors (NRTIs) (especially stavudine (d4T) and didanosine (ddI)). High rates of these conditions are being reported in the SA public sector ART programme. We undertook this study to document the incidence, risk factors and outcomes of severe SHL (defined as serum lactate  $\geq 5$  mmol/l) at one referral facility in Cape Town, G F Jooste Hospital. We also assessed the safety of re-challenge with AZT (an NRTI that carries a lower risk for mitochondrial toxicity than d4T) in a select group of patients with less severe presentations. G F Jooste Hospital is a public sector hospital which serves a population of approximately 1.5 million people. There are 6 primary care ART clinics which refer to G F Jooste. There were two parts to the study. The first was a retrospective observational study on patients referred to G F Jooste Hospital with severe SHL. All patients with a lactate  $\geq 5$  mmol/l attributed to NRTIs during the period 1 August 2003 to 30 November 2005 were included. We calculated cumulative exposure to ART among patients attending the 6 ART clinics to derive an estimated rate of referral. Secondly, a matched case-control study which used incidence density sampling and was based on the cases sampled in the observational study outlined above was conducted. For practical reasons, controls were randomly selected from the same cohort (the same month commencing ART and at the same clinic – i.e. matched on facility and duration on ART) as each case.

#### MAJOR FINDINGS

##### OBSERVATIONAL STUDY

Seventy-three patients were diagnosed with severe SHL during the study period. During this period there was a cumulative

exposure to ART of 7 080 patient years at all 6 ART clinics in the referral area, resulting in an estimated rate of referral for severe SHL of 10/1 000 years of treatment.

Sixty-nine patients (95%) were female. All 73 patients were on d4T-containing regimens, or had been switched off d4T in the preceding few weeks. The median duration on ART was 10 months (IQR = 8 - 11.3). The median serum lactate was 7.6 mmol/l. Thirteen patients (18%) had standard bicarbonate (SBC)  $\geq 20$  mmol/l, 49 patients (67%)  $< 20$  mmol/l (i.e. lactic acidosis), and in 11 (15%) SBC was not measured.

Eleven patients died acutely (15%), and 1 patient died 4 months later. SBC below 15 mmol/l was the only risk factor consistently associated with acute mortality in univariate and multivariate modelling (OR = 35,  $p = 0.004$ , adjusted for age).

##### MANAGEMENT AND OUTCOME

All patients were managed acutely according to a management guideline which included general supportive therapy, vitamin supplementation and treatment of complications. ART was immediately interrupted in 66 patients. In the other 7 patients d4T was switched to AZT and ART was not immediately interrupted. However, in 5 of these 7 patients there was continued clinical or biochemical deterioration necessitating ART interruption. Thus 71 patients in total interrupted ART.

Of the 62 initial survivors, 3 were lost to follow-up and 59 patients were re-established on safer ART regimens. These included the 2 successful switches, plus 57 patients rechallenged with safer ART regimens once lactate levels had normalised after a mean 87-day treatment interruption.

The outcomes of those re-established on ART were: 29 patients with less severe presentations (all these patients had lactates  $< 10.4$  mmol/l and SBCs  $> 14$  mmol/l and none had pancreatitis) were restarted on an ART regimen which contained AZT and 3TC, with lactate monitoring. One of these patients was lost to follow-up. The remaining 28 patients were still in care on the same regimen at the time of data censure, with no recurrence of hyperlactataemia. A cumulative follow-up of 1 137 weeks (mean = 39 weeks) was available on these patients without recurrence of SHL. The other 30 more severe cases were re-initiated on a tenofovir-containing regimen or an NRTI-

sparing regimen (i.e. Kaletra + NNRTI). There were no recurrences among these patients.

## MATCHED CASE-CONTROL STUDY

### DEMOGRAPHIC AND CLINICAL FEATURES AT BASELINE ASSOCIATED WITH SUBSEQUENT SHL

In multivariate analysis, compared with people with a baseline weight below 60 kg, those between 60 and 74 kg were 5 times more likely to experience severe SHL (95% CI 1.6 - 15.4) while those over 75 kg had an increased 19-fold risk (95% CI 4.8 - 77.1). The odds ratio for females was 44.2, with a wide confidence interval due to the paucity of men in the study (95% CI 6.4 - 303.8).

### CLINICAL VARIABLES ASSOCIATED WITH SHL DURING FOLLOW-UP

In a regression model, patients having at least one of the listed major symptoms (abdominal pain, diarrhoea, nausea, and vomiting) within 80 days prior to case presentation were 18 times (95% CI 3.5 - 97.6) more likely to present with severe SHL. Weight gain of  $\geq 6$  kg in the first 3 months on ART was a further clinical association with severe SHL, with an odds ratio of 11 (95% CI 1.9 - 67.5). Patients with weight loss of  $\geq 3$  kg during the last 3 months prior to diagnosis were 12 times (95% CI 2.2 - 62.1) more likely to present with severe SHL. Concurrent peripheral neuropathy was also found to be independently associated with developing severe SHL (OR 8.4, 95% CI 1.4 - 51.8).

## WHAT ARE THE IMPLICATIONS FOR CLINICAL PRACTICE?

The rate of severe SHL (referral rate of 10/1 000 patient treatment years) was higher than that reported from many developed-world settings. This is likely due to uniform use of d4T in first-line therapy, but may also be related to the risk factor profile of patients starting ART in SA. In order to minimise the morbidity and mortality related to this condition it is important to develop strategies to address prevention, earlier diagnosis and appropriate management.

Preventive measures may include:

- Changes in drug regimens on a programme level: an example would be substituting d4T with tenofovir, a drug which has not by itself been associated with lactic acidosis.
- Dose reduction of d4T: the WHO now recommends d4T be dosed at 30 mg bd in all patients regardless of weight. This is anticipated to reduce mitochondrial toxicity rates.
- Strategies focusing on high-risk patients: our study identified female gender, higher weight and rapid weight gain after starting ART as risk factors for severe SHL. One strategy that has been advocated and is supported by these data is to start overweight women on AZT (or tenofovir) rather than d4T and to switch those who become overweight on ART from d4T to AZT (or tenofovir).

To facilitate early diagnosis it is essential that a high index of suspicion for SHL is maintained. Clearly those at highest risk (overweight women) should be monitored most closely. Our study shows that symptoms such as abdominal pain, diarrhoea, nausea, and vomiting, weight loss  $\geq 3$ kg as well as

symptoms of neuropathy are important heralds of the condition. Also, most patients (85%) in this study presented with severe SHL after having been on ART for between 6 and 14 months. This period is thus the critical time to monitor for symptoms of SHL and weight loss.

Management of patients with severe SHL involved stopping drugs and a range of supportive measures.<sup>1</sup> It is worth noting that in 7 patients d4T was switched to AZT and ART was not immediately stopped. This strategy however failed with 5 of these patients deteriorating and requiring that ART be stopped. It is thus advisable that in all patients presenting with SHL and lactate  $> 5$  mmol/l ART be immediately stopped.

Once ART was stopped it took a mean of 3 months for the lactate to normalise so that ART could be re-initiated. We were encouraged to find that our practice of re-challenging with an AZT-containing regimen in a group of 29 patients with less severe presentations (all had lactate  $< 10.4$  mmol/l and SBC  $> 14$  mmol/l and none had pancreatitis) was well tolerated with no recurrences of SHL. Lactate levels and symptoms were closely monitored in these patients on rechallenge. This provides a Kaletra-sparing alternative, while we wait for tenofovir to be available in the public sector, for carefully selected patients who can be monitored on rechallenge.

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## ASSESSING THE RISK OF CONTAMINATION BETWEEN SAMPLES DURING THEIR EXCISION FROM DRIED BLOOD SPOTS FOR HIV-1 DNA PCR TESTING

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It is now well established that early infant diagnosis is critical for implementation of early antiretroviral (ARV) treatment, stratification of health care services, monitoring the success of prevention of mother-to-child transmission (PMTCT) programmes, and reducing maternal anxiety. The diagnosis of HIV below 15 - 18 months of age requires the use of virological nucleic acid testing strategies. Qualitative DNA-based assays such as the Roche DNA polymerase chain reaction (PCR) assay have stood the test of time in South Africa, being conducted at 6 weeks of age with sensitivities and specificities of 98.8% and 99.4%, respectively.<sup>1</sup> Local work has also revealed that the collection method of choice for clinical sites is the dried blood spot (DBS), which facilitates easy collection by relatively unskilled staff and reduces transport difficulties. The performance of the Roche PCR assay using DBS has been evaluated and demonstrates comparable results to those obtained on liquid blood samples.<sup>2</sup> DBS prepared from capillary (e.g. heel prick) versus venous blood in 206 children also yields highly accurate HIV DNA PCR results with a sensitivity of 98.3% and specificity of 98.7%.<sup>3</sup>

Concerns have been raised over the following aspects of large-scale implementation of this technology in South Africa: (i)

## EARLY MORTALITY AMONG PATIENTS WITH HIV-ASSOCIATED TUBERCULOSIS IN AFRICA: IMPLICATIONS FOR THE TIME TO INITIATION OF ANTIRETROVIRAL TREATMENT

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the need for automation of this methodology; and (ii) concerns of contamination when spots are punched out either manually or via an automated punch. The issue of automation has been addressed at several levels within the laboratory: (i) automation of the punching step, using the BSD1000 GenePunch; (ii) at the extraction step, by conducting DNA extraction from both liquid blood samples and DBS using automated extraction systems such as the Roche MagNapture instrument; and (iii) investigation of more automated amplification and detection systems, such as the Roche Taqman DNA qualitative assay, which are ongoing studies.

We investigated the second concern expressed by laboratory scientists, that of contamination. In this study, the risk of contamination between samples was assessed during the excision of DBS for HIV-1 DNA PCR testing using a manual punch and an automated punching system.

For both the manual and the automated punch, a spot from a known HIV-negative patient was excised after a spot from a known HIV-infected patient. For the hand-held punch, 3 to 4 × 6 mm discs (± 50 µl) from a total of 372 samples using three different cleaning methods applied between each sample was evaluated. Cleaning methods included: (i) punch swabbed with Virkon/ethanol; (ii) punching a clean card; and (iii) no cleaning (N = 124 for each method). For the automated punch investigation, 7 × 3.2 mm discs were excised per spot (± 75 µl) from 202 samples and a clean card punched between each sample. This was followed by genomic DNA extraction from the discs followed by amplification and detection using the Roche Amplicor HIV-1 DNA assay version 1.5.

The manual punching method produced no false-positive HIV DNA PCR results. We obtained 1 and 3 equivocal results on HIV-negative samples with cleaning interventions (i) and (ii), respectively. This may represent a degree of contamination that was insufficient to affect the assay's specificity. The automated punch yielded 2 equivocal and 2 false-positive results (specificity 98%). Of the latter, 1 sample of DNA extracted from the same disc produced a negative HIV DNA PCR result, confirming that contamination had arisen downstream from the excision step. The other sample for which a false-positive result was obtained could not be retested (specificity 99%).

We concluded that available punching methodologies for DBS excision present very low risks of contamination. The automated punch option, although working well, is complicated by cost and significant space requirement. This work, together with publications cited above, suggest there can no longer be any reason for not scaling up early infant diagnosis in South Africa.

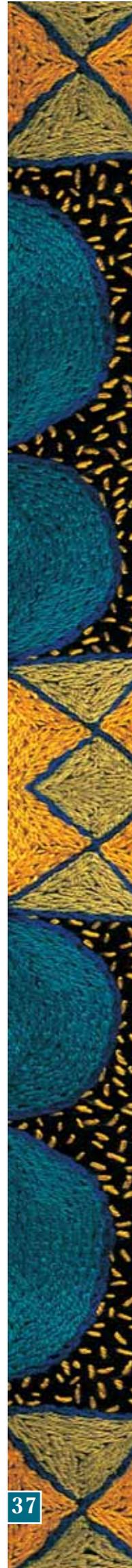
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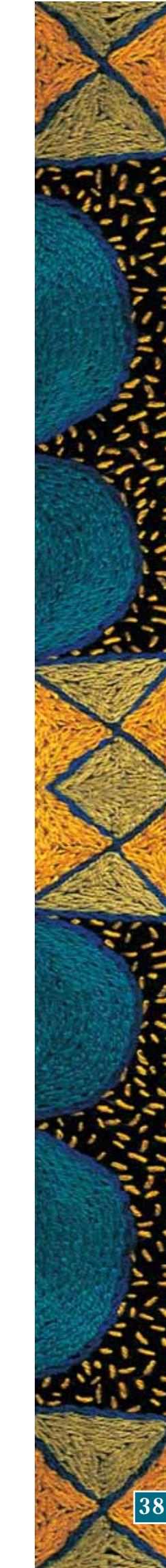
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The HIV epidemic has been associated with major increases in tuberculosis (TB) notification rates in South African townships over the past 10 years, principally among young adults.<sup>1</sup> Not surprisingly, as antiretroviral treatment (ART) clinics have been established in these communities, TB has emerged as a key challenge. The burden of TB within ART clinics is very high. We have previously reported from an ART service in Gugulethu, Cape Town, that among patients enrolling in the clinic, 52% have previously been treated for one or more episodes of TB, 26% have an active diagnosis of TB and a further 10% develop TB during the first year of ART.<sup>2</sup> Overall, during the first year from enrolment, approximately one-third of patients receive concurrent antituberculosis treatment and ART.

The impact of this huge burden of TB within ART services in sub-Saharan Africa has not been fully characterised, but it might be expected to contribute to the high early mortality in programmes in the region.<sup>3</sup> Indeed, we have previously reported from the clinic in Gugulethu that patients who have TB at entry to the programme have a 2-fold greater mortality risk in the first year of ART compared with those who remain TB-free.<sup>2</sup> While not altogether surprising, this observation nevertheless warrants careful examination to gain a better understanding of the factors contributing to this high early mortality as some deaths may be preventable.

Firstly, it might be expected that among patients receiving concurrent TB treatment and ART, pharmacokinetic drug interactions and impaired treatment adherence (due to high pill burden, reduced regimen tolerability and overlapping toxicity profiles) would undermine responses to ART and thereby increase mortality risk. However, we and others have found this not to be the case, with good immunological and virological responses being observed among patients receiving rifampicin-containing TB treatment and efavirenz-based ART in standard dosages.<sup>2,4,5</sup> Secondly, concurrent use of rifampicin with nevirapine, another non-nucleoside reverse transcriptase inhibitor (NNRTI), has been associated with reports of severe hepatotoxicity.<sup>6</sup> However, in Gugulethu where efavirenz is the NNRTI routinely used and where monitoring of serum hepatic transaminases is available, no deaths attributable to efavirenz/rifampicin co-toxicity have occurred.<sup>7</sup> Thirdly, initiation of ART among patients receiving treatment for TB is commonly associated with immune reconstitution disease (frequently referred to by clinicians as immune reconstitution





inflammatory syndrome or 'TB IRIS'). However, although we have found that IRIS is common among patients with advanced immunodeficiency starting ART early in the course of TB treatment in the Gugulethu clinic, it is most commonly self-limiting and is an infrequent cause of death.<sup>8</sup> Indeed, IRIS associated with cryptococcal disease is a far more important cause of mortality than TB IRIS in this setting.<sup>9,10</sup>

None of the above factors therefore account for the high early mortality among TB patients accessing ART. A further critical question, though, is whether the timing of initiation of ART affects mortality risk among TB patients. This is potentially a very important variable, as it is one over which the clinician has direct control. We have previously shown that the mortality rate of patients entering the Gugulethu ART service is extremely high and even short delays in ART initiation may potentially be associated with appreciable mortality risk.<sup>7,11</sup> We therefore decided to study in more detail the early mortality among TB patients accessing ART with a focus on the timing of treatment initiation and presented these data at CROI.<sup>12</sup>

### WHY WAS THIS STUDY DONE?

The aim of this study was to determine the mortality risk associated with TB among patients enrolling in the ART service in Gugulethu and to assess the association between mortality and the timing of ART initiation during TB treatment. These analyses were done to provide insights into the appropriate timing of ART in patients with HIV-associated TB.

### WHAT DID THE RESEARCHERS DO AND FIND?

Mortality occurring in the two intervals between programme enrolment, initiation of ART and the first 16 weeks of treatment was prospectively studied among patients with ( $N = 213$ ) and without ( $N = 675$ ) TB accessing the ART service in Gugulethu, Cape Town. The mortality rate among those with TB was 1.8-fold (95% confidence interval (CI) = 1.62 - 2.80) greater than that of patients who were TB-free (40 versus 21 deaths/100 person-years;  $p = 0.003$ ). When the TB patients were subdivided into those with active TB ( $N = 73$ ) or inactive TB ( $N = 140$ ), the mortality rate was significantly greater in both groups (44 and 37 deaths/100 person-years, respectively) compared with those who were TB-free (21 deaths/100 person-years) ( $p < 0.01$  for each comparison).

Patients with TB, however, had lower CD4 cell counts than those who were TB-free, and this was potentially an explanation for the higher mortality rates observed. We therefore did multivariate analysis to assess risk factors for mortality in the whole cohort. In this analysis, TB (either active or inactive) was no longer significantly associated with mortality risk. Instead, mortality risk was only independently associated with baseline CD4 cell count  $< 100$  cells/ $\mu$ l (adjusted hazards ratio (AHR) = 2.85, 95% CI = 1.52 - 5.34) and WHO clinical stage 4 disease (AHR = 2.94, 95% CI = 1.80 - 4.82). This analysis therefore showed that the high excess mortality risk among patients with TB was largely explained by advanced immunodeficiency and not with TB disease activity or even with diagnoses of TB *per se*. This is consistent with findings in cohorts in Zambia and Malawi.<sup>13,14</sup> Since mortality risk is predominantly associated with immunodeficiency, delays in the initiation of ART should be minimised.

We next examined the association between the timing of ART initiation in TB patients and mortality risk. Of patients with TB diagnosed within the programme prior to ART initiation ( $N = 73$ ), 48 had received ART by data censorship after a median of 42 days from TB diagnosis. A total of 14 patients died. Just 4 deaths occurred after initiation of ART of which 2 were due to immune reconstitution disease. However, 10 deaths (71%) occurred in patients waiting to commence ART, most ( $N = 8$ ) within the first 6 weeks of antituberculosis treatment. All those who died waiting to commence ART had either a CD4 cell count  $< 100$  cells/ $\mu$ l or WHO stage 4 disease.

These data are observational (non-randomised) and therefore potentially subject to bias. However, baseline patient and disease characteristics did not differ when comparing those who died with those who survived or when comparing those who did or did not receive ART. Mortality risk was only associated with ART status. However, if ART were commenced earlier, it is not known what proportion of deaths might be prevented and also to what extent TB IRIS would become a greater problem.

### WHAT DOES THIS MEAN?

These data are important with regard to the optimal timing of commencement of ART in patients with TB. There is no consensus between various national and international guidelines in respect of this timing. Moreover, it will be several years before the results of randomised controlled trials will become available to address this issue definitively. In the interim, these observational data therefore provide important insights for patients being treated in South Africa and elsewhere in sub-Saharan Africa.

Collectively these data indicate that patients with TB have high mortality risk due to advanced immunodeficiency and that with the current median delay of 42 days in this programme between TB diagnosis and starting ART, many patients die waiting to start ART. Clearly, the mortality risk associated with delays in ART in this service greatly exceeded any mortality risk associated with early initiation of ART (e.g. due to TB IRIS). Those at greatest risk of death were those with CD4 cell counts  $< 100$  cells/ $\mu$ l and those with WHO stage 4 disease. Such patients should be prioritised in respect of early initiation of ART.

The optimal time for ART initiation cannot be determined from these non-randomised data. However, the current WHO guidelines for resource-limited settings recommend that TB patients with CD4 cell counts  $< 200$  cells/ $\mu$ l should commence ART between 2 and 8 weeks of ART.<sup>15</sup> Data from this study strongly suggest that those with CD4 cell counts  $< 100$  cells/ $\mu$ l and WHO stage 4 disease should commence ART as early as possible within this time-frame (i.e. after 2 weeks of TB treatment).

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## ADOLESCENTS AND HIV VACCINE TRIALS

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The incidence and prevalence of HIV infection among adolescents and young adults in South Africa is extremely high, particularly among girls. Data from national seroprevalence surveys estimate the prevalence of HIV to be 9.4% among 15 - 19-year-old girls and 23.9% in women aged 20 - 24.<sup>1</sup> Four young women between the ages of 15 and 24 are infected for every man in the same age group. Despite current efforts, including HIV prevention programmes targeted to youth, HIV infection rates have shown no sign of decreasing. The lack of a substantial decline in HIV incidence among young women in South Africa may be attributed to the marked changes in society seen since the transition from apartheid with concomitant rapid rates of urbanisation, disintegration of family structures, and a general lack of understanding of adolescent sexuality, current youth attitudes and practices. Currently HIV prevention programmes within South Africa have not been context-specific, which may have contributed to the lack of appreciable behaviour changes seen, despite high levels of HIV/AIDS awareness. As South Africa will be the first country to enrol adolescents aged 16 - 18 years into a phase IIb efficacy HIV vaccine trial (the HVTN 503 study), it is imperative to understand some of the challenges to adolescent enrolment in HIV vaccine trials.

Studies performed to date in Soweto include a cross-sectional survey of knowledge and attitudes to HIV/AIDS and HIV vaccines and willingness among adolescents and their

stakeholders to participate in a hypothetical study, as well as a small longitudinal cohort study that assessed sexual risk behaviour and knowledge and attitudes to HIV among Soweto youth aged 12 - 21.

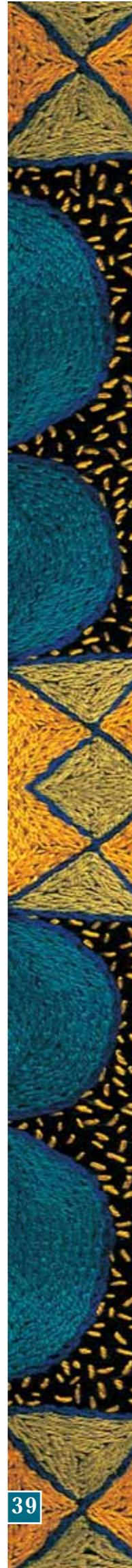
### ADOLESCENT INVOLVEMENT IN VACCINE TRIALS

A two-stage sampling procedure was used. The first-stage sampling units were all 72 public high schools in Soweto. Ten schools were randomly selected and the first four were approached regarding participation. All pupils in the selected schools from whom parental consent and child assent could be obtained were eligible for participation. A self-administered, facilitated questionnaire was completed by participants. Two hundred and seventy-seven school-going youth (mean age 16.2 years, range 10 - 25, 53.1% female) participated in this survey. Of 240 responses to the willingness item, 84 (35%) indicated they were probably and 126 (52.5%) definitely willing to join a study of a vaccine to prevent HIV. There were no significant differences in willingness by gender, age, school grade, or institution. Factors rated as 'very important' in determining willingness included receiving current information about HIV research ( $N = 209$ , 88.2%), getting free counselling and testing every 6 months ( $N = 168$ , 70%), indicating that participants would be doing something to honour people who have HIV/AIDS or have died of AIDS ( $N = 168$ , 70%), and that participants may receive some protection against HIV infection from the vaccine ( $N = 167$ , 70.5%). Some misconceptions regarding vaccine research were common, particularly regarding placebo and potential eligibility criteria for vaccine trials. Soweto school-going youth report high degrees of willingness to participate in HIV vaccine trials. Whether hypothetical willingness translates into participation will await data from adolescent HIV vaccine trials.

### ADULTS' PERCEPTIONS REGARDING ADOLESCENT PARTICIPATION IN VACCINE TRIALS

This study was conducted in Soweto between August 2005 and March 2006. The participants for this study were recruited using convenience and snowballing techniques as well as through community outreach. A self-administered questionnaire in English was completed by participants, with facilitation by study staff. The sample size was 64 (mean age, 41.3 years), 57% of participants were women, 55 were parents and 9 were teachers. Regarding adolescent sexual risk, of the 64 adults who participated in this research, 31 (55%) believed that the sexual debut of Soweto children may be as early as age 9 and 39 (70%) believed that these children were vulnerable to HIV infection. Sixty per cent ( $N = 33$ ) of participants believed that adults spoke to their 9 - 10-year-olds about HIV. Most adults indicated that they would want to know if their child is sexually active (91%) and that should their child participate in an HIV vaccine trial, they would want to know their HIV test results.

A great concern to researchers involved in HIV vaccine research is the issue of 'social harm' that trial participation may cause. Potential social harms that may impact on trial participation include the perception of being at high risk of HIV acquisition, or being thought to have AIDS. In this study, more than half the adults sampled did not believe that the perceived stigma of HIV vaccine trial participation would impact on their decision to allow their adolescents to



participate in a HIV vaccine trial (Table I). The relationship between trial participation and stigma in this research did not distinguish between participation in phase I, II or IIb/III trials and the potential distinction made in the trial eligibility criteria with regard to the sexual risk profile of participants. Moreover, the figures presented here reflect attitudes of an uninitiated population. It is not beyond reason that attitudes towards HIV vaccine trial participation may change with better understanding of the clinical trial process.

Willingness to participate in a hypothetical HIV vaccine trial may not reflect participation in an actual trial. This notwithstanding, 44 (80%) indicated willingness ('Probably would' (27%) or 'Definitely would' (53%)) to allow adolescent participation and 51 (91%) indicated that they would also want to be involved in the research. Of the respondents 48 (86%) indicated that they have confidence in medical research; however, 9 (16%) thought that HIV vaccine research was unsafe and 18 (32%) were unsure whether vaccine research is unsafe. Factors cited as important for deciding on trial participation included potential benefits of participation, such as access to information and counselling; potential impact on risk behaviour; and potential protection against HIV from the vaccine. Participation was also seen as altruistic and socially beneficial. Access to counselling and testing, current information, and potential impacts on improving motivation to reduce risk behaviour were very important for determining willingness to participate.

The Soweto community, located within Gauteng province where an estimated 14.5% of its population is estimated to be HIV infected,<sup>1</sup> is heavily affected by HIV. It is therefore not surprising to see the high level of HIV discourse occurring and the low levels of reported stigma. Most (49/50) of the adults we sampled had spoken to others about HIV, 43/48 had spoken to family members, 30/50 had ever been tested for HIV, and only 9/50 felt afraid of people with HIV while 12/50 felt uncomfortable around people with HIV.

#### ADOLESCENT HIV PREVENTION AND VACCINE PREPAREDNESS STUDY

This study was conducted to demonstrate that adolescents could be retained in a longitudinal cohort study. This study also assessed over time: risk behaviour; willingness to participate in vaccine trials; knowledge and attitudes towards

TABLE I. STIGMA AND HIV VACCINE TRIAL PARTICIPATION

Factor	How important to decision? (N (%))			
	Very	Somewhat	Slightly	Not at all
My child may be discriminated against at school	12 (22)	4 (7)	9 (16)	30 (55)
People may avoid my child	12 (22)	6 (11)	7 (13)	30 (55)
People may think my child has HIV or AIDS	5 (9)	8 (15)	4 (7)	38 (69)
People may think my child is at high risk of HIV or AIDS	5 (9)	9 (16)	10 (18)	32 (57)
People may not want to have sex with my child	15 (27)	5 (9)	9 (16)	27 (58)

HIV/AIDS; and documented for the first time in an African setting, family and social networks of participants, enabling a more dynamic delineation of risk. From July 2005 to November 2005, 52 'index' adolescents (adolescent initially contacted and enrolled) and 19 'alter' adolescents (adolescents referred by the index from their family or social network) were enrolled into the study (Table II). Overall, 69 participants were eligible for follow-up. More than 50% of participants were female. Most (56) participants were in school, with the median year of schooling being approximately 10 years. Only 5 participants had previously had access to HIV testing and counselling. Most participants (56/65) were willing to undergo HIV testing and counselling. Twenty-six (37.7%) participants reported that they had had penetrative sex. However, only 9 (35%) reported consistent condom use, with 3 (11.5%) reporting that they had never used a condom, and 5 (19%) reporting use of condoms for more or less half the time.

Birth control was not widely used, with only 10 (26%) participants using condoms as a birth control method, 1 participant using withdrawal as a contraceptive method, and 1 participant using hormonal contraception. One participant reported ever having been pregnant. Preliminary data available from the 2-week post-prevention intervention session designed to deal with HIV vaccine research demonstrated that adolescents need more information and education to understand some of the fundamentals of HIV vaccine research. The concept of placebo was not fully understood; some adolescents (15%) believed that an HIV vaccine could give one HIV. The issue of vaccine-induced seropositivity appeared to be understood by most participants. Approximately two-thirds of adolescents were willing to participate in HIV vaccine trials and almost 70% were comfortable about referring other adolescents or family members for participation in such trials. Interestingly, most adolescents were willing to participate in future vaccine research. There appeared to be few barriers cited for vaccine trial participation. Motivators for being involved in vaccine trials were mostly related to altruism or a desire to learn more about HIV/research. Almost a quarter of participants indicated that receiving reimbursement for trial participation would be a reason to be involved in research. Thirty per cent of adolescents indicated they would be wary of trial participation because of potential side-effects, 20% indicated that they needed more information before they could make up their minds regarding trial participation, and 60% agreed that they were at risk of HIV acquisition and therefore would consider trial participation.

TABLE II. DEMOGRAPHICS OF ADOLESCENTS PARTICIPATING IN THE HIV PREVENTION AND HIV VACCINE PREPAREDNESS STUDY

	Index (N = 50)	Alter (N = 19)
Gender	25 female	11 female
Median (yrs)	16.68	17.05
Soweto residents	45	17
Ethnicity	Black = 50	Black = 18*
Scholars	37	19
Median years of education (IQR)	9.78	10.44
Sexually active	18	8
Drug use	6	1
Alcohol use	32	14

\*Missing = 1.

## CONCLUSION

Taken together, these preliminary studies indicate a high degree of willingness to participate in HIV vaccine trials among Soweto youth and adults. The need for regular risk reduction counselling was highlighted as an important benefit by both youth and adults, indicating the perceived need for developmentally and culturally tailored tools, as well as for services for youth. The adolescent vaccine preparedness study has been critical to developing site expertise in studies with adolescent participation. This programme also highlighted several key developmental issues that would require adaptation of existing risk reduction counselling formats and content to fully address the needs of Soweto youth.

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## EMERGING HIV-1 DRUG RESISTANCE PATTERNS FROM TWO JOHANNESBURG CLINICS ON THE SOUTH AFRICAN ARV ROLL-OUT PROGRAMME

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The South African government began the antiretroviral (ARV) roll-out programme in April 2004, and over 250 000 AIDS patients have been enrolled to date. The programme uses two standardised regimens for all patients accessing care, with the first-line regimen consisting of lamivudine (3TC), stavudine (d4T), efavirenz (EFV)/nevirapine (NVP) and the second line consisting of didanosine (ddI), zidovudine (AZT) and Kaletra. There are already reports of treatment failures in South African HIV-1 subtype C-infected patients accessing ARV drugs, attributed to the emergence of drug-resistant viruses. However, there are currently no published data available on the development of ARV drug resistance (DR) in South African HIV-1 infected individuals on the chosen public sector regimens. Routine resistance testing is currently not available in South Africa owing to prohibitive costs of current sequencing-based assays.

There is no consensus yet in the literature about the possible effects of the genetic diversity of HIV-1 on the development of DR, although several publications describe subtype-specific DR mutations/polymorphisms. ARV DR studies conducted in subtype C-infected individuals failing therapy from Zimbabwe,<sup>1</sup> Brazil,<sup>2</sup> Ethiopia,<sup>3</sup> and Botswana<sup>4,5</sup> revealed that HIV-1 subtype C developed similar ARV mutation profiles to HIV-1 subtype B. However, comparisons of subtype C reverse transcriptase (RT) and protease (PR) sequences to subtype B sequences, and corresponding clinical data have revealed subtype C-specific polymorphisms that impact on treatment outcome. For example, V106M has previously been shown to be a subtype C-specific mutation in patients failing EFV,<sup>6,7</sup> and is a result of a natural polymorphism that occurs at this codon. The K65R mutation may emerge at a higher frequency in HIV-1 subtype

C-infected patients on certain nucleoside reverse transcriptase inhibitor (NRTI)-containing regimens,<sup>5</sup> and its rapid emergence has been shown to confer resistance to tenofovir in cell culture.<sup>7</sup> The K103N mutation occurred at a greater frequency and higher levels in women infected with subtypes C and D as opposed to subtype A.<sup>8</sup> In addition, the pathways leading to non-nucleoside reverse transcriptase inhibitor (NNRTI) and protease inhibitor (PI) resistance may be subtype-specific. For example, Grossman *et al.*,<sup>9</sup> and Douella-Bell *et al.*,<sup>5</sup> showed that HIV-1 subtype C-infected patients on nelfinavir-containing regimens developed resistance to this PI through distinct mutational pathways from subtype B. A number of other studies have confirmed the presence of baseline polymorphisms in subtype C in the protease regions.<sup>10,11</sup> These data confirm the need for continued evaluation of drug resistance patterns in HIV subtype C.

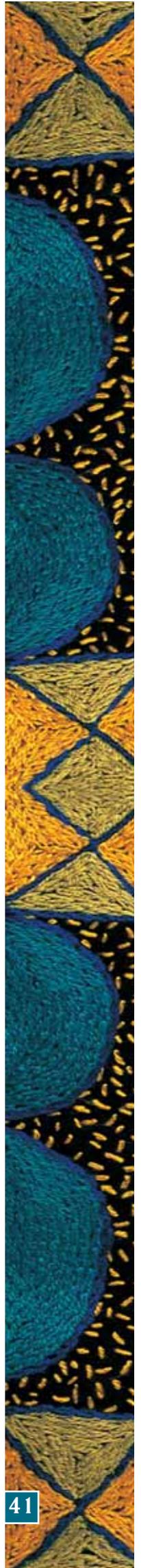
In an effort to begin to understand the evolution of HIV-1 subtype C drug resistance (HIVDR) in South Africa, a pilot study was performed in patients demonstrating treatment failure in two Johannesburg clinics. This study looked at HIVDR patterns emerging in patients failing either the first- or second-line regimens or a combination thereof.

One hundred and fifteen patient samples from the two Johannesburg clinics were sent for HIVDR testing. Clinics 1 and 2 defined virological failure differently. Clinic 1 defined it as HIV RNA levels greater than 1 000 copies/ml on two consecutive visits, whereas at clinic 2 it was classified by a repeated viral load greater than 5 000 copies/ml. The average viral load from patients failing therapy at clinic 2 was found to be 0.8 log higher than clinic 1. The RT mutation patterns seen in our two cohorts have been documented previously in subtype B, with a few exceptions. The mutations that occurred at the highest frequencies (>10%) at both clinics were M184V, K103N, V106M, G190A, D67N and can be directly attributed to the ARV drug pressure exerted from the prescribed regimens in South Africa. However, the ARV DR mutation profiles in patients failing therapy were different in the two clinics, with clinic 2 appearing to have significantly more complex resistance profiles for the RT-associated mutations. These complex mutation patterns may be a result of leaving patients on a failing regimen for an extended period of time.

The higher frequency of the K65R mutation, 7.87% and 11.54% at clinics 1 and 2, respectively, is unusual as it is not commonly associated with the prescribed regimens. Two factors may potentially be contributing to this increase in K65R. Firstly, studies have shown that patients left on failing regimens accumulate several mutations, one of which is K65R, which may be the case in clinic 2. Secondly, the bases at this codon are different from those in HIV-1 subtype B and may have resulted in a faster switch to the K65R mutation.

There was also an increase of thymidine analogue mutations (TAMs) at clinic 2, which accumulate over time and confer cross-resistance to most NRTIs, making salvage therapy difficult. Previous studies have suggested that a delayed switching causes complex resistance patterns to arise (as seen in clinic 2), resulting in reduced susceptibility to most if not all NRTIs and the PIs being the only effective drug in the second-line regimen.

As in other studies, both clinic 1 and 2 showed a high prevalence of secondary mutations and several naturally occurring polymorphisms in the PR region. The presence of these polymorphisms in HIV-1 subtype C PR region may have



an impact on the efficacy of the PI drugs that will be included in future drug regimens.

## CONCLUSION

ARV drug treatment failure in HIV-1 subtype C-infected patients is associated with the development of DR, and these mutation patterns are similar to subtype B. The data suggest that the longer the treatment is continued in the presence of drug-resistant viruses, the more DR mutations accumulate. Viral load monitoring of HIV treatment may therefore be important even in resource-poor countries. High-level DR mutations that lead to broad-class DR for NRTIs were demonstrated, providing concern for the use of tenofovir in second-line treatment, and strategies of recycling NRTIs in second-line treatment. Finally, these preliminary findings need to be further investigated and confirmed on a larger sample size in a controlled study.

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## OUTCOMES OF CHILDREN ON NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR VERSUS PROTEASE INHIBITOR HAART REGIMENS IN RESOURCE-LIMITED SETTINGS

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Few data exist as to the most effective highly active antiretroviral therapy (HAART) regimens for children with

vertically acquired HIV infection. Most countries in southern Africa have limited regimens based on nucleoside analog reverse transcriptase inhibitor (NRTI) backbones combined with either a non-NRTI (nNRTI) or a protease inhibitor (PI). The World Health Organization (WHO) recommends nNRTI-containing regimens as the first line for children despite the fact that nevirapine (NVP) is often used for vertical transmission prevention (PMTCT) in these settings. In addition, recent evidence suggests that recommended dosing for NVP and efavirenz (EFV) in children may be too low.

## WHY WAS THIS STUDY DONE?

1. To evaluate the outcomes of children on HAART in a resource-limited setting.
2. To compare laboratory and clinical outcomes of children on PI versus nNRTI-containing regimens.
3. To determine the correlates of virological suppression (VS).

## WHAT DID THE RESEARCHERS DO AND FIND?

This study describes the outcomes of a cohort of 389 children on HAART treated in a public sector hospital-based antiretroviral programme. Children were started on HAART that included two NRTIs, and either an nNRTI (NVP or EFV) or a PI (lopinavir/ritonavir or ritonavir). Approximately 50% of the children received a PI ( $N = 199$ ) v. nNRTI ( $N = 188$ ). Few children in this group were exposed to NVP for PMTCT.

The median age at baseline was 26.3 (intraquartile range (IQR) 12.4 - 53.8) months, the median baseline CD4 percentage 13% (IQR 8.0 - 17.0%) and median viral load 5.5  $\log_{10}$  (IQR 4.8 - 6.0  $\log_{10}$ ). The median baseline weight-for-age Z-score (WAZ) was -2.5. There was no significant difference in baseline characteristics of patients on the different regimens with viral load, CD4 percentage or WAZ; however infants initiating treatment with an nNRTI were older (34.3 v. 21.8 months,  $p < 0.01$ ).

There were obvious improvements in the infants in all parameters after the initiation of HAART. The overall increase in CD4% from baseline to 24 months on treatment was 13% (double that at baseline), and increase in WAZ of 1.8. An overall viral load log drop of 2.6  $\log_{10}$  occurred at 24 months of treatment. However, when comparing PI versus nNRTI regimens, a significantly better virological response was seen among those children receiving a PI at all times through 48 months, although there were no differences in CD4%, WAZ or in survival.

In a multivariate analysis predicting VS, PI-based regimens were independently associated with VS, as was CD4 percentage, WAZ, and age. This association was evident even when adjusting for year of starting HAART.

## WHAT DO THESE FINDINGS MEAN?

HAART greatly improves the health of children in resource-limited settings. However, despite profound improvements in outcomes, this study found nNRTI-based regimens inferior to PI regimens in achieving and maintaining virological suppression. This difference did not seem to have clinical implications during the study period, since there was no difference on growth, immunological outcomes, or survival. Nevertheless, the implications of detectable viraemia in

children are that resistance may develop and switches to second-line regimens may therefore occur sooner. In developing country settings where regimens are limited, the goal of HAART should be to reach and maintain undetectable viral loads for as long as possible. The effectiveness of regimens containing nNRTIs may be decreased in this setting because of less than optimal dosing, drug-drug interactions (such as tuberculosis therapy), or PMTCT programmes. There is an urgent need to further explore optimal regimens, dosing, and pharmacokinetic interaction studies for children on HAART in these settings.

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## RECRUITMENT AND SEXUAL RISK ASSESSMENT OF HIV-NEGATIVE ADOLESCENTS AGED 14 - 17 YEARS IN PREPARATION FOR HIV VACCINE TRIALS

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The majority of new HIV infections in sub-Saharan Africa occur between ages 15 and 24 years, so adolescents are important targets for HIV preventive vaccination and must be included in HIV vaccine trials. To date no HIV vaccine trials have included adolescents, in part because of the challenges associated with HIV prevention research in young people, including issues of recruitment, informed consent, confidentiality, stigma, and potential for behavioural disinhibition. Adolescents are likely to be 'hard to reach', and may be difficult to recruit into long-term studies. Phase I to III vaccine trials require participants of specific sexual risk, but adolescents may not freely admit their sexual behaviour. More information is needed to assess the feasibility of recruiting adolescents of varying sexual risk into HIV prevention research.

### WHY WAS THIS STUDY DONE?

- To assess the feasibility of recruiting HIV-negative 14 - 17-year-olds, with parental consent, from a community in which HIV vaccine trials are planned.
- To compare different methods of recruiting adolescents into HIV prevention research.
- To assess self-reported HIV risk-related perceptions, knowledge and behaviours in this group.

### WHAT DID THE RESEARCHERS DO AND FIND?

Adolescents aged 14 - 17 were recruited from a peri-urban Xhosa-speaking community. Consent was obtained from all adolescents and a parent or legal guardian. HIV and syphilis testing was performed, and pregnancy testing where

applicable. Participants completed interviewer-assisted paper questionnaires on demographics, sexual risk behaviour, HIV knowledge, and perceived risk for HIV.

There were 107 adolescents screened; 3 failed screening due to HIV infection and 3 due to pregnancy, and 1 was underage. The study was fully enrolled in 4 months. Challenges arose in obtaining consent from parents, and required after-hours home visits. Of the 100 adolescents enrolled, 98 were recruited through outreach activities.

The mean age of the cohort was 15 years, and 70% were female. All participants were attending school with a median level of education of 8th grade. In general, HIV knowledge was high, except that only 78% knew that an infected person could test negative on routine HIV testing, and only 93% thought a person with HIV could look healthy.

Risky sexual behaviour was reported by the participants, with 43% reporting sexual activity, and 30% of these reporting more than one partner in the past year. Only 19% knew the HIV status of their partner, yet 21% had never used a condom in the past 6 months. The adolescents perceived themselves to be at low risk of infection with HIV despite this behaviour, with only 3% reporting that they were at risk, and 63% believing that their bodies could fight off HIV. However, one-third felt that getting HIV would be bad for their futures and their family relationships. Among the sexually active adolescents, condom use was not associated with perceived risk ( $p = 0.32$ ). Perceived risk was not associated with HIV knowledge ( $p = 0.30$ ). Females were more likely to perceive themselves as at high risk for HIV ( $p = 0.06$ ); however, this association did not persist when adjusting for sexual activity. In a multivariate analysis predicting sexual activity, adjusting for HIV knowledge, perceived personal impact of HIV, and gender, only perceived risk for HIV was positively associated with sexual activity.

### WHAT DO THESE FINDINGS MEAN?

Recruitment for this study was relatively rapid, but required flexibility in clinic hours. Recruitment via outreach activities seems to be far more effective than through voluntary counselling and testing (VCT), a method often used for recruitment of adults. This may be due to the poor VCT attendance of adolescents in this community. The high level of sexual risk-taking in this population suggests that young people are at substantial risk of HIV infection, yet these adolescents do not perceive themselves to be at high risk for HIV. This is not due to poor HIV knowledge, as the level of HIV knowledge among these adolescents was high. HIV prevention intervention studies will therefore need strong age-appropriate risk-reduction counselling. As shown in prior studies, young females are more often sexually active, and therefore at higher risk for HIV, than young males. Yet these young women are also more aware of their HIV risk. There is an ethical imperative to facilitate inclusion of adolescents in HIV vaccine trials in order to ensure rapid licensure of a successful vaccine for this high-risk group.

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