Meeting reports

5th International Workshop on HIV Transmission – Principles of Intervention
15-16 July 2010, Vienna, Austria

2nd International Workshop on HIV Pediatrics
16-17 July 2010, Vienna, Austria
# Upcoming Workshops

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Reviews in Antiviral Therapy

Reviews in Antiviral Therapy is the official journal of abstracts and conference reports from International Workshops on the clinical management of viral diseases.

Reviews in Antiviral Therapy publishes peer-reviewed articles relating to viral diseases including HIV, Hepatitis and emerging viruses. Featured topics include clinical management, drug resistance, diagnostic applications, pharmacology, transmission & prevention. Each edition will be dedicated to a specific aspect of viral infection, focusing on the presentations from the latest international meeting on the topic.

Reviews in Antiviral Therapy aims at translating the latest key scientific and clinical findings in antiviral therapy into tangible and applicable knowledge to assist readers in routine clinical management.

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5th International Workshop on HIV Transmission – Principles of Intervention
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Antiretrovirals as an HIV prevention tool

Treatment as prevention

Dr Cohen (University of N Carolina, USA) discussed the concept of using antiretroviral treatment (ART) as an HIV prevention tool. He started his lecture by stating that he is an ‘advocate of this approach’ but stressed that its success is dependent on ‘paying attention to all of the details of the strategy’. There are many questions that need to be answered before the test and treat strategy can be implemented on a wide scale: Dr Cohen cited a few examples:

- Can ART reliably prevent HIV transmission? There is a body of evidence of its biological plausibility, as well as data from discordant couples and ecological studies, but no absolute proof.
- How should infected people and discordant couples be counselled about the capacity of ART to prevent transmission? This depends on the answer to the question above.
- How important is acute infection in the spread of HIV throughout the population? The precise role is very hard to define because identifying all cases of acute infection in a timely manner is challenging.
- If test and treat does result in HIV prevention at the population level – how can this be demonstrated?
- What impact does ART have on HIV infected cells, especially those in semen and the vaginal tract? What is the relationship between the presence of HIV RNA in the genital tract and viral infectiousness? This is technically very difficult to assess because semen and vaginal secretions inhibit cell growth in vitro. New drugs may be needed to control HIV replication in these cells.

Of the five recent prospective observational cohort studies in discordant couples, four have generated positive results while one has given a negative result. In one of the positive studies, the protection offered by ART and condoms was not complete: the reduction in the risk of HIV transmission was 80%. In a study of 1,927 discordant couples living in Henan, China, the 84 seroconversions that occurred were distributed equally amongst couples in which the HIV positive person was taking or not taking free ART. Dr Cohen commented that this study resembled ‘real world’ conditions and that lessons should be learned from it before rolling out test and treat on a wide scale.

HPTN 052 is an ongoing randomised clinical trial (RCT) in which the effects of ART in preventing HIV transmission from an HIV positive person to their HIV negative partner are being evaluated. HIV positive individuals are randomised to receive ART when the CD4 cell count is <250 cells/mm3 (deferred therapy) or immediately upon enrolment in the study (CD4 cell count 350-550 cells/mm3). The study is entering its second year and the results are eagerly awaited. Dr Cohen pointed out that the costs of ART per patient per year in this study were $10,000 and that ART was administered for at least five years, resulting in the pharmaceutical industry contributing >$15,000,000 over seven years. HPTN 052 was first discussed in 1989-2000, when it seemed impossible to supply ART to African countries. In the meantime, two new beliefs have emerged: that treatment will result in HIV prevention; and that early ART is better than late ART.

Dr Cohen cautioned that the outcomes of the models used to support the test and treat strategy are highly dependent on their key assumptions. If it is possible to identify all HIV infections and treat everyone without ARV resistance emerging, the models predict that the HIV epidemic will disappear in 20 years. Dr Cohen described these assumptions as ‘utopian’ and warned that the consequences of imperfectly implementing test and treat are unknown. Since measuring HIV incidence is problematical, monitoring changes in incidence during test and treat implementation will be very challenging. He added that there is evidence that ART does not reliably stop viral shedding in the
genital tract, even if viral replication in the blood is suppressed. Acute infection appears to play an important role in HIV transmission. Transmission of ARV resistant virus and imperfect adherence will diminish the efficacy of treatment regimens.

“Test and treat has the potential to work if we avoid hyperbole and shortcuts,” declared Dr Cohen, “We must apply rigour to the challenge.”

Microbicides and condoms

Using antiretrovirals to prevent HIV transmission is complicated by the fact that no individual pharmacokinetic (PK) measure is optimal for predicting efficacy, Dr Kashuba (University of North Carolina, USA) explained. Hence, pharmacodynamic (PD) targets for HIV prevention have not yet been established. In order to prevent HIV infection with ARVs, it will be necessary to deliver the right ARV(s) in the appropriate concentration(s) to the appropriate biological site(s) for the appropriate length of time, while avoiding the development of drug resistance.

Oral dosing with ARVs results in different drug exposure levels in the genital tract, even within the same ARV class; for example, indinavir achieves high levels (200% of plasma levels) while lopinavir is only present at very low levels (8%). The time course of mucosal infection means that ARVs need to enter the genital tract rapidly and be retained there for several days. For some drugs, such as tenofovir, the critical factor is the intracellular concentration that is achieved. The vaginal and rectal environments differ, but it is essential to ensure that any ARV-based prevention method is effective in both situations. It is not yet known if ARV protein binding differs at the mucosal surface compared to within the genital tract tissues. However, it has been established that maraviroc protein binding is 7% in female genital tract secretions compared to 15% in male genital secretions. No data are available on ARV protein binding in rectal secretions at present.

Dr Kashuba summarised the available information on compartment specific PK of ARVs with potential usage as HIV prevention agents (Table 1). She called for further studies so that informed decisions can be made about the ARVs and doses that should be used for HIV prevention.

Dr Kashuba has been involved in a study of raltegravir PK in the blood plasma and cervicovaginal fluid of HIV positive and HIV negative women; her colleague, Dr Patterson (University of North Carolina, USA) presented their results. The HIV negative women (n=7) tended to be younger; have lower body mass indices (BMI); and all were Caucasian. By contrast, the HIV positive women (n=6) were representative of the HIV epidemic in SE USA: they were older, had greater BMIs and 5/6 were black. Although raltegravir penetrated the cervicovaginal fluid in both HIV positive and negative women, there was considerable variability in the levels achieved in this fluid and in the plasma. There was no predictable relationship between blood plasma levels of raltegravir and female genital tract exposure to the ARV; in some cases there was a log difference between the levels of exposure in the two compartments.

One of the concerns about using microbicides that contain ARVs is the risk of sub-optimal levels of ARVs developing in the tissues. The window of protection offered by a gel containing tenofovir has not yet been defined, even though the high tissue drug levels and long intracellular half life of tenofovir (60-120 hr) suggest that it may be protracted. A macaque model of SHIV infection has been used to determine if a single vaginal 1% tenofovir gel, administered three days before exposure to the virus, can protect against transmission. The monkeys were challenged with physiological viral inocula twice a week and protection was measured over a ten week (approximately two menstrual cycles) period. Animals were considered

<table>
<thead>
<tr>
<th>Properties</th>
<th>Vagina/Cervix (n=20)</th>
<th>Rectum (n=1-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARV exposure within 2 hours of dosing</td>
<td>Yes</td>
<td>Yes (MRV)</td>
</tr>
<tr>
<td>ARV exposure variable and may not be easily predicted</td>
<td>Yes</td>
<td>Yes (MRV, FTC, TDF)</td>
</tr>
<tr>
<td>Less Protein Binding</td>
<td>Yes</td>
<td>Unknown</td>
</tr>
<tr>
<td>Long ARV “Tail”</td>
<td>Yes (MRV, RAL, 3TC, TDF)</td>
<td>Yes (FTC, TDF)</td>
</tr>
<tr>
<td>Using topical formulations, is there drug exposure in the opposite compartment?</td>
<td>Yes 10% (TDF gel)</td>
<td>Yes 2% (TDF gel)</td>
</tr>
</tbody>
</table>

Source: Dr Kashuba, Univ. of North Carolina, USA.
infected if there was evidence of SHIV RNA by RT-PCR, proviral DNA or seroconversion. Infected animals continued to receive the tenofovir gel to monitor the emergence of systemic and local drug resistance. Animals were considered protected if all tests remained negative during the efficacy study and throughout 10 weeks of follow up. When the gel was administered 30 minutes before exposure to the virus, protection was 100%. The efficacy was 63% when the gel was administered three days before exposure. This appears to be due to lower drug levels in vaginal lymphocytes. Lymphocyte turnover in vaginal tissues may reduce the prophylactic window of the tenofovir gel. A strong correlation between high tissue drug levels and protection was noted.

Both of the breakthrough infections that occurred were wild type. There was no evidence of K65R emergence in animals that became infected, despite the 10 weeks of exposure to tenofovir gel. This observation is important because it suggests that use of the tenofovir gel by an HIV infected person is unlikely to result in the emergence of tenofovir-resistant virus and the subsequent limitations in their future treatment options. Dr Heneine (CDC, USA) concluded that these data highlight the importance of high levels of adherence if the tenofovir gel is going to be used in a coital-dependent manner.

Cell associated virus plays a role in HIV transmission but most microbical activity is directed against cell free virus. Dr Selhorst (Institute of Tropical Medicine, Belgium) has investigated the in vitro activity of candidate microbicides against cell associated HIV in an assay that utilises resting peripheral blood monocytes (PBMC), activated PBMCs and macrophages. Only entry inhibitors showed a small (3-10 fold) loss of activity against cell associated virus as compared with cell free virus. C34-chol and griffithsin (both entry inhibitors) were 10-100 times more potent than the reference ARV, dapsone. Infected macrophages and activated PBMCs were more difficult to inhibit than resting PBMCs. Only protease inhibitors (PIs) were able to completely inhibit p24 production by infected donor cells.

Impact of semen on HIV infection
Seminal fluid has been shown to enhance HIV infection and impair the antiretroviral efficacy of ARVs and microbicides by Dr Münch et al (University Clinic of Ulm, Germany). Amyloidoergic PAP fragments, known as semen enhancer of viral infection (SEVI, Figure 1), were identified in a semen derived peptide library. SEVI enhanced HIV-1 infection by increasing the attachment rate of the virus to the membrane and encouraging virus-cell fusion.

Concentrations of semen >1% in cell culture are cytotoxic; it is therefore difficult to set up meaningful in vitro HIV-1 infection assays using semen. Dr Münch explained that his group had developed a protocol to reduce the final concentration of semen by pre-treating virions so that the infectious effects of semen can be analysed under non-cytotoxic conditions. In this assay, semen has been shown to potently enhance HIV-1 infection of cells, independently of pH or the presence of vaginal secretions. PRO2000 and other polyanion microbicides, as well as ARV-based microbicides, are inactive against HIV treated with semen. A novel strategy has been proposed to reduce HIV-1 transmission: antagonising semen’s ability to enhance HIV-1 infection. A number of compounds, for example surfen that inhibits the binding of SEVI to host cells, are being examined to determine if they could function as microbicides in this manner.

Behaviour change for HIV prevention
The results of the Kenya Demographic and Health Survey 2008-9 showed that only 32% of women and 37% of men who reported having had sex with two or more partners in the previous 12 months had used a condom. Although promotion of condom usage is a principle of the Kenyan National HIV and AIDS Strategic Plan, it is often hard to obtain condoms, especially in rural communities because of social stigma and embarrassment, Mr Rugiata (Strategic Management Options, Kenya) explained. More than 80% of Kenyans are Christian and the use of condoms is commonly seen as a moral issue. In
addition, the condoms distributed by government agencies or NGOs are often perceived as inferior in quality to purchased condoms because of their poor packaging. Obtaining condoms from health facilities or family planning clinics is not popular, especially among men. A peer to peer model of educational services and free condom provision was set up by the Embu District Youth Development Initiative in order to facilitate access to condoms and educate youths about their correct use. The initiative was rolled out to 50 youth organisations. Between April 2008 and July 2010, the initiative has distributed 40 cartons, each containing 4,800 condoms.

Several lessons have been learned from the initiative. Access to condoms for youths should always be accompanied by health educational programmes. Condom distribution programmes can be conducted on a wide scale at reasonable cost in rural areas if local networks are utilised. In Kenya, sexual decision making is male dominated and so it is important to reach men with effective condom promotion messages. Female condoms are not widely available in Kenya and are very expensive. Male condoms are now seen primarily as a protective, rather than a contraceptive, device. Condom provision and education form part of a successful primary HIV prevention programme.

Ms Tumusiime (Catholic Relief Services, Uganda) described a behaviour change programme that has been designed as a HIV prevention measure. The results of the 2005 Uganda Behavioural HIV seroprevalence survey showed that 74% of new infections occurred in adults aged >25 years: 42% of people with new infections were married. The Catholic Relief Services/AIDSRelief developed the concept of the faithful house as a metaphor for marriage (Figure 2). The four pillars of the faithful house are true love; faithfulness; respect for human life and dignity; and communication.

A questionnaire was used to determine benchmark values in the community. Based on these results, 72 role model couples were selected. They were trained as faithful house curriculum facilitators and then they trained other couples in the community. The impact of training was assessed. The following changes were observed: increased awareness of HIV risk behaviour; enhanced capacity to influence peers in their HIV risk assessment and in accessing HIV testing and counselling as couples; and increased likelihood of being asked to help resolve marital conflicts and advise on strategies to enhance a family’s social well being. Role model couples formed support groups to sustain their HIV prevention efforts and reported that their standing within their communities had increased. Ms Tumusiime concluded that the faithful house model could be used in rural communities to reverse the trend towards increased rates of HIV transmission amongst married people.

Prevention of mother to child transmission (PMTCT)

During the past 20 years, the risk of mother to child transmission (MTCT) of HIV has fallen from ~40% to 1-3% if ARVs are used during pregnancy, delivery and breastfeeding. The plasma viral load (pVL) correlates well with cervical, vaginal and breast milk VLs, as well as with the risk of HIV transmission. Co-
infections that increase recruitment of inflammatory cells or breaches in the tissues, such as genital ulcers or mastitis, are associated with increased MTCT. The landmark ACTG 076 study was the first demonstration that administering zidovudine (ZDV) to the mother and infant could protect against MTCT of HIV. Since then, several short and long course ARV regimens have been evaluated for their PMTCT efficacy (Figure 3). For each regimen, reducing the pVL resulted in a reduction in the MTCT rate. Different regimens had different effects; for example, single dose nevirapine reduced the VL in breast milk significantly more than short course zidovudine.

The main disadvantage of short course PMTCT regimens, especially the single dose nevirapine option, was the emergence of ARV resistant virus, which compromised subsequent ART efficacy in both women and children. Adding a ‘tail’ of two ARVs (ZDV/3TC or tenofovir/FTC) to single dose nevirapine so that replication was suppressed until nevirapine was cleared from the mother’s bloodstream or using combination regimens substantially decreased the risk of viral resistance. Increasing coverage of PMTCT options is still a major challenge: in 2007, only 18% of pregnant women in sub Saharan Africa received an HIV test, which is the entry point for PMTCT interventions, but this has now increased to 45%. Dr John-Stewart (University of Washington, USA) pointed out that increasing HIV positive women’s access to contraception would have a huge impact on preventing the birth of HIV infected babies. Recent data has shown that both men and women are at increased risk of acquiring HIV during pregnancy, possibly because genital mucosal and hormonal changes make transmission more likely. However, interventions to prevent HIV transmission during pregnancy have not been well studied. In 2008, approximately 65,000 infant infections were averted through the use of ART prophylaxis to HIV positive women. “We need to increase PMTCT coverage and access to contraception in order to eliminate vertical transmission of HIV-1,” declared Dr John-Stewart.

Protection during breastfeeding
Protecting babies born to HIV positive mothers during breastfeeding is essential in resource limited settings because the risks of formula feeding in these situations include increased morbidity and mortality. ART has been shown to suppress breast milk HIV-1 RNA but not DNA11. Data from a number of studies have shown that maternal ART during breastfeeding is associated with a low risk of transmission (~1%). Maternal ART not only decreases breast milk HIV but also provides prophylaxis to the infant. An alternative option is to provide the infant with ARV prophylaxis during breastfeeding; in the BAN study, this option resulted in a similar MTCT rate as that observed with maternal ART (1.6%). This option may be particularly relevant for HIV positive women with CD4 cell counts >350 cells/mm3 who may no longer be eligible for ART when they have delivered their baby, based on the national guidelines. It is not yet known whether HIV positive mothers with high CD4 cell counts should

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**Figure 4.** PEPI-Malawi study design
Source: Dr Taha, Johns Hopkins Hospital, USA12

<table>
<thead>
<tr>
<th>Control Suspended Aug 2007</th>
<th>Intra-partum*</th>
<th>Birth</th>
<th>Post-partum</th>
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<td>NVP x1*</td>
<td>Infant NVP x1</td>
<td>Infant ZDV x1 wk</td>
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<table>
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<th>Intra-partum*</th>
<th>Birth</th>
<th>Post-partum</th>
</tr>
</thead>
<tbody>
<tr>
<td>NVP x1*</td>
<td>Infant NVP x1</td>
<td>Infant ZDV x1 wk</td>
<td>Infant: NVP x 14 wks</td>
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</tbody>
</table>

<table>
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<th>Extended NVP + AZT</th>
<th>Intra-partum*</th>
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<th>Post-partum</th>
</tr>
</thead>
<tbody>
<tr>
<td>NVP x1*</td>
<td>Infant NVP x1</td>
<td>Infant ZDV x1 wk</td>
<td>Infant: NVP + ZDV x 14 wks</td>
</tr>
</tbody>
</table>

*If mothers diagnosed in time for intra-partum prophylaxis

Mothers counseled to exclusively breastfeed and wean by 6 months
stop ART after delivery or at the end of breastfeeding, or if they should continue to take ARVs lifelong. Dr John-Stewart commented that there are resistance and toxicity issues with both approaches but stated that it is simpler and less expensive to give ARVs to the baby than to the mother. The new WHO guidelines support both approaches so that policy makers can choose the appropriate option for each situation.

Updated results of the PEPI-Malawi trial of post-exposure ARV prophylaxis of breastfeeding infants were presented by Dr Taha (Johns Hopkins Hospital, USA). In the PEPI-Malawi study, infants received daily ARV prophylaxis (ZDV plus nevirapine or nevirapine alone) for the first 14 weeks of life (Figure 4): postnatal HIV-1 transmission was reduced by >65% during the prophylaxis period and by ~50% at 9 months of age. No substantial differences between the monotherapy and combined ART regimens were noted in terms of efficacy. By 24 months, the transmission rate was 15.6% in the control group compared to 10.8% in the nevirapine arm and 11.2% in the ZDV/nevirapine arm. Dr Taha said that these data indicate that a longer period of prophylaxis is needed to protect HIV exposed infants during breastfeeding.

**Biological aspects of transmission**

**HIV-2 transmission**

"HIV-2 infection could be described as a naturally attenuated human retrovirus infection", said Dr Rowland Jones (Oxford University, UK). HIV-2 was isolated in 1986, three years after HIV-1 had been identified. Although Guinea Bissau appears to be the epicentre of the HIV-2 epidemic in West Africa, HIV-2 infections have also been recorded in Portugal, Angola, Mozambique, Goa, India, Brazil, S. Korea and Japan. The high prevalence of HIV-2 in Guinea Bissau in the 1980s-90s (8-10%) was associated with the war of independence; female genital mutilation; and parenteral treatment for tuberculosis (TB) and trypanosomiasis. Unlike the rising rate of HIV-1 infection in W Africa, the seroprevalence of HIV-2 in the region is stable or falling (0.5-1%) and infections are more common amongst older people (five fold higher in people >45 years) than younger individuals, who are more likely to be infected with HIV-1 than HIV-2. It is not known whether the higher frequency of HIV-2 infection in older women is due to a cohort effect or increased susceptibility to HIV-2 with age. Progression to AIDS only occurs in ~20% of HIV-2 infected individuals. Dual HIV-1/2 infections can occur as simultaneous primary infection with both viruses or as a HIV-1 super-infection of HIV-2 positive people. Sexual transmission of HIV-2 is less efficient (3.5 fold less) than for HIV-1 and vertical transmission is infrequent, with a mother to child transmission (MTCT) rate of 4%. These observations may be due to the low pVL in HIV-2-infected people. HIV-2 shedding into the semen or female genital occurs at lower rates than for HIV-1. Dr Rowland Jones observed. She added that the replicative capacity of HIV-2 in vitro is lower than that of HIV-1. HIV-2 does not result in productive infection of dendritic cells, which is one of the main portals of HIV-1 infection.

"One reason for studying HIV-2 is that responses in HIV-2 infected long term non-progressors (LTNP) are similar to those sought by investigators who are trying to develop a vaccine against HIV-1," said Dr Rowland Jones, "Strong immune responses against HIV-2 and low pVLs are observed in LTNP: there is an interaction between viral, host genetic and immune factors." A number of investigators have sought to understand why HIV-2 infection results in more LTNP than HIV-1 infection. Control of HIV-2 VL has been shown to be strongly associated with T cell responses to a single, highly conserved region in the capsid protein. T cells that recognise this region express unusually high avidity with restricted T cell receptor usage and do not select viral escape variants. They proliferate well and are not 'exhausted', despite the long period of HIV-2 infection. Dr Rowland Jones concluded by posing the question: "Is this the sort of immune response we should be looking for when testing HIV-1 vaccines?"

**Vertical transmission of HIV-2**

Only three cases of HIV-2 vertical transmission have been reported in Portugal since 1998. Dr Barosa (University of Lisbon, Portugal) reported that two new cases of potential transmission were studied using phylogenetic analytical techniques. In Case 2, the diagnosis was made at 2.5 years of age, while it was made within the first month of life for Case 3 (in 1998). Genetic sequences were obtained from the mother and Case 2 and the genetic distance between them was 8.3%. Both viruses were CCR5 positive. Unfortunately, both mother and child were lost to follow up.

Case 3’s mother was taking ART when blood samples were taken from her and her child in 2000 and 2003. The child started ART in November 2003 and is currently clinically stable. The pVL and CD4 cell counts from mother and child indicated an acute and progressive infection in the child and a chronic infection in the mother. During the follow up period, the child’s infection switched from a mixed CCR5/ CXCR4 HIV-2 population to a CXCR4 infection. The evolutionary rate of the HIV-2 env gene in the child’s viral population was unexpectedly high when compared to that in the maternal viral infection.
Figure 5. Molecular genetics of HIV-2
Source: Dr Ibe, Nagoya Medical Center, Japan

(CXCR4 virus). Increasing antibody responses to the envelope glycoproteins were observed during the follow up period: Dr Barosa suggested that the humoral immune system was a major driver of the early evolution of HIV-2 in this child.

First report of a circulating recombinant form of HIV-2
The first circulating recombinant form of HIV-2, CRF01_AB, has been identified in Japan. Eight HIV-2 strains, named A to H, and two AB recombinants had been identified prior to this report (Figure 5). Between 1994 and 2008, 843 patients with HIV infection were cared for by the Nagoya Medical Center: 80.9% were Japanese and only 3.3% were African in origin. The majority (88.1%) were men. The most common risk group was men who have sex with men (MSM) (61.6%), while 23.8% had been infected via heterosexual sex. Five patients (two women and three men) were infected with HIV-2 via heterosexual sex but no direct relationships between the five were established. There was no evidence of dual HIV-1/-2 infection. Two of the men originated from Nigeria and one from Ghana. Both of the women were Japanese. HIV-2 pVLs ranged from 25,000 to 680,000 copies/mL. Four of the patients had AIDS and the fifth was asymptomatic. Gag and env polymerase chain reaction (PCR) products were obtained from the AIDS patients but not from the asymptomatic case.

Three isolates formed a cluster with an AB recombinant isolate, 7312A. Full length genome sequencing was undertaken and a common mosaic genome structure was identified: Dr Ibe (Nagoya Medical Center, Japan) announced that this was the first identification of a circulating recombinant form of HIV-2 CRF01_AB. The strain has a chimeric env gene and appears to have emerged between 1964 and 1973, several decades after the A and B groups had separated. Given the high proportion of LTNPs in HIV-2 infected patients, the investigators were surprised that all of the CRF01_AB isolates were obtained from AIDS patients. It is not yet clear if this reflects a more pathogenic nature of this strain compared to other HIV-2 strains.

Risk factors for HIV acquisition
HIV that is transmitted from women to men tends to contain a modestly underglycosylated env gene. Underglycosylation was most pronounced in acutely infected men and the difference between the level of glycosylation in virus from chronically infected women was significant (p<0.0001). Two thirds of the underglycosylation occurred in the variable loops (V1/V2 and V4/V5) and position 442 is significantly underglycosylated. Two hypotheses have been proposed to explain the female to male transmission of underglycosylated virus:

- Compartmentalisation of virus within the female genital tract versus in the plasma or mucus.
- Altered viral migration within the cervical mucus, due to interactions with sialic acid on complex carbohydrates or with a lectin-like molecule.

In the subtype B epidemic in the US, HIV positive individuals tend to transmit virus at an earlier stage in their infection than individuals infected with subtype C virus in sub Saharan Africa. The reasons for this are unknown at present but it has been suggested that HIV positive individuals are having more frequent sex in the US than in sub Saharan Africa or that therapy is started earlier in the US.

Risk of infection during menstrual cycle
Very little information is available about the effects that the natural hormonal fluctuations, which occur during the menstrual cycle, have on retroviral infection. It is known that progesterone can affect the integrity of the vaginal epithelium, as well as mucosal innate and adaptive immune factors. In addition, women are less likely to acquire a sexually transmitted infection (STI) during the follicular (early) phase of the menstrual cycle when the epithelium of the vaginal wall is thicker than in the luteal (late) phase and the immune reaction is more vigorous.

An animal model of repeat, low dose SHIV infection in 19 female pig tailed macaques was used to test the hypothesis that infection was more likely during the luteal phase of the menstrual cycle than the follicular phase. Macaques have lunar menstrual cycles, similar to human beings. In 15 macaques, the SHIV challenge was twice a week and it was once a week in the other four monkeys. The detection limit...
of the assay was 50 RNA copies/mL and so there was a 7-14 day delay between the infection occurring and its detection (eclipse period). When the eclipse period was taken into account, the window of highest susceptibility to SHIV was shown to be the late luteal phase (days 22-29) i.e. when the vaginal epithelium is at its thinnest and the immune response is attenuated. Dr Kersh (CDC, USA) cautioned that although these data are very interesting, it is not yet clear if such variations in susceptibility occur in women during their menstrual cycles. She suggested that HIV prevention methods designed for women should be tested in female monkey species with lunar menstrual cycles. In addition, characterisation of mucosal immune factors that are influenced by hormones could identify natural resistance factors to HIV.

Usage of hormonal contraceptives
Hormonal contraceptives are used by >150 million women, >100 million of whom take combined oral contraceptives (COCs) and >50 million use DMPA injectable contraceptives. DMPA use is common in the young and in South Africa, Dr Morrison (Family Health International, USA) explained. However, there are concerns that the biological mechanisms by which hormonal contraceptives work could increase the risk of HIV acquisition. These include vaginal wall thinning; altered cervical structure; genital tract infections; local and systemic immune changes, including increases in the number of inflammatory cells; and upregulation of HIV replication. The Hormonal Contraception and Risk of HIV Acquisition Study (HC-HIV), a prospective cohort study, enrolled 6,109 HIV negative women aged 18-35 years in Uganda, Zimbabwe and Thailand. All of the women had used low dose COCs, DMPA, a non-hormonal contraceptive method or no contraception for at least three months. They were tested for HIV, syphilis and HSV-2 every 12 weeks for 15-24 months (mean follow up: 21.9 months). A total of 213 HIV infections per woman year (wy) occurred: an incidence of 2.75 per 100 wys. The incidence rate in the COC group was 2.59/100 wys; it was 3.11/100 wys in the DMPA group; and 2.55/100 wys in the non-hormonal group. Data from the study were re-analysed using marginal structural modelling to account for time dependent confounding factors, such as the fact that exposure to hormonal contraceptives and HIV risk factors varied over time (Table 2).

Table 2: Adjusted hazard ratios for HIV acquisition by contraceptive group: original and marginal structural modelling reanalysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Original Analysis(^1) Hazard Ratios (HR) (95%) P</th>
<th>MSM Reanalysis HR(^2) (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All data without weight P</td>
<td>All data with weight P</td>
</tr>
<tr>
<td>Non-HC</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>COC</td>
<td>0.99 (0.69, 1.42) 0.94</td>
<td>1.05 (0.73, 1.52) 0.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.19 (0.80, 1.76) 0.39</td>
</tr>
<tr>
<td>DMPA</td>
<td>1.25 (0.89, 1.78) 0.20</td>
<td>1.25 (0.89, 1.77) 0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.48 (1.02, 2.15) 0.04</td>
</tr>
</tbody>
</table>

\(^1\)Adjusted for time-varying contraceptive group, site, living with partner, age, time-varying participant behavioral risk, time-varying primary partner risk, time-varying coital frequency and time-varying consistent condom use.

\(^2\)Adjusted for time-varying contraceptive group, site, living with partner, age, baseline participant behavioral risk, baseline primary partner risk, baseline coital frequency and baseline any condom use.

Source: Dr Morrison, Family Health International, USA

Overall, DMPA, but not COC use, was significantly associated with HIV acquisition. Young women (<25 years) and HSV-2 negative women who used hormonal contraceptives were at increased risk of HIV infection while older women and HSV-2 positive women using hormonal contraception were not. Dr Morrison called for additional high quality data to be collected so that this crucial public health question can be addressed. He stressed that hormonal contraceptives are widely used in resource limited settings and have many positive benefits but their potential as a risk factor for HIV acquisition should be assessed in more detail to determine their risk:benefit ratio in high HIV prevalence settings.
**Contribution of early infections to the HIV epidemic**

The risk of transmitting virus during acute (AHI) or early HIV infection (EHI) is very high due to a combination of a high viral load, greater infectivity of the viral particles and the individual's low awareness of their infection status\(^1\). Detecting AHI, however, is relatively uncommon and requires complex and expensive RNA or p24 antigen tests. The role of AHI and EHI in HIV transmission has not been fully characterised; if it has a considerable impact, interventions targeted at people with chronic HIV infection may fail to prevent HIV transmission, while if it has a small impact, devoting resources to detecting AHI and EHI may not be worthwhile on a public health basis.

Behavioural and VL data from Kamuzu Central Hospital in Lilongwe, Malawi, have been used to estimate the proportion of HIV transmissions attributable to index cases with EHI and to predict the reduction in HIV prevalence that could be achieved if EHI cases were detected. A deterministic mathematical model determined the risk of HIV transmission within steady pairs; by a paired individual to a non-paired individual; and by singles. Transmission rates within EHI, asymptomatic HIV infection, early AIDS and late AIDS were modelled. A Bayesian melding procedure was used to account for uncertainty in relation to sexual behaviour and infectivity. The proportion of new cases due to transmission by EHI cases in 2010 was estimated to be 38% (lower estimate: 19%; higher estimate: 58%). This was similar to estimates by other authors, albeit a little higher. If interventions were only targeted to people with EHI, the HIV epidemic would be reduced but not eliminated, even if 100% coverage was achieved. Interventions targeted towards people with chronic HIV infection would only lead to elimination if 100% coverage were achieved. Since this is unlikely, it is recommended that interventions be targeted towards people with EHI and with chronic infection; even if coverage of the interventions to these two groups is only 75%, the epidemic could be eliminated in 20 years.

**HIV transmission epidemiology**

**Sexual networks**

Network theory states that, even within the most complex systems, connections are not random and so networks behave in ways that can be modelled and predicted, Dr Little (University of California San Diego, USA) began her lecture by saying\(^2\). Transmission probabilities can be estimated by identifying which network parameters are the most useful predictors of HIV infection risk. Defining the structure of a sexual network is challenging. An individual's position within the network and his/her risk behaviour influences his/her risk of acquiring HIV or another sexually transmitted infection (STI); for example, the type of sex act that he/she favours may be more important than his/her total number of sex partners. The position of a person within a network affects the risk of acquiring STIs: an individual at the centre of a network is at higher risk than a person at the periphery. Concurrent relationships are associated with an enhanced risk of STIs in sexual networks, even if the total number of contacts is relatively small.

The San Diego primary infection cohort includes 562 patients (enrolled from 1996 until present day). Between one and 21 viral samples per patient have been sequenced and this has revealed that 46.3% of them belong to a 1%-delineated phylogenetic cluster. The San Diego HIV network appears to be linear (96% correlation) with a characteristic exponent of -3, which is consistent with a scale free network. Dr Little explained that this type of network could be generated by a ‘preferential attachment’ or ‘a friend of your friend is my friend’ model. Her group has used the cohort data to estimate the size of the HIV-1 infection network in San Diego. The observed network includes 562 nodes (individuals) who comprise 378 clusters with 756 edges (contacts). Since sampling in the cohort is biased to recruit partners of people with primary HIV infection, an allowance was made in the model for this factor. The closest fit for the observed number of clusters and edges in the San Diego cohort was a network of 9,500 individuals. Dr Little cautioned that understanding sexual networks is complicated by the number of ‘missing’ contacts, e.g. if the person does not know/is unwilling to provide partners’ names. The emergence of social networking applications, either via the internet or using GPS technology to find casual sexual partners in the vicinity, is a further challenge to effective contact tracing since the sex partners may possess very little information about each other. The potential contribution of AHI to HIV transmission is considerable since people with acute HIV infection are 12-25 times more infectious during seroconversion and for up to three months after seroconversion than those with established infection. Identifying people with AHI is a major challenge. Two recent studies, one in London and one in Quebec, have shown that early infection is a major driver of onward HIV transmission. “We need to understand sexual networks so that we can determine HIV migration patterns within communities and devise appropriate response strategies,” concluded Dr Little.

Phylogenetic, epidemiological and clinical data have been combined in order to characterise the local HIV epidemic in Ghent, Belgium\(^3\). Between January 2001 and March 2009, 699 new HIV-1 infected, ARV
naïve patients were registered at the ARC, Ghent. Of these, 570 met the inclusion criteria for the study. Subsequently, an additional 71 patients were added to the cohort between April and December 2009. Two groups of patients were identified: a subtype B infected population of whom >90% were male, >90% were Caucasian, and >80% were infected by homosexual sex; and a non-subtype B infected population who were more likely to be infected by heterosexual sex (only 9% were infected via homosexual sex). In the subtype B population, 19 clusters (143 patients) were identified with an average size of 7.5 and 20 transmission pairs. One cluster included 57 patients. In the non-subtype B population (n=204), seven clusters were identified, with an average size of 3.7 and 15 transmission pairs. Clustered patients were more likely to present early with high CD4 cell counts, primary HIV infection and sexually transmitted infections (STIs) (a surrogate for risk taking behaviour). Monitoring of the clusters over time indicated that the 57 member cluster had grown to 63 patients by the end of 2009. These data suggest that high risk taking MSM are an important source for onward HIV transmission in Ghent, despite indications that they are aware of their risk of HIV infection and tend to seek care earlier than people infected with non subtype B virus.

The results of a similar study in Stockholm has shown that an outbreak of HIV infection in intravenous drug users (IDU) in the city was due to the introduction and rapid spread of a new HIV variant: CRFO1_AE23. An increase in HIV diagnoses in Stockholm was noted in 2006-07, Dr Albert (Karolinska Institute, Sweden) explained. Circulating HIV strains amongst 74 IDUs diagnosed with HIV infection between 2004 and 2007 were identified using phylogenetic and phylodynamic (changes in incidence over time) techniques. Two parallel IDU epidemics were observed: the ongoing spread of subtype B virus and the introduction and rapid spread of a new variant, CRFO1_AE, which originated in Helsinki, Finland. Similar VL set points were observed in both groups, suggesting that the rapid spread was not due to intrinsic viral characteristics. Dr Albert expressed the belief that the variant had spread so rapidly because it had been introduced into a network of IDUs with risky needle sharing behaviour.

Transmission of drug resistant HIV
At least one drug resistance related mutation has been detected in 5-10% of all new HIV infections in developed countries and transmission of drug resistance mutations is increasingly being reported in developing countries24. The mutation M184V is common in long term treated patients (70%) but relatively rare (10%) in untreated patients with AHI. When allele specific PCR (AS PCR) was used, a greater number of strains carrying M184V was detected (an additional 13.3%). There are two possible reasons why M184V is relatively uncommon compared to other drug resistance associated mutations: M184V may reduce the transmissibility of the virus; or M184V rapidly reverts to wild type when it is transmitted to a ARV naïve person and the wild type outgrows the mutant strain. Sequential samples from AHI individuals that were analysed with AS PCR showed that M184V disappeared between 32 and 52 weeks after infection. If M184V was linked to other
mutations, it tended to persist for longer periods of time. Dr Wainberg commented that these data were consistent with the ‘reversion to wild type’ theory. Transmission of K65R has not been observed in N America using bulk sequencing. Higher levels of K65R mutations were observed when AS PCR was performed on subtype C viral samples from patients failing ART (23.3% vs. 10% with bulk sequencing); but there was no difference when subtype B viruses were analysed (10% with both methods). Dr Wainberg warned that K65R is a more important mutation in subtype C than subtype B viruses and that this could have an effect on tenofovir-based prevention and treatment strategies in areas where subtype C virus predominates.

The impact of transmitted drug resistance on the prognosis of Danish patients with HIV-1 infection between 2001 and 2008 was discussed by Dr Audelin (Statens Serum Institut, Denmark)25. Data were analysed from two Danish cohorts: the SERO project and the Danish HIV Cohort. In the SERO project, 5.8% of newly diagnosed patients (70/1,197) were infected with drug resistant virus. The majority of infections were subtype B, regardless of whether they carried transmitted resistant mutations (73%) or not (68%). There were no differences between the patients infected with resistant or susceptible virus in terms of route of transmission: 47% of cases infected with drug susceptible virus had been infected by male homosexual sex compared to 46% of cases infected with drug resistant virus; for heterosexual transmission, the figures were 42% and 46%, respectively. VLs and CD4 cell counts did not differ significantly between the two groups.

Phylogenetic analyses were undertaken using the Danish HIV sequence database and documented cases of transmitted drug resistance. In 45% of cases (n=40) of transmitted drug resistance could be linked phylogenetically to either a Danish viral sequence obtained from the Danish database or another transmitted drug resistance sequence. Twelve clusters involved subtype B virus; one cluster involved subtype D; and one cluster involved subtype G. Twenty percent of the transmitted drug resistant group formed a cluster; all of the patients were MSM. Seventy patients in the transmitted drug resistance group were followed for 1 month to 4 years: 10 did not receive HAART; 21 received sub optimal HAART; and 39 received HAART that was tailored to the patient’s specific resistance profile. Therapeutic virological failure occurred in 7% of patients. Transmitted drug resistance had no impact on the patients’ response to HAART in relation to viral load, CD4 cell count and time to development of AIDS/death.

The majority of transmitted drug resistance mutations are single mutations but it is not known if they represent the ‘tip of the iceberg’ of resistance or true singletons26. Dr Pingen (Erasmus Medical Center, the Netherlands) and colleagues performed 454 amplicon sequencing on viruses isolated from 10 patients who had been identified as being infected with viruses

**Figure 7.** Drug resistance trends in Japan 2003-09
Source: Dr Sugiura, Nagoya Medical Center, Japan27
that carried a single mutation at a resistance-related codon by population sequencing (10-20% detection limit). Six of the 10 patients were infected with virus carrying a mutation at position 215 of the RT gene (215E, 215L, 215S). Dr Pingen noted that it is not possible to completely rule out the possibility of more drug resistant viruses being in other compartments. Despite the presence of singleton resistance mutations, the patients responded well to ART: viral rebound occurred in only one patient. “We could not find evidence of transmission of more extensively resistant virus in these patients who were identified as being infected with virus carrying a single mutation,” concluded Dr Pingen.

National studies on transmitted drug resistance mutations

An increase in the prevalence of transmitted drug resistant virus has been observed in Japan in recent years (2003-09), according to the Japanese Drug Resistance HIV-1 Surveillance Network27. Between January 2003 and December 2009, viral samples from all newly diagnosed cases of HIV infection were genotyped and subtyped. The majority of cases were male, Japanese MSM who were infected with subtype B virus. Over the period 2003-09, the prevalence of drug resistance mutations increased from 5.9% to 8.6% (Figure 7). The T215X, K103N and M46I/L mutations have been detected throughout the follow up period and it appears that viral strains carrying these mutations have become circulating strains.

A highly sensitive PCR method has been used to monitor low frequency drug resistant HIV strains in Japan28. The assay used primers from the RT region of the genome29. Four newly diagnosed patients were identified as being infected with drug resistant subtype B HIV (one incidence of M41L, two of K65R, one of K70R and one of M184V) in 2008. In one case, three mutations were detected (M41L/K70R/ M184V). Three cases of CRF01_AE strains carried M41L. In 2009, four cases of drug resistant subtype B HIV (one K65R, one K70R, one K103N and one T215L) were detected. Using the highly sensitive PCR methodology, the prevalence of drug resistant HIV in Japan was 9.7--11% in subtype B virus.

The prevalence of transmitted drug resistance in recently HIV infected individuals and newborns in Panama has been assessed by Dr Castillo and colleagues (Instituto Conmemorativo Gorgas de Estudios de la Salud, Panama)30. In Panama, the first line ARV regimen is zidovudine/3TC (administered as Combivir®) plus efavirenz while pregnant women receive Combivir® plus lopinavir/ritonavir. Stored plasma samples from 48 recently infected adults and 26 newborns with confirmed HIV infection were genotyped. The Stanford HIV Drug Resistance algorithm was used to score the ARV-associated mutations. The prevalence of mutations conferring resistance to RT inhibitors was 8.3% in adults and 11.5% in newborns. M184V was not detected in either group. Protease gene polymorphisms were detected but they had no clinical significance.

The prevalence of drug resistance in HIV-1 infected children born in Honduras and Belize between 2001 and 2004 was reported by Dr Parham (National Autonomous University of Honduras, Honduras)31. The estimated HIV-1 prevalence in 2007 was 0.7% in Honduras and 2.1% in Belize; the relevant figures for pregnant women were 0.3% and 0.9%. PMTCT programmes began in Honduras in 2001 using ZDV or nevirapine monotherapy, and in 2002 in Belize where nevirapine was used. Dried blood spots were obtained from 66 HIV infected infants (55 from Honduras and 11 from Belize). None of the infants had received ARVs but 18% of the Honduran mothers and 72% of the Belizean mothers in the study had received ARV prophylaxis. Mutations associated with drug resistance were detected in 15% (10/66) children; all of the mutations observed in the Belize cohort were associated with resistance to NNRTIs. In the samples from the Honduran infants, resistance to PIs were detected in 28.5% of samples; to NRTIs in 28.5% of samples; and to NNRTIs in 43% of samples. Dr Parham commented that these rates of resistance were high compared to the frequency of drug resistance observed in viral samples from ARV naïve Honduran adults. It is not yet known if the drug resistant viruses identified in these children were due to unreported prior and inconsistent use of ARVs by their mothers or the transmission of drug resistant virus.

MARK - THE - DATE

6th International Workshop on HIV Transmission – Principles of Intervention
14 - 15 July 2011 - Rome, Italy

Abstracts and Presentations of the 5th International Workshop on HIV Transmission – Principles of Intervetion are available online at www.virology-education.com
References


Report

2nd International Workshop on HIV Pediatrics
16-17 July 2010, Vienna, Austria
Prevention of Mother To Child HIV Transmission

In the 20 countries with the largest number of HIV-infected women in 2008, only two—South Africa and Botswana—have made significant progress toward reaching goals for prevention of mother-to-child transmission (PMTCT) by 2015, according to UNAIDS data reported by Dorothy Mbopi-Ngacha (United Nations Children’s Fund). Six countries are making progress toward meeting that goal—Kenya, Lesotho, Namibia, Swaziland, Tanzania, and Zambia. The remaining 12 countries are off track.

In 2010 the World Health Organization (WHO) updated recommendations on PMTCT and on treating HIV-positive women with antiretrovirals. Reviewing the update at the Pediatrics Workshop, Elaine Abrams (Columbia University, New York) noted two principal objectives: (1) maximally reduce the risk of MTCT, and (2) improve maternal and infant survival. To realize these goals, WHO outlines two approaches to PMTCT: (1) lifelong antiretroviral therapy (ART) for pregnant women who need treatment for their own health, or (2) antiretroviral prophylaxis to prevent MTCT during pregnancy, delivery, and breastfeeding for HIV-infected women not in need of treatment.

As for all HIV-infected adults and adolescents, WHO now recommends ART for pregnant woman with a CD4 count at or below 350 cells/mm³ regardless of WHO clinical stage and for all with a CD4 count above 350 cells/mm³ and stage 3 or 4 disease. When a pregnant woman has stage 1 or 2 disease and a CD4 count above 350 cells/mm³, WHO calls for antiretroviral prophylaxis for mothers and exposed infants. Thus CD4 testing is critical to identify women eligible for ART. A study of 3736 pregnant women in the MTCT-Plus Initiative found that 1959 (52%) were eligible for ART by CD4 count and/or WHO stage. Among those 1959 eligible women, CD4 count identified 96% as eligible, while WHO stage identified only 20%. In the Mma Bana study of 560 HIV-infected breastfeeding mothers with a CD4 count above or below 200 cells/mm³, maternal ART limited MTCT to 1% in the first 6 months of life.

WHO-preferred regimens for ART-eligible women are nevirapine or efavirenz plus zidovudine/lamivudine, though efavirenz should be avoided in the first trimester. Alternative nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) are tenofovir plus emtricitabine. For PMTCT prophylaxis, WHO proposes two options, which are thought to be equivalent in efficacy and carry regimen-specific risks and toxicities (Figure 1). Factors that may determine which option is more appropriate include feasibility of use, access, cost equity, health system capacity, and current approaches to PMTCT.

Two-year PEPI-Malawi data confirm 14-week infant PEP benefit

After 24 months of follow-up in the randomized PEPI-Malawi trial, 14 weeks of infant postexposure prophylaxis (PEP) yielded sustained benefits in HIV infection incidence and HIV-free survival when compared with single-dose nevirapine (sdNVP) plus 1 week of zidovudine. Children continued to become infected after the initial 9-month follow-up point, a result indicating a need for longer infant prophylaxis.

PEPI-Malawi was an open-label phase 3 trial that randomized HIV-negative infants born to HIV-infected, breastfeeding mothers to one of three prophylactic regimens: (1) sdNVP plus 1 week of zidovudine (the control arm, 1090 infants), (2) the control regimen plus daily nevirapine through 14 weeks (1160 infants), or (3) the control regimen plus daily nevirapine and zidovudine through 14 weeks (1147 infants). Mothers were counseled to breastfeed exclusively and to wean by 6 months.

In the 9-month primary-endpoint analysis, estimated Kaplan-Meier probability of HIV infection in infants was 11.1% in the control arm, 5.0% with extended nevirapine, and 6.0% with extended nevirapine/zidovudine. The difference between each extended prophylaxis arm and the control arm was statistically significant, but new infection rates did not differ significantly between the two extended arms. At 24 months, respective estimated HIV infection rates were 15.6%, 10.8%, and 11.2%.
Probability of death at 9 months was 9.6% in the control arm, 6.2% in the extended-nevirapine arm, and 6.0% in the extended nevirapine/zidovudine arm. Respective estimated mortality rates at 24 months were 16.5%, 12.8%, and 12.5%. Probability of infection or death at 9 months was 16.9% in the control arm, 10.1% in the extended-nevirapine arm, and 10.6% in the extended-nevirapine/zidovudine arm. At 24 months, respective estimated probability of infection or death stood at 24.7%, 19.8%, and 19.9%. At 24 months protective efficacy of extended prophylaxis compared with the control arm was 31% with extended nevirapine and 28% with extended nevirapine/zidovudine. At no point were differences between the two extended-prophylaxis arms statistically significant.

Proportional hazards modeling showed that extended nevirapine lowered the risk of HIV infection 40%, while extended nevirapine/zidovudine lowered the risk 35% (Table 1). Lower maternal CD4-cell count and late maternal presentation each independently raised

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**Figure 1.** In its 2010 guidelines, WHO proposes two options for PMTCT prophylaxis.² Because the options appear to be equal in efficacy, toxicity profiles and site-specific variables will determine which is more appropriate for patients. Source: Dr Abrams (Columbia University, New York)

Probability of death 9 months was 9.6% in the control arm, 6.2% in the extended-nevirapine arm, and 6.0% in the extended nevirapine/zidovudine arm. Respective estimated mortality rates 24 months were 16.5%, 12.8%, and 12.5%. Probability of infection or death 9 months was 16.9% in the control arm, 10.1% in the extended-nevirapine arm, and 10.6% in the extended-nevirapine/zidovudine arm. At 24 months, respective estimated probability of infection or death stood at 24.7%, 19.8%, and 19.9%. At 24 months protective efficacy extended prophylaxis compared with the control arm was 31% with extended nevirapine and 28% with extended nevirapine/zidovudine. At no point were differences between the two extended-prophylaxis arms statistically significant.

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**Table 1.** Risk factors for infant HIV infection or mortality at 24 months in PEPI-Malawi

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Adjusted HR (95% CI)</th>
<th>P</th>
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<tbody>
<tr>
<td>Risk of infant HIV infection alone</td>
<td></td>
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<tr>
<td>Extended nevirapine versus control</td>
<td>0.60 (0.46 to 0.78)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Extended nevirapine/zidovudine versus control</td>
<td>0.65 (0.50 to 0.85)</td>
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<tr>
<td>Every 100-cell lower maternal CD4 count</td>
<td>1.22 (1.16 to 1.29)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Late versus early maternal presentation</td>
<td>1.27 (1.00 to 1.60)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

| Risk of infant HIV infection or death          |                      |         |
| Extended nevirapine versus control             | 0.71 (0.58 to 0.87)  | 0.001   |
| Extended nevirapine/zidovudine versus control  | 0.73 (0.60 to 0.90)  | 0.003   |
| Every 100-cell lower maternal CD4 count        | 1.14 (1.10 to 1.18)  | < 0.0001|
| Higher birth weight                            | 0.63 (0.52 to 0.76)  | < 0.0001|

Source: Dr Taha (Johns Hopkins University, Baltimore)³
the risk of infant HIV infection (Table 1). Extended nevirapine, extended nevirapine/zidovudine, and higher birth weight also independently lowered the risk of HIV infection or death, while lower maternal CD4 count raised the risk of HIV infection or death.

The rate of serious adverse events possibly related to treatment was significantly higher in the extended nevirapine/zidovudine group (78%) than in the control group (44%) or the extended-nevirapine group (47%) (P = 0.01). Rates of serious adverse events probably related to treatment did not differ significantly between the three study arms.

Protective efficacy of nevirapine exceeded 70% during the intervention (up to week 14). After 9 months, the protective efficacy of nevirapine was 55%, after 12 months 47%, and after 24 months 31%. Taha Taha (Johns Hopkins University, Baltimore) and PEPI-Malawi colleagues concluded that “longer prophylaxis [is] therefore needed for HIV-exposed infants who continue breastfeeding.”

High rate of false-negative confirmatory HIV tests in children on ART

Rapid antibody tests and polymerase chain reaction (PCR) for HIV DNA in dried blood spots were often false-negative after 18 months of age in children receiving ART. These findings from a retrospective study in a single Lesotho clinic deserve particular attention now that WHO guidelines advise beginning ART in all HIV-infected children under 1 year old.

The WHO recommends HIV antibody testing after 18 months of age for children in whom a single virologic test detected HIV before that age. To assess the value of that recommendation, Anthony Garcia-Prats (Baylor College of Medicine, Houston) and colleagues retrospectively analyzed data from children initially seen from December 2005 to February 2009 at an HIV clinic in Maseru, Lesotho. All children had a positive HIV DNA PCR before 18 months of age and parallel rapid tests after 18 months.

Among 109 children studied, 60 (55%) had positive confirmatory rapid tests after 18 months, 27 (25%) had discordant rapid test results, and 22 (20%) had negative rapid tests. Among the 27 children with discordant rapid test results, 7 were positive and 2 negative by dried blood spot PCR after 18 months. Among the 22 children with negative rapid test results, 5 were positive and 9 negative by PCR after 18 months.

Univariate logistic regression analysis determined that children younger than 9 months when they began ART had more than a 4 times higher risk of discordant or negative rapid test results after 18 months (odds ratio [OR] 4.25, P = 0.02). Children receiving ART for 9 months or longer had a 5 times higher risk of discordant or negative rapid test results (OR 4.96, P < 0.001). Factors not associated with discordant or negative confirmatory results in this analysis were gender, PMTCT status, baseline immunologic status, and acute malnutrition.

Garcia-Prats and colleagues concluded that false-negative confirmatory rapid test results are not rare. They proposed confirming HIV diagnosis in children younger than 18 months with an immediate repeat PCR after an initial positive result. False-negative confirmatory results, they cautioned, could lead to inappropriate discontinuation of ART.

Birth defect rate low in women on ARVs in first trimester

Among women taking antiretrovirals during the first trimester of pregnancy, birth defect rates did not differ substantially from rates in women taking antiretrovirals in the second or third trimester or from rates in a general population surveillance group.

These reassuring findings come from analysis of 11,261 live births in 11,867 women exposed to antiretrovirals from January 1989 through January 2010 and tracked in the Antiretroviral Pregnancy Registry. Physicians prospectively register women in this ongoing international registry before the birth outcome is known and record exposure throughout pregnancy.

Approximately 85% of women in this analysis were registered in the United States, with smaller percentages from the United Kingdom (3.3%), South Africa (1.9%), France (1.2%), and 62 other countries. Median age at registration was 28 years and ranged from 13 to 55. While 30.3% of registered women had a CD4 count at or above 500 cells/mm3, 46.2% had a count between 200 and 499 cells/mm3, and 17.7% had fewer than 200 cells/mm3. CD4 counts were unknown in the remaining women. Almost half of the women studied, 47%, took antiretrovirals in the first trimester of pregnancy.

Of the 11,261 live births evaluated, 299 infants (2.7%) had at least one birth defect. Birth defect rates did not vary much by trimester: 2.8% in the first trimester and 2.5% in the second or third trimester. The risk of birth defects after first-trimester antiretroviral exposure did not significantly exceed the risk in the second and third trimester (prevalence ratio 1.10, 95% CI 0.88 to 1.37). Birth defect prevalence in the first trimester did
not differ significantly from the rate in a population-based surveillance system.

The Antiretroviral Pregnancy Registry Advisory Committee concluded that “the defects reported show no apparent increases in frequency and no pattern to suggest a common cause.” Although the population monitored to date is not large enough to detect an increased risk of some rare birth defects, the Advisory Committee proposed that these findings “should provide some assurance when counseling patients.”

**Tenofovir during pregnancy linked to lower infant weight at 1 year**

HIV-negative infants of mothers taking tenofovir during pregnancy had birth weights similar to those of neonates whose mothers took other antiretrovirals. But 1 year after birth, the Pediatric HIV/AIDS Cohort Study (PHACS) detected a higher rate of low weight in tenofovir-exposed infants.

Because adult use of tenofovir is increasing in the United States and elsewhere, and because animal studies suggested that tenofovir exposure affects fetal growth, PHACS researchers analyzed the potential impact of tenofovir exposure on HIV-exposed but uninfected infants in the United States. As part of the Surveillance Monitoring for Antiretroviral Toxicities (SMARTT) protocol of PHACS, this analysis involved 1887 neonates assessed for birth weight, 739 neonates assessed for birth length and head circumference, and 532 1-year-olds assessed for weight, length, and head circumference.

George Siberry (National Institutes of Health, Bethesda) and SMARTT colleagues defined low birth weight at below 2.5 kg. They defined small size at 1 year of age as z scores for weight, length, and head circumference below -1.5 (approximately the 7th percentile). The investigators limited the analysis to children of women taking or not taking tenofovir as part of a regimen including three or more antiretrovirals from two or more antiretroviral classes.

Maternal tenofovir use increased from 15% of the study population in 2003 to 39% in 2009: 12% of women took tenofovir in the first trimester, and 21% of infants were exposed to tenofovir. Siberry and coworkers recorded low birth weight in 20% of neonates, with no significant difference between those exposed to tenofovir (20.7%) and those not exposed (19.5%). A statistical model that adjusted for high maternal viral load, low maternal CD4 count, and alcohol or substance abuse found no association between tenofovir use and low birth weight (adjusted odds ratio [AOR] 1.03, 95% CI 0.75 to 1.40, \( P = 0.87 \)). In the same analysis, tenofovir exposure did not raise the risk of short length or small head circumference at birth.

Unadjusted analysis found a higher risk of low weight at 1 year of age in infants exposed to tenofovir in utero (OR 1.76, 95% CI 1.01 to 3.05). That association remained marginally significant even after adjustment for gestational age. Tenofovir exposure was not associated with short length or small head circumference in the unadjusted model or the adjusted model.

Why tenofovir exposure in utero would have no impact on birth weight but would affect weight at 1 year remains unclear. Siberry noted that other studies have detected a delayed clinical impact associated with perinatal exposure to other antiretrovirals, including lower CD8-cell count, mitochondrial dysfunction and neurologic development, febrile seizures, and central nervous system cancer. The investigators will continue to monitor these children to determine whether low weight at 1 year has clinical consequences.

**Treatment of Pediatric HIV Infection**

In revising ART guidelines for infants and children in 2010, WHO stressed the urgency of determining the HIV exposure status of all infants with an unknown or uncertain status and testing all HIV-exposed infants at age 4 to 6 weeks or as soon as possible. If you don’t test, you will never know,” noted Lynne Mofenson (National Institutes of Health, Bethesda) in reviewing the revised pediatric guidelines at the workshop. Infants with an initial positive virologic test (such as HIV DNA PCR) should begin ART without delay, but a second specimen should be collected to confirm the initial positive test. HIV-exposed infants with negative virologic tests who are well should have an HIV antibody test at age 9 months (the last immunization visit). If that test is reactive, the child should have an HIV viral test. Breastfeeding children with an initially negative viral test at age 4 to 6 weeks should be retested 6 weeks after breastfeeding stops completely, or earlier if symptoms suggesting HIV develop.

WHO guidelines now call for antiretroviral treatment of all HIV-infected children younger than 2 years regardless of CD4 or clinical criteria. Older children with WHO stage 3 or 4 disease should start ART regardless of CD4 count or percent. Children 24 to 59 months old with stage 1 or 2 disease should begin treatment at a CD4 count below 750 cells/mm3 or a CD4 percent...
For older children with stage 1 or 2 disease, the treatment threshold is the same as in adults: start at a CD4 count below 350 cells/mm³. With these changes, 60% to 80% of HIV-infected children would be eligible for ART.

Which antiretrovirals to start with depends on the child’s age, prior exposure to a nonnucleoside reverse transcriptase inhibitor (NNRTI) for PMTCT, and tuberculosis (TB) or hepatitis B virus (HBV) coinfection status (Table 2). For all children, the preferred order of NRTIs is zidovudine/lamivudine, followed by abacavir/lamivudine, then stavudine/lamivudine.

The updated guidelines stress that inability to perform laboratory monitoring, including CD4 count or viral load assessment, should not prevent children from receiving ART.9 The guidelines say viral load monitoring is desirable but not essential before starting ART. When available, viral load monitoring should be used to confirm clinical or immunologic failure before switching to a new regimen. CD4 counts should be measured at the time of diagnosis, every 6 months thereafter, more frequently as the CD4 count or percent approaches the treatment threshold in children over 2 years, before starting ART, and every 6 months after initiation. CD4 monitoring is also recommended if a new clinical staging event develops.

**WHO 2010 guidelines increase number of ART-eligible children**

If 2010 WHO pediatric antiretroviral guidelines (summarized above) were applied in a Ugandan cohort, they would significantly increase the number of children eligible for treatment at enrollment, even though not dramatically when compared with WHO 2008 guidelines.17 This study also found that, by applying existing guidelines, clinicians would put only half of eligible children on ART.

The analysis involved 985 HIV-positive children enrolled in the Tukula Fenna project in Kampala since 2003. Median age at enrollment was 5.8 years (interquartile range [IQR] 1.7 to 10.1). Martina Penazzato (Padua Hospital) and colleagues considered ART eligibility as the first-met criterion in the following order: age, WHO stage, CD4 count or percent. The investigators estimated the proportion of ART-eligible children according to WHO guidelines issued in 2006, 2008, and 2010.

The proportion of children eligible for treatment rose from 70% by the 2006 guidelines to 82% by the 2008 guidelines and to 87% by the 2010 guidelines (Figure 2). This increase was mainly attributed to the WHO recommendation to treat all HIV-positive children under 12 months old in 2008 and all positive children under 24 months old in 2010. Among children eligible because they were younger than 12 months (by the 2008 guidelines), only 12% would have met WHO clinical or immunologic criteria at any time during the

### Table 2. WHO 2010 first-line antiretroviral guidelines of infants and children

<table>
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<tr>
<th>Age and NNRTI, TB, or HBV status</th>
<th>Recommendation</th>
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| < 2 years, known PMTCT NNRTI exposure | 2 NRTIs + lopinavir/ritonavir (if available)  
If no lopinavir/ritonavir, start nevirapine |
| < 2 years with no or unknown NNRTI exposure | 2 NRTIs + nevirapine |
| > 2 years but under 3 years | 2 NRTIs + nevirapine |
| All others (regardless of nevirapine exposure) | 2 NRTIs + nevirapine or efavirenz  
Efavirenz preferred for TB cotreatment |
| < 3 years and needs TB treatment | 2 NRTIs + nevirapine (200 mg/m²)  
Or zidovudine or stavudine + lamivudine + abacavir |
| > 12 years with hepatitis B requiring treatment | Tenofovir + lamivudine or emtricitabine + efavirenz or nevirapine |

Source: World Health Organization.9
observation period. Among children eligible because they were younger than 24 months (by the 2010 guidelines), only 26% would have met WHO clinical or immunologic criteria.

Depending on whether the 2006, 2008, or 2010 guidelines are applied to the Tukula Fenna cohort, 40%, 57%, or 60% of children would have been eligible for ART at enrollment. Respective proportions of children still antiretroviral-naive 2 years after enrollment would be 24%, 16%, and 12%.

After 2008, when age eligibility began, 33 of 42 age-eligible children (78.6%) had not begun ART in this cohort. In contrast, only 12 of 48 children (25%) eligible by clinical stage did not begin therapy, and only 3 of 11 (27.3%) eligible by CD4 criteria had not started treatment. Overall, only 53 of 101 children (52.5%) began ART. Before 2008, clinical stage was the least applied eligibility criterion (OR 2.0, 95% CI 1.2 to 3.2), whereas age was the most missed criterion thereafter (OR 10.5, 95% CI 3.8 to 31.1).

Penazzato and coworkers suggested these latter findings indicate physician skepticism about applying WHO age criteria for beginning ART in this cohort. The investigators proposed that early infant diagnosis and better guideline dissemination should be enhanced in an effort to start infants and young children on treatment in a timely manner. They concluded that adopting 2010 WHO pediatric antiretroviral guidelines would significantly increase the number of children eligible for treatment at enrolment, though not significantly when compared with WHO 2008 criteria. However, they noted, a different impact may be expected in pediatric programs with strong links to PMTCT services because such programs are more likely to enroll infants and young children into care.

Virologic response to ART improving in European infants

Among infants starting ART before the age of 1 year in the nine-cohort European Pregnancy and Paediatric HIV Cohort Collaboration (EPPICC), virologic response improved substantially in more recent years. This EPPICC analysis presented by Claire Townsend (University College London) also found a better virologic response among infants starting a four-drug NNRTI regimen than in those starting a three-drug NNRTI regimen.

The study focused on 414 infants born from 1996 through 2008 who began ART before reaching their first birthday and who had at least 9 months of follow-up. The greatest proportion of infants (38%) was from the UK and Ireland, followed by Italy (23%), and France (18%). Most infants (76%) began ART before the age of 6 months, 36% were breastfed, and 29% had a pretreatment AIDS diagnosis. The proportion of infants starting a boosted protease inhibitor (PI) rose from 8% in 2000-2002 to 24% in 2003-2005 and to 41% in 2006-2008, while the proportion taking an NNRTI plus 3 NRTIs remained consistent over those three periods at 15%, 20%, and 20%. Slightly higher proportions received an NNRTI plus two NRTIs: 25% in 2000-2002, 30% in 2003-2005, and 30% in 2006-2008.
Defining virologic suppression as plasma viremia below 400 copies/mL, Townsend and colleagues charted a rising proportion of 12-month responders over four periods of ART initiation: 52% in 1996-1999, 56% in 2000-2002, 71% in 2003-2005, and 85% in 2006-2008. Multivariate analysis determined that every later year of ART initiation raised the chance of 12-month virologic response about 20% (OR 1.2, 95% CI 1.0 to 1.3, P < 0.01). Twelve-month response was not associated with any of the other variables considered: age at initiation, cohort, gender, ethnic group, maternal ART, neonatal prophylaxis, breastfeeding, or pretreatment AIDS, CD4 z score, or viral load.

Infants who began a four-drug NNRTI regimen had a 2.7-fold increased risk of 12-month virologic suppression compared with infants starting a three-drug NNRTI regimens (OR 2.7, 95% CI 1.1 to 6.8, P = 0.02), although almost all infants on four-drug NNRTI regimens were from the UK and Ireland. Change in CD4 z score at 12 months was associated with baseline CD4 z score (coefficient -0.8, 95% CI -0.9 to -0.7, P < 0.001) and maternal ART during pregnancy (coefficient -1.0, 95% CI -1.5 to -0.5, P < 0.001).

The investigators suggested that the correlation between later year of ART initiation and virologic response could reflect improvements in regimen efficacy as well as individualized HIV management leading to better adherence. They called for further study of the association between a four-drug NNRTI regimen and virologic response.

Mortality and loss to follow-up still high in Malawian children starting ART

In the first 3 months of ART at a pediatric HIV clinic in Blantyre, mortality and loss to follow-up did not improve substantially from 2006 to 2008. Younger age, Kaposi sarcoma, and wasting were independently associated with early death or loss to follow-up.

Malawi has approximately 90,000 HIV-positive children under the age of 16, and HIV accounts for 15% of childhood deaths. To assess the impact of ART on mortality and loss to follow-up, Peter Moons (Queen Elizabeth Central Hospital, Blantyre) and colleagues retrospectively studied 814 children who began ART with split-dose adult Triomune tablets ( stavudine, lamivudine, and nevirapine) between January 2006 and December 2008. Boys made up just over half of the study group (51.6%), 16.7% were 0 to 18 months old, 35.0% 18 months to 5 years old, 26.9% 5 to 10 years old, and 21.4% 10 to 15 years old. Almost two thirds of children, 62.8%, had WHO stage 3 or 4 disease. Half of the children were underweight, 73.1% were stunted, and 24.7% were wasted.

Among 448 children with 24 months of follow-up, 44.7% were alive and on treatment, 26.9% had transferred out of the clinic, and physicians stopped ART in 0.7%. Moons noted that the percentage of patients transferred out is roughly double that of clinics in similar setting, which probably explains the lower percentage of patients still in care at the clinic.

Of the 51 children who died in the first year of treatment, 71% died within 3 months of starting and 76% within the first 6 months. Among children lost to follow-up in the first year, 80% were lost in the first 3 months. Despite earlier HIV testing, elimination of an ART waiting list, and access to CD4 monitoring

| Table 3. Death and loss to follow-up among children starting ART in a Blantyre clinic |
|---|---|---|---|---|
| Source: Dr Moons (Queen Elizabeth Central Hospital, Blantyre) |
| Number of children | 2006 | 2007 | 2008 | Complete study period |
| Early death | 4.6% | 3.5% | 5.3% | 4.4% |
| Early loss to follow-up | 11.7% | 16.2% | 9.2% | 12.5% |
| Combined | 16.3% | 19.7% | 14.5% | 16.9% |

For Table 3, please refer to the source mentioned in the context.
after 2006, mortality and loss to follow-up within 3 months of starting ART did not change substantially from 2006 through 2008 (P > 0.1) (Table 3).

Multivariate analysis determined that younger age, wasting, and Kaposi sarcoma independently raised the risk of death or loss to follow-up within 3 months of starting ART at the following odds ratios (and 95% confidence intervals [CIs]):

- 0 to 18 months old (versus 5 to 15 years): OR 2.20 (1.18 to 4.11)
- 18 months to 5 years old (versus 5 to 15 years): OR 3.53 (2.08 to 5.99)
- Kaposi sarcoma: OR 12.34 (4.52 to 33.33)
- Wasting: OR 4.76 (2.93 to 7.75)

Multivariate analyses considering death and loss to follow-up separately yielded similar results. Moons listed three limitations to this analysis: no causes of death were recorded, there were no CD4 or viral load outcomes, and outcomes of children who transferred out of the clinic were unknown.

CD4 counts have limited value in identifying antiretroviral-treated adults with virologic failure. To assess that correlation in children, Brian Eley and coworkers in the IeDEA Southern Africa Collaboration used data from seven IeDEA sites to determine sensitivity, specificity, positive predictive value, and negative predictive value of WHO and US definitions of incomplete viral suppression during the first year of ART and viral rebound in children.

All children were younger than 16 years old and starting ART with three or more drugs. All South African IeDEA sites measured CD4 and viral load at least every 6 months. Eley and colleagues defined incomplete viral suppression as failure to achieve at least one viral load measurement below 400 copies/mL during the first year of ART. They defined rebound as a confirmed viral load above 5000 copies/mL after at least 18 months of treatment in children who attained viral suppression during the first year of treatment. Table 4 shows WHO and US definitions of immunologic failure in children.

Among 3640 children with at least 1 year of follow-up after starting ART, 3115 (86%) had one or more viral load assays in the first year of therapy, and 392 of those 3115 (12.6%) had incomplete virologic suppression. Among children with incomplete viral suppression, 90 of 2714 (3.3%) met any 2006 WHO immunologic failure criterion, 61 of 2380 (2.6%) met any 2010 WHO criterion, and 355 of 2585 (13.7%) met any US criterion. Although specificity and negative predictive value of immunologic criteria were high in identifying incomplete virologic suppression, sensitivity and positive predictive value were low:

- **WHO 2006 immunologic criteria:** sensitivity 10%, specificity 97%, positive predictive value 31%, negative predictive value 90%
- **WHO 2010 immunologic criteria:** sensitivity 6%,
specificity 98%, positive predictive value 20%, negative predictive value 92%

- US immunologic criteria: sensitivity 26%, specificity 87%, positive predictive value 20%, negative predictive value 91%

False-negative rates were high with all immunologic criteria: 250 of 2581 paired samples with 2006 WHO criteria, 179 of 2256 paired samples with 2010 WHO criteria, and 197 of 355 paired samples with US criteria.

Among 2513 children who achieved initial virologic suppression and had follow-up data 18 months after starting ART or later, cumulative probability of viral rebound in the following 2 years was 5.5%. Again, specificity and negative predictive value of immunologic criteria in identifying viral rebound were high, but sensitivity and positive predictive value were low:

- WHO 2006 immunologic criteria: sensitivity 4%, specificity 99%, positive predictive value 19%, negative predictive value 96%
- WHO 2010 immunologic criteria: sensitivity 4%, specificity 99%, positive predictive value 18%, negative predictive value 96%
- US immunologic criteria: sensitivity 21%, specificity 99%, positive predictive value 9%, negative predictive value 96%

There were 172 false-negatives in 4361 paired samples according to 2006 WHO immunologic failure criteria, 182 false-negatives in 4534 paired samples with 2010 WHO criteria, and 130 false-negatives in 3857 paired samples with US criteria.

Eley and colleagues concluded that “current definitions of immunologic failure are poor at detecting failure to suppress viral load and viral rebound” in children.

Viral load above 5000 predicts clinical events in Latin American children

A viral load above the WHO-defined failure threshold of 5000 copies/mL predicted WHO stage 3 or 4 events independently of CD4-defined immunosuppression, hemoglobin, and other variables in a large cohort study. George Siberry and NISDI Pediatric Study Group 2010 colleagues argued that “detection at this viral load threshold is feasible and desirable in all settings providing HAART to children.”

Although earlier research established that viral load has prognostic value in children before ART and in adults before and during ART, the potential value of viral load monitoring in predicting morbidity in children had not been addressed until the NISDI group established that baseline viral load predicts significant clinical events in ART-treated children, independently of pretreatment immunologic status.

The new study aimed to determine how well viral load or immunosuppression predicts clinical events in children when using time-varying values for viral load and CD4.

The NISDI pediatric study is an ongoing observational cohort study that began enrolling HIV-exposed and infected children in Argentina, Brazil, Jamaica, Mexico, and Peru in September 2002. This analysis focused on perinatally infected children enrolled between September 2002 and April 2007 who were younger than 15 years old and had received at least 6 months of continuous ART. Proportional hazards modeling updated CD4 and HIV RNA values to the most recent value before diagnosis of a stage 3 or 4 WHO event.

The 584-child study group was 54% female; 37% were 0 to 4 years old, 43% were 5 to 9, and 20% were 10 to 15. These children had taken ART for an average of 2.1 years, and 30% were taking their first antiretroviral regimen. Ninety-two children (16%) had a new stage 3 or 4 event, usually oral candidiasis (18.5%), unexplained diarrhea for at least 14 days (18.5%), recurrent bacterial pneumonia (15%), or low weight-for-age z score (15%).

The model considering time-dependent measures of viral load (above or below 5000 copies/mL), CD4 immunologic status (no immune suppression, mild, advanced, or severe), and hemoglobin determined that viral load above 5000 copies/mL independently raised the risk of a WHO stage 3 or 4 event 1.81 times (95% CI 1.05 to 3.11, P = 0.033). Every 0.5 log higher viral load increased the risk 1.14 times (95% CI 1.03 to 1.27, P = 0.016). A viral load at or above 400 copies/mL versus below 400 copies/mL did not independently raise the risk of progression (adjusted HR 1.61, 95% CI 0.88 to 2.96, P = 0.123).

Severe immunosuppression versus no immunosuppression raised the risk of a WHO stage 3 or 4 event 3.37 times (95% CI 1.66 to 6.84, P = 0.001), mild/advanced immunosuppression versus no immunosuppression raised the risk 2.39 times (95% CI 1.34 to 4.25, P = 0.001), and every 1 g/dL lower hemoglobin raised the risk 1.32 times (95% CI 1.11 to 1.55, P = 0.002).

The investigators noted several limitations of their analysis: This was an observational study in which awareness of viral load results may have prompted greater adherence counseling or regimen modification. Viral load was monitored only every 6 months, resistance testing was not available, and
study participants could be taking their first or a later regimen.

HIV clinics in many countries rely on clinical and CD4 criteria to predict HIV disease progression, as directed by the WHO. Siberry and colleagues proposed that “better identification of virologic failure through viral load monitoring could improve determination of need for increased adherence counseling or change of HAART regimen.”

First-line PI vs NNRTI and switch strategies in PENPACT 1
Viral suppression after 4 years of ART did not differ between children randomized to a first-line PI or NNRTI in the international PENPACT 1 trial.23 Nor did virologic response differ between children randomized to switch to a second-line regimen at a viral load above 1000 copies/mL versus 30,000 copies/mL. One potentially meaningful difference did emerge in this study: Through 5 years of follow-up, NRTI mutations developed more often in children randomized to an NNRTI than in those randomized to a PI, particularly those randomized to an NNRTI and switching above 30,000 copies/mL.

PENPACT 1 was a collaborative trial of the PENTA and PACTG groups (PENTA 9 and PACTG 390). The trial had two primary objectives: a long-term comparison of first-line PI-based therapy versus NNRTI-based therapy, and a long-term comparison of switching to second-line therapy at two viral load thresholds, more than 1000 and more than 30,000 copies/mL. Gareth Tudor-Williams (Imperial College, London) and colleagues randomized 266 ART-naive children to a PI plus two NRTIs or an NNRTI plus two NRTIs. Those groups were then randomized to switch to a second regimen if they had a viral load above either 1000 or 30,000 copies/mL after 24 weeks (or a CDC stage C diagnosis). Study physicians picked the antiretrovirals prescribed for each child. Minimum follow-up was set at 4 years, and the primary endpoint was change in viral load from baseline to 4 years.

Three children were excluded from analysis, and 218 (83% of 263 analyzed children) were in follow-up at the end of the study in August 2009. Median follow-up at that point was 5.0 years (range 5 weeks to 6.7 years). Median age of the 263 analyzed children at study entry was 6.5 years (range 1 month to 17.8 years), 52% were boys, 49% black, 26% white, and 18% Hispanic; 51% had CDC stage B or C disease. Median baseline CD4 percent was 17% (IQR 10% to 25%) and median viral 5.0 log10 copies/mL (IQR 4.5 to 5.7). In the PI group, almost everyone took lopinavir/ritonavir (49%) or nelfinavir (48%). In the NNRTI group, more children took efavirenz (61%) than nevirapine.

At the end of follow-up, 188 children (71%) remained on their initial regimen, and time to switch did not differ between the PI arm and the NNRTI arm. (By the end of the study, only 3% of children needed a third-line combination.) Change in viral load after 4 years—the primary endpoint—did not differ significantly by regimen (PI -3.16 log versus NNRTI -3.31 log, P = 0.26) or by viral load switch threshold (1000 copies/mL -3.26 log versus 30,000 copies/mL -3.20 log, P = 0.56). Nor did most other outcomes differ by randomization group, including proportion with a viral load below 50 copies/mL, change in CD4 percent, new CDC stage C events, or grade 3 or 4 adverse events.

PENPACT 1 researchers genotyped samples collected from children with a viral load above 1000 copies/mL at regimen switches, at 4 years, and at the end of follow-up. Cumulative resistance at the end of follow-up did not differ between the two switch strategies when analyzed as number of PI mutations.

Table 5. Cumulative NRTI resistance at the end of follow-up in PENPACT 1

<table>
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<tr>
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<th>1-2 mutations</th>
<th>&gt;3 mutations</th>
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<tbody>
<tr>
<td>PI 1000-copy switch</td>
<td>8 (12%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>PI 30,000-copy switch</td>
<td>5 (8%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>NNRTI 1000-copy switch</td>
<td>10 (15%)</td>
<td></td>
</tr>
<tr>
<td>NNRTI 30,000-copy switch</td>
<td>9 (14%)</td>
<td>7 (11%)</td>
</tr>
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</table>

Source: Dr Tudor-Williams (Imperial College, London)23
number of NNRTI mutations, samples with high-level resistance to etravirine, or number of children with resistance mutations. However, prevalence of NRTI mutations was higher in children randomized to an NNRTI than in those randomized to a PI (Table 5), largely because of a higher rate of three or more mutations in the NNRTI 30,000-copy-switch arm (11%) than in the PI 30,000-copy-switch arm (2%) or the PI 1000-copy switch group (2%).

Tudor-Williams and colleagues proposed that “frequent viral load testing (particularly if performed infrequently) is unlikely to have an impact on development of NNRTI resistance,” since resistance to this class occurs at low levels of viral rebound. They also suggested that “time spent addressing adherence at first virological failure is time well-spent” because the risk of selecting resistance mutations is low and adherence counseling at this point may promote a better response to second-line therapy.

### Complications of HIV and its Treatment

HIV infection, even when well controlled, and antiretroviral therapy both cause long-term complications. These problems are a special concern in children, many of whom face decades of HIV infection and treatment. Yet HIV- and ART-related complications are not as well studied in children and adolescents as in adults.

Alessandra Viganò (Luigi Sacco University Hospital, Milan) reviewed metabolic complications of HIV and its treatment at the Pediatrics Workshop, focusing on lipodystrophy, cardiovascular disease, and bone abnormalities. She reported that an ongoing prospective study of HIV-infected children and young adults (93% of them vertically infected) determined that 29% of them have body fat redistribution without dyslipidemia, 13% have dyslipidemia without body fat redistribution, and 14% have both complications. Alterations in body fat are generally not severe until puberty and are associated with duration of ART, with use of stavudine or PIs, and with markers of disease severity.

Although small pediatric cohort studies documented an association between ART and increased carotid artery intima-media thickness (cIMT), a marker of subclinical atherosclerosis, the relative impact of ART and HIV infection on cIMT remains poorly defined in children. To address this question, Viganò and coworkers conducted a case-control study comparing 23 HIV-infected adolescents and young adults receiving ART (mean age 20 years) and 19 healthy controls matched by age, gender, and body mass index. Both cases and controls underwent clinical, anthropometric, and laboratory assessments, including Echo-Doppler for cIMT.

cIMT was significantly greater in HIV-infected cases than in controls (mean 0.5 versus 0.4 mm, P < 0.001). Regression analysis documented an association between HIV infection and cIMT (regression coefficient 0.13, 95% CI 0.09 to 0.17, P < 0.001) and between male gender and cIMT (regression coefficient 0.08, 95% CI 0.03 to 0.12, P < 0.001). Applying age-specific standard deviation scores of cIMT for 10- to 20-year-old Caucasians to 30 youths in this study group who were 20 years old or younger, Viganò found that the mean standard deviation score corresponded to the 98th percentile in HIV-infected study participants and to the 46th percentile in controls. This finding, Viganò argued, confirms that the cIMT difference between cases and controls in her study is clinically meaningful.

Further analysis found a significant association between cIMT and duration of PI- or NNRTI-based ART after treatment with one or two NRTIs, that is, in study participants with the longest antiretroviral treatment history (P = 0.019). But this association was not significant in HIV-infected study participants who had not been treated with one or two NRTIs. Viganò concluded that prolonged antiretroviral exposure may place HIV-infected children at very high risk of premature atherosclerosis.

#### Cardiac manifestations in ART-naive and experienced children in Tanzania

One third of HIV-infected children had one or more markers of cardiovascular disease in a 96-child Tanzanian study. Before the study, clinicians had suspected cardiac abnormalities in only 2 of these 32 children.

Between September 2008 and March 2009, Werner Schimana (Elizabeth Glaser Pediatric AIDS Foundation, Moshi) and coworkers recruited a convenience sample of 96 children under 14 years old at the Kilimanjaro Christian Medical Center in northern Tanzania and assessed prevalence of cardiovascular manifestations by history, physical exam, echocardiography, and electrocardiography. They extracted clinical and lab data from medical records. The investigators excluded children with congenital heart disease. They defined left ventricular dysfunction as shortening fraction less than 28%, dilated cardiomyopathy as left ventricular systolic dysfunction with left ventricular enlargement, and pericardial effusion as effusion greater than 4 mm.
The study group included 65 children receiving ART and 31 who were not. Age averaged 7.5 years in the ART group and 6.4 years in the non-ART group, and equivalent proportions were boys (54% ART, 55% non-ART). The only difference between groups was the proportion with WHO clinical stage 3 or 4 disease: 62 children (95%) receiving ART and 13 (42%) not receiving ART ($P = 0.001$).

Thirty-two children—25 on ART (38.5%) and 7 not on ART (22.6%)—had any cardiovascular manifestation, but this difference between groups was not statistically significant. Nor did the groups differ significantly in rates of dilated cardiomyopathy (3% versus 0%) or pericardial effusion (16.7% versus 16.1%). The ART group did include a significantly larger proportion of children with left ventricular dysfunction (19, 29.2%) than did the non-ART group (3, 9.7%) ($P = 0.015$).

More advanced WHO clinical stage (but not CD4 percent) was significantly associated with cardiovascular manifestations ($P = 0.017$; Table 6). Numerous clinical signs and symptoms predicted cardiovascular manifestations in univariate analysis: dyspnea on exertion (OR 40.7, 95% CI 5.1 to 323.7), dyspnea at rest (OR 3.4, 95% CI 1.1 to 10.9), easy fatigability (OR 6.8, 95% CI 2.4 to 19.5), hepatomegaly (OR 13.8, 95% CI 1.6 to 115), tachycardia (OR 11.7, 95% CI 1.4 to 99.1), and cardiomegaly by x-ray (OR 4.9, 95% CI 1.4 to 16.9).

The study group included 36 children taking zidovudine in their first regimen and 19 taking stavudine. Schimana and colleagues detected left ventricular dysfunction in 15 children (42%) taking zidovudine and 4 (21%) taking stavudine, a significant difference ($P = 0.012$). But in univariate analysis, the confidence interval for the odds ratio measuring the relative impact of zidovudine versus stavudine crossed 1.0 (OR 3.75, 95% CI 0.77 to 18.2).

Schimana and colleagues concluded that cardiovascular manifestations are common, and often occult, in Tanzanian children with HIV infection. They suggested that physicians caring for such children “should consider regular cardiac evaluation, including screening echocardiography, in settings where this is available.”

### Risk factors for metabolic abnormalities in a pediatric European cohort

Nearly one third of 389 children in a European cohort had one or more metabolic abnormalities in a cross-sectional study. Ritonavir use independently raised the risk of metabolic abnormalities, while atazanavir use independently lowered the risk.

This analysis involved 389 children from 14 clinics in Belgium, Poland, and Italy that are part of the European Pediatric HIV Lipodystrophy Study. Children and youth from 2 to 18 years old were enrolled in the cohort, which excluded children taking corticosteroids. Findings reported at the Pediatrics Workshop by Naufil Alam (University College London) are cross-sectional data collected at enrollment. The investigators defined a metabolic abnormality as at least one episode of glucose intolerance or of gender- and age-determined hypercholesterolemia or hypertriglyceridemia.

Of the 389 study participants, 46% were male, 69% white, and 27% black. 37% were at Tanner stage I (prepubertal) and 25% at Tanner stage V (mature). Median age stood at 12.1 years (IQR 9.0 to 14.9). Most enrollees, 95%, were vertically infected, and 92% had CDC stage N or A disease at enrollment. All study participants were taking ART for a median duration of 8.4 years (IQR 5.4 to 10.7); 38% had a viral load above 50 copies/mL. At enrollment, about 60% were taking a PI (lopinavir/ritonavir in about 45%), and about 35% were taking an NNRTI (usually efavirenz).

Alam and colleagues found that 31% of study participants had a metabolic abnormality, 42% had fat redistribution, and 57% had lipodystrophy syndrome (metabolic abnormality and/or fat redistribution (Figure 3). Among all enrollees, 24% had hypertriglyceridemia, 13% hypercholesterolemia, and 1% glucose intolerance. Only 170 of 389 enrollees (44%) had no lipodystrophy symptoms.

Logistic regression models adjusted for age at

### Table 6. Cardiovascular manifestations and WHO stage in Tanzanian children

<table>
<thead>
<tr>
<th>WHO stage</th>
<th>Cardiovascular manifestation</th>
<th>With</th>
<th>Without</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>0</td>
<td>7 (10.9%)</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>3 (9.4%)</td>
<td>11 (17.2%)</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>9 (28.1%)</td>
<td>26 (40.6%)</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>20 (62.5%)</td>
<td>20 (31.3%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>32</td>
<td>64</td>
</tr>
</tbody>
</table>

Source: Dr Schimana (Elizabeth Glaser Pediatric AIDS Foundation, Moshi)27
recruitment, duration of antiretroviral exposure, and clinical site aimed to determine risk factors for (1) any metabolic changes, (2) any hypercholesterolemia, (3) any hypertriglyceridemia, and (4) both hypercholesterolemia and hypertriglyceridemia. The final model contained covariates significant at P < 0.05. These analyses revealed four factors independently associated with one or more outcomes at the following adjusted odds ratios (and 95% CIs):

**Any metabolic abnormality**
- White versus black race: AOR 2.3 (1.1 to 4.7), P < 0.05
- Current ritonavir: AOR 6.5 (3.6 to 11.7), P < 0.001
- Current atazanavir: AOR 0.1 (0.0 to 0.9), P < 0.05

**Hypercholesterolemia**
- Viral load > 50 copies/mL: AOR 0.4 (0.1 to 0.9), P < 0.05
- Current ritonavir: AOR 3.0 (1.5 to 6.4), P < 0.05

**Hypertriglyceridemia**
- White versus black race: AOR 3.0 (1.3 to 7.0), P < 0.05
- Viral load > 50 copies/mL: AOR 2.0 (1.0 to 3.7), P < 0.05
- Current ritonavir: AOR 12.3 (5.5 to 27.5), P < 0.001

**Hypercholesterolemia and hypertriglyceridemia**
- Current ritonavir: AOR 11.9 (2.7 to 52.4), P < 0.001

Alam and coworkers suggested that children taking ritonavir and ritonavir-boosted PIs may need increased monitoring for metabolic abnormalities, whereas children taking atazanavir may have a decreased risk of these complications. They called for further study of lipodystrophy syndrome in children because “metabolic abnormalities may be important in the early stages of development of cardiovascular disease.”

Severe on-treatment anemia linked to more advanced HIV in Asian children

Severe anemia was the most common adverse event during the first 6 months of ART in a study of 2127 children in the TREAT Asia Pediatric HIV Observational Database (TApHOD).29 Severe pretreatment anemia, use of zidovudine, WHO stage 4 disease, pretreatment CD4 percent below 5%, and male gender raised the risk of grade 3 or 4 anemia (hemoglobin below 7.5 g/dL) in these children.

This observational study involved 2127 children under 18 years old who began ART after 1 January 2002 and had at least 1 year of follow-up after at least 6 months of treatment. Torsak Bunupuradah and TApHOD collaborators defined adverse events as any adverse change in health or drug-related side effects after treatment began, graded 1 to 4 according to the US DAIDS 2004 system. The investigators analyzed the earliest adverse event in each child.

The study group had a median age of 6.7 years (IQR 3.9 to 9.6) and had equal proportions of girls and boys. Median weight was 16.0 kg (IQR 11.5 to 20.8), median CD4 percent 8% (IQR 2% to 14%).
median viral load 5.2 log10 copies/mL (IQR 4.8 to 5.7), and median hemoglobin 10.4 g/dL (IQR 9.3 to 11.5); 5.2% of children began ART with a hemoglobin level below 7.5 g/dL. Before treatment, 9% of children had WHO stage 1 disease, 28% had stage 2, 36% stage 3, and 27% stage 4. Only 10% of children had taken antiretrovirals before starting their first triple regimen; 92% began treatment with an NNRTI-based combination, usually nevirapine plus stavudine/lamivudine (44%) or nevirapine plus zidovudine/lamivudine (23%). Among the 177 children starting a PI regimen, 77% took lopinavir/ritonavir.

Two children died from severe hypersensitivity reactions and one from anemia. Table 7 lists incidence rates per 100 child-years for anemia, hypersensitivity reaction and rash, elevated alanine aminotransferase, and clinical lipodystrophy, which were the most common adverse events. No adverse events resulted in death. Proportions of children with abnormal lipid and glucose levels before and after ART began were 6% and 47% for total cholesterol at or above 200 mg/dL (P < 0.001), 40% and 71% for triglycerides at or above 130 mg/dL (P < 0.001), 61% and 32% for high-density lipoprotein cholesterol below 35 mg/dL (P < 0.001), and 4% and 9% for glucose at or above 110 mg/dL (P = 0.08).

Multivariate analysis identified five factors independently associated with grade 3 or 4 anemia and two factors associated with grade 2 or 3 lipodystrophy at the following hazard ratios (HR) (and 95% CIs):

| Clinical lipodystrophy defined as: grade 1—detectable by study participant or by caregiver for young children; grade 2—detectable on physical exam by health care provider; grade 3—disfiguring or obvious changes on casual visual inspection. | Source: TREAT Asia Pediatric HIV Observational Database.29 |
| Number of cases | Incidence / 100 child-yrs | 95% CI |
| Grade 3 or 4 anemia (hemoglobin < 7.5 g/dL) | 57 | 5.7 | 4.4 to 7.4 |
| Grade 3 or 4 hypersensitivity and rash | 18 | 2.0 | 1.3 to 2.2 |
| Grade 3 or 4 elevated alanine aminotransferase | 16 | 1.6 | 1.0 to 2.6 |
| Grade 2 or 3 clinical lipodystrophy* | 68 | 1.1 | 0.9 to 1.4 |

Bunupuradah and colleagues noted that the incidence of severe anemia in these children paralleled incidence in a cohort of Asian adults. They proposed that beginning ART before disease progression could reduce the frequency of some adverse events in children, and they endorsed transition from use of stavudine, as recommended by the WHO. The investigators suggested that standardized lipodystrophy assessments are needed for resource-limited settings to help track metabolic toxicities.

| Severe anemia (grade 3 or 4) |
| Baseline hemoglobin below 7.5 g/dL: HR 13.2 (6.9 to 25.4), P < 0.001 |
| Currently taking zidovudine: HR 4.5 (2.4 to 8.4), P < 0.001 |
| WHO stage 4: HR 3.7 (1.5 to 9.2), P = 0.006 |
| Baseline CD4 percent below 5%: HR 3.0 (1.0 to 8.9), P = 0.047 |
| Male: HR 1.8 (1.01 to 3.1), P = 0.046 |

| Clinical lipodystrophy (grade 2 or 3) |
| Stavudine use for 6 or more months: HR 9.7 (3.9 to 24.3), P < 0.001 |
| Age 13 years or older: HR 4.9 (1.9 to 12.8), P = 0.001 |

Table 7. Adverse event incidence in 2127 TREAT Asia cohort children on initial combination ART
Bone mineral density and growth parameters in Brazilian adolescents

One third of vertically infected adolescents in a prospective, single-center Brazilian study had low bone mineral density. Analysis of correlations between bone density and weight, height, and lean mass suggested that reversing nutritional risk in HIV-infected adolescents can benefit those with low bone mineral density.

Annie Schtscherbyna and colleagues at Clementino Fraga Filho University Hospital in Rio de Janeiro planned this observational study to estimate bone mineral density and bone mineral content, and to determine risk factors for low bone density, in adolescents who had been vertically infected with HIV. The investigators used DEXA scans to evaluate bone density. They defined lower-than-expected bone density for age as a z score below -2 for L1-L4 spine density and/or for total body bone mineral density.

The study group consisted of 57 adolescents and young adults (56% female) whose age averaged 16.3 years (+ 1.9 standard deviation [SD]). Weight averaged 51.6 kg (+ 9.5 SD), height 1.59 m (+ 0.09 SD), body mass index 20.3 kg/m² (+ 3.2 SD), and body fat 20.9% (+ 11.2 SD). Almost three quarters of cohort members (73%) had CDC immunological class C disease, 70% were taking PI-based ART, half were taking tenofovir, 30% had a CD4 percent below 15%, and 34% had a viral load below 50 copies/mL. Thirty-seven study participants (65%) had a bone mineral density z score above -2, while 20 had a z score at or below -2. Those with a higher z score weighed significantly more (56.6 kg + 9.1 SD versus 49.5 kg + 7.6 SD, P= 0.004) and had a significantly higher body mass index (21.7 kg/m² + 3.1 SD versus 19.5 kg/m² + 2.7 SD, P = 0.027). The two groups did not differ significantly in height, lean body mass, total body fat, cortisol, insulin-like growth factor-1, parathyroid hormone, or calcium.

The investigators documented vitamin D deficiency in 23% of the study group, and average vitamin D measured as 25OHD was nonsignificantly lower in the group with a z score below -2 (36.9 versus 44.0 ng/mL, P = 0.079). Consumption of calories, protein, fat, carbohydrates, calcium, and vitamin D did not differ significantly between the two groups. Nor did the groups differ in proportions with an undetectable viral load, taking tenofovir, or taking a PI.

Schtscherbyna and coworkers found significant positive correlations between lumbar spine bone mineral density and weight (r = 0.492), and between total body bone mineral density and weight (r = 0.496), body mass index (r = 0.448), and total body fat percent (r = 0.268) (all P < 0.05). Gains in weight, height, and lean mass through 1 year correlated positively with spine and total body bone mineral density. For total body bone mineral density, these correlations were r = 0.50 for weight, r = 0.523 for height, and r = 0.672 for lean mass (all P < 0.05).

The investigators believe their findings suggest that “attempts to reverse the state of nutritional risk [in HIV-infected youth] can be beneficial to those with low bone mineral density.” They suggested that adolescents with a low z score for weight or body mass index should be screened for low bone mineral density.

Tregs may limit HIV-specific immune response in children

T cells with regulatory activity (Tregs) suppress immune responses and thus may contribute to failure of the immune system to control HIV infection. At the same time, Tregs may influence chronic immune activation, a characteristic of poorly controlled HIV infection.

To explore the impact of Tregs on immune response in children taking antiretrovirals, Riccardo Freguja (University of Padova) and coworkers compared levels of Tregs and other CD4-cell and CD8-cell subsets in 89 children with HIV and in 10 HIV-exposed but seronegative age-matched children. Children’s ages ranged from 6 to 18 years. The investigators divided HIV-infected children into three groups: (1) 49 children with a viral load below 50 copies/mL, (2) 19 children with a viral load from 50 to 1000 copies/mL, and (3) 21 children with a viral load above 1000 copies/mL.

Freguja defined Tregs as T cells that express CD25 and the transcription factor FoxP3 but have low expression of CD127. They defined naive recent thymic immigrants as expressing CD45RA and CD31, naive peripherally expanded cells as expressing CD45RA but not CD31, memory cells as not expressing CD45RA, and activated cells as expressing CD45RA, and activated cells as expressing CD38.

Group 3, the children with the highest viral loads, had a lower median CD4 count (718 cells/mm3, IQR 590 to 1029) than group 2 (752 cells/mm3, IQR 557 to 1261), group 1 (908 cells/mm3, IQR 736 to 1191), or the uninfected children (1155 cells/mm3, IQR 768 to 1296) (overall P = 0.048). CD8 cell levels were significantly higher in children with than without HIV (P = 0.03), largely because of an expansion of memory CD8 cells: median 134 cells/mm3 (IQR 77
to 231) in children with HIV versus 72 cells/mm3 (IQR 50 to 112) in seronegative children (P = 0.023). In children with HIV, levels of activated CD8 cells were higher in each viral load stratum: 56 cells/mm3 (IQR 24 to 79) in group 1, 151 cells/mm3 (IQR 64 to 270) to group 2, and 151 cells/mm3 (IQR 64 to 270) in group 3 (P < 0.001).

Numbers of Tregs were highest in group 3, the group with the highest viral loads, at a median of 162 cells/mm3 (IQR 125 to 266) versus 134 cells/mm3 (IQR 83 to 274) in group 2 with intermediate viral loads and 123 cells/mm3 (IQR 79 to 235) in group 1 with undetectable viral loads (P = 0.06 for group 3 versus group 1). Treg levels correlated positively with levels of activated CD8 cells (r = 0.366, P < 0.001).

Freguja and colleagues believe their results suggest that chronic immune activation persists in children with a poor response to ART and may contribute to Treg expansion. They proposed that “Tregs may limit the HIV-1 specific response, but do not suppress immune activation in HIV-1 infected children.”

**HIV Infection and Adolescents**

Late HIV diagnosis remains a problem in children and adolescents, a concern complicated by delayed entry to care and a high dropout rate, according to one US study. Groups in Nigeria and Benin reported effective methods for identifying HIV-infected children not diagnosed soon after birth. Unprotected sex is common among some HIV-infected and exposed children, a US study found. Systematic review of global adherence studies in children and adolescents found better overall adherence in Africa, Asia, and Europe than in North America or Latin America. But the study also suggested the unreliability of caregiver reports as an adherence index.

**Systematic review finds better pediatric ART adherence in low/middle- income countries than in high-income countries**

Systematic review of 60 studies assessing ARV adherence among children and adolescents under 18 years of age found better overall adherence in low- and middle-income countries than in high-income countries, although that difference did not reach statistical significance. Factors that influenced adherence included caregiver characteristics, child health and psychosocial functioning, medication characteristics, and HIV status disclosure.

Lisa Butler (University of California, San Francisco) and colleagues searched electronic databases for articles that assessed ART adherence in children and adolescents (0 to under 18 years old). They included observational studies published up to 1 September 2009. Two reviewers independently screened and coded candidate studies. The investigators stratified articles by country income level (low/middle or high income according to World Bank definitions), assessment period (under 3 months, 3 months or longer, unknown), and assessment method (caregiver report, pill count, MEMs caps,).

After excluding 2265 articles, Butler and colleagues focused on 60 nonintervention studies, including 42 studies from high-income countries (7427 children) and 18 from low- and middle-income countries (3729 children). In the 42 high-income countries, on average 61% of children (95% CI 54% to 68%) were adherent to at least 80% of their ART doses. In the 18 low- and middle-income countries, 73% of children (95% CI 66% to 80%) were adherent to at least 80% of their doses. Thus, the proportion of children adherent to at least 80% of their doses was higher in studies conducted in low- and middle-income countries, though the difference did not reach statistical significance (P = 0.06).

In high-income countries, the proportion of children adherent to at least 80% of their doses was similar in studies with an assessment period shorter than 3 months (62%, 95% CI 55% to 69%) and in studies with an assessment period of 3 or more months (65%, 95% CI 51% to 78%), but lower in studies with an unspecified assessment period (53%, 95% CI 37% to 69%). Similarly, in low- and middle-income countries, the proportion of children who were adherent to at least 80% of their doses did not differ significantly between studies with an assessment period shorter than 3 months versus those with an assessment period of 3 or more months (76%, 95% CI 69% to 83% versus 74%, 95% CI 69% to 79%). Results analyzed by adherence assessment method suggested overestimates in 38 studies relying on caregiver report (73%, 95% CI 68% to 77%), compared with six studies relying on estimates by pill count (50%, 95% CI 38% to 62%) and three studies using MEMs caps to measure adherence (26%, 95% CI 15% to 37%).

Butler outlined an array of factors that affected adherence in these studies:

**Caregiver characteristics**

- Sociodemographic factors
- Relationship to child
- Understanding of antiretroviral administration
- Understanding benefits of taking antiretrovirals
- Cognitive and psychosocial functioning
- Self-efficacy
- Outcome expectancy
Other relevant variables were treatment or medication characteristics and disclosure of HIV status, though Butler and colleagues noted that the evidence for the effect of those and additional factors was mixed with respect to their effect on adherence. Further, their examination of the literature revealed no universal risk factor for poor adherence in children and adolescents.

Butler and colleagues concluded that antiretroviral expansion in low- and middle-income countries should not slow because of concerns about adherence. The researchers recommended work to standardize methods for measuring adherence in children and adolescents. They called for development and evaluation of “culturally appropriate, sustainable, strategies to improve and maintain optimal levels of adherence throughout childhood and adolescence.”

Multivariate analysis identified several factors that independently affected the risk of delayed entry into care: Living alone almost doubled the risk (adjusted OR 1.89, 95% CI 0.94 to 3.81), while living within 5 miles of the HIV clinic nearly tripled the risk (adjusted OR 2.87, 95% CI 1.36 to 6.03). Minniear noted that the 5-mile radius around the clinic captures three ZIP Codes with the highest poverty rates (30% to 60%) in Memphis. He suggested proximity to the clinic is probably a surrogate for unmeasured socioeconomic factors influencing public health.

Young people with a stable residence were half as likely to have delayed entry (adjusted OR 0.49, 95% CI 0.24 to 0.98). And youth with less than a college education were less likely to have delayed entry (adjusted ORs 0.32 for high-school graduates, 0.20 for high-school students, and 0.30 for high-school dropouts). Minniear suggested that faster entry into care by less educated youngsters reflects their greater likelihood of living at home where a parent or guardian may encourage care soon after HIV diagnosis. Busy college students living without immediate parental control may find reasons to put off their first clinic visit.

Race, sexual identity, age, and distance from the clinic did not differ significantly between people who failed to remain in care; nor did CD4 count, CD4 percent, viral load, or clinical stage. In multivariate analysis, not having insurance (versus private insurance) raised the risk of failure to remain in care more than 5 times (adjusted OR 5.6, 95% CI 1.58 to 20.11). Less formal education also raised the risk of dropping out of care: Compared with college students, adjusted ORs were 5.88 for high-school graduates, 8.64 for high-school students, and 11 for high-school dropouts. Regular drug use did not independently affect failure to remain in care, while having custody of a child tended to raise the risk of dropping out of care (adjusted OR 2.65, 95% CI 0.95 to 7.42).

People who failed to remain in care gained significantly fewer CD4 cells during follow-up than those who did (mean 107 versus 164 cells/mm³, P = 0.003). Their viral load fell less than people who remained in care (mean 2.4 versus 4.4 log10 copies/mL, P = 0.004). And those failing to remain in care had a higher latest viral load (mean 7.1 versus 5.5 log10 copies/mL, P = 0.006).

Minniear and colleagues called for “distinct
interventions targeting adolescents from unstable environments and college students diagnosed with HIV. . . to ensure prompt linkage into HIV services.” The investigators recommended “preemptive interventions” to stop youngsters with less than a college education or without insurance from dropping out of care.

Risk behavior in perinatally HIV-infected and exposed youth

Nearly half of participants in a study of perinatally HIV-exposed and infected adolescents met criteria for one or more behavioral health problems, most often a mental health problem (30%).34 Onset of sex and recent substance use (usually alcohol or marijuana) were each reported by 15%. Among sexually active youth, half or more reported unprotected sex. And among sexually active HIV-infected children, half had a detectable viral load and one third reported recent nonadherence to ART (any missed doses in the past 7 days according to caregiver or child).

Those findings emerged from a study of 254 perinatally HIV-infected or exposed 10- to 16-year-olds in the US-based Pediatric HIV/AIDS Cohort Study (PHACS). George Siberry and colleagues planned this analysis to examine co-occurrence of behavioral health outcomes, including mental health problems, sexual behavior and substance abuse, and ART adherence. All children analyzed in this study were born to an HIV-infected mother, and all HIV-infected youth were in medical care and had an ART history available.

The study group included 185 children with HIV and 69 HIV-exposed but seronegative children. About half in each group were boys, and 63% of those with HIV were 13 or older compared with 32% of those without HIV. Blacks accounted for 74% of the HIV group and 65% of the HIV-negative group; respective proportions in families with an annual income at or below $20,000 were 41% and 68%.

Across groups, 46% of participants met criteria for at least one behavioral health outcome, and 12% met criteria for two or more behavioral health outcomes. Among HIV-infected youth, the most frequently combined health behavior outcomes were nonadherence and mental health problems (in 34%), and nonadherence plus substance use and sexual activity (in 17%).

A model adjusting for race/ethnicity, household income, and (for HIV-infected youth only) a CDC class C diagnosis found the following independent predictors of two or more behavioral health outcomes (versus none):

- Male gender (in HIV-negative only): OR 9.87, 95% CI 1.45 to 67.1
- Age 13 or older (in HIV-negative only): OR 23.4, 95% CI 3.47 to 157
- Birth mother caregiver (in HIV-positive only): OR 2.86, 95% CI 1.04 to 7.88

Figure 4. Nearly half of 254 perinatally infected and exposed children in a US cohort had one or more indicators of behavioral problems.

Siberry and colleagues noted that HIV-negative children of HIV-infected mothers are often a forgotten group with less access to service programs. As this study shows, however, high proportions of these children need such support. The association between two or more behavioral outcomes and having a birth mother as caregiver may seem counterintuitive. But the finding could indicate that these children receive poor care from mothers coping with violence, substance abuse, and other problems.

The investigators proposed that the high rate of nonadherence in children with a detectable viral load and multiple behavioral problems “indicates a significant public health need for integrated prevention and intervention services targeting comorbid conditions.”

In Benin 21% of siblings of infants born in PMTCT program have HIV

Consistent testing of siblings of infants born to mothers in a PMTCT program in Cotonou, Benin revealed that 21% were infected with HIV.36 Children of women who had an earlier miscarriage or stillbirth, or whose child died, had a higher risk of HIV infection.

With a population of 8 million, the West African country of Benin had an HIV prevalence of 1.7% in 2007. The estimated proportion of HIV-infected women enrolling in PMTCT programs rose from 28% in 2006 to 70% in 2009, and 13% of women accessing PMTCT visit an antiretroviral treatment site within the following year. One of the first pediatric ART sites was the Military Teaching Hospital, where Alain Azondekon and colleagues began to encourage mothers accessing PMTCT to bring their other children for HIV antibody testing. This process continues until the mother brings her other children for testing or until the newborn is 2 years old.

This analysis involved 179 mothers, 113 (63%) of whom had at least one other child besides the newborn. Seventy-one of these 113 mothers (63%) had a history of miscarriage, stillbirth, or death of a child. The median number of siblings was 2 (range 1 to 5), and 18% of mothers had more than 3 children.

The investigators identified 221 siblings with a median age of 4 years (range 13 months to 14 years), and 188 of these siblings (85%) had an HIV antibody test. Median time from when mothers were first encouraged to bring children for testing until they did so was 6 months (range 1 to 23). Thirty-nine of 188 tested siblings (20.7%) had a positive test. HIV prevalence was 26% in children of mothers with a history of miscarriage, stillbirth, or a child who died versus 11% in other children (P = 0.01). Siblings with a positive HIV test represent 9.2% of HIV-positive children in care at the center. Seventeen of the 39 HIV-infected children (43.6%) were eligible for ART according to national guidelines. Among the 33
siblings not tested, 14 had been tested before.

Azondekon and colleagues suggested that this strategy “should be used as a key element in improving access to care and treatment” of HIV-infected children. They raised the question whether sibling testing should begin as soon as HIV-positive pregnant women are identified.

**Comprehensive Pediatric HIV Care**

Although mortality is low in children starting ART compared with untreated HIV-infected children, loss to follow-up ranges from 4% to 22% in the first 18 months of therapy, according to results of a 54-site analysis of the IeDEA pediatric cohort. Further analysis of the PEPI-Malawi study confirmed that stopping breastfeeding raises the risk of morbidity and malnutrition in HIV-exposed infants. Scrutiny of the Antiretroviral Pregnancy Registry over two decades disclosed higher risks of preterm birth and low birth weight with exposure to PI-based PMTCT combinations.

**Loss to follow-up 17%, mortality 5% in first 18 months of pediatric ART**

A 13,611-child analysis of the multinational IeDEA pediatric cohort recorded a 5.4% death rate and 16.6% loss to follow-up rate, with marked differences between the regions studied: Asia, East Africa, West Africa, and Southern Africa. Younger age and more advanced HIV infection raised the risk of both death and loss to follow-up.

The IeDEA analysis included children up to 15 years old when they began ART with three or more drugs. Children could have exposure to perinatal antiretrovirals for PMTCT. Valeriane Leroy (University Bordeaux) and IeDEA colleagues studied 1454 children from 11 Asian sites, 3114 from 23 East African sites, 6162 from 10 Southern African sites, and 2881 from 10 West African sites. Across these four regions, 86% or more had free access to lab tests. Rates of access to opportunistic infection prophylaxis were 77% in Asia, 100% in East Africa, 96% in Southern Africa, and 41% in West Africa. To track children lost to follow-up (defined as not making clinic visits for more than 6 months), home visits and phone tracing were used by 81% of Asian sites, 91.5% of East African sites, 64% of Southern African sites, and 30% of West African sites.

More than 90% of children in Asia and East Africa started ART with an NNRTI and two NRTIs, compared with 66% of children in Southern Africa and 69% in West Africa. Rates of children starting ART before 2005 were 40% in Asia, 6% in East Africa, 22% in Southern Africa, and 32% in West Africa. Median CD4 percent and age were 7% and 7 years in Asia, 12% and 6 years in East Africa, 14% and 4 years in Southern Africa, and 13% and 5 years in West Africa. Mortality at a median of 18 months after ART began varied from 4.3% to 7.4% across regions, while loss to follow-up varied even more widely (Table 8).

**Table 8. Mortality and loss to follow-up after starting ART* in IeDEA pediatric cohorts**

<table>
<thead>
<tr>
<th>Region</th>
<th>Number</th>
<th>Median follow-up (m)</th>
<th>Deaths (%)</th>
<th>Loss to follow-up (%)</th>
<th>Transferred out (%)</th>
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</thead>
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<td>Asia</td>
<td>1453</td>
<td>34</td>
<td>5.4</td>
<td>4.1</td>
<td>8.0</td>
</tr>
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<td>East Africa</td>
<td>3114</td>
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<td>4.3</td>
<td>14.0</td>
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<tr>
<td>Southern Africa</td>
<td>6162</td>
<td>17</td>
<td>5.7</td>
<td>9.0</td>
<td>16.4</td>
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<tr>
<td>West Africa</td>
<td>2881</td>
<td>21</td>
<td>7.4</td>
<td>21.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Total†</td>
<td>13,611</td>
<td>18</td>
<td>5.7</td>
<td>12.3</td>
<td>8.6</td>
</tr>
</tbody>
</table>

*Median 18 months after starting.
†Between-region heterogeneity P < 0.01.
Source: Dr Leroy (University Bordeaux)
Kaplan-Meier method was highest in West Africa (8.9%, 95% CI 7.8 to 10.1), followed by Southern Africa (6.4%, 95% CI 5.8 to 7.1), Asia (5.8%, 95% CI 4.7 to 7.2), and East Africa (5.5%, 95% CI 4.6 to 6.5) (P < 0.0001). Estimated 18-month loss to follow-up was 24.7% (95% CI 23.0 to 26.4) in West Africa, 17.0% (95% CI 15.6 to 18.6) in East Africa, 11.5% (95% CI 10.6 to 12.5) in Southern Africa, and 4.5% (95% CI 3.5 to 5.8) in Asia (P < 0.0001). Statistical analysis adjusted for several risk factors identified five predictors of mortality and 10 predictors of loss to follow-up at the following adjusted risk ratios (aRR) (and 95% CIs):

**Mortality**
- Age < 12 months (versus 10 to 15 years): aRR 2.8 (2.5 to 3.6), P < 0.01
- AIDS or stage 4 disease: aRR 2.0 (1.7 to 2.4), P < 0.01
- CD4% < 10% (versus > 20%): aRR 2.9 (2.2 to 5.8), P < 0.01
- Missing CD4%: aRR 1.5 (1.2 to 2.1), P < 0.01
- Severe anemia (hemoglobin < 7 g/dL): aRR 2.8 (2.1 to 3.6), P < 0.01

**Loss to follow-up**
- Age 1 to 2 years (versus 6 to 10 years): aRR 1.2 (1.0 to 1.5), P = 0.01
- Age < 12 months (versus 6 to 10 years): aRR 1.8 (1.5 to 2.2), P < 0.01
- 1 PI plus 2 NRTIs (versus 1 NNRTI plus 2 NRTIs): aRR 1.5 (1.3 to 1.7), P < 0.01
- Other regimen (versus 1 NNRTI plus 2 NRTIs): aRR 1.6 (1.3 to 2.1), P < 0.01
- AIDS or stage 4 disease: aRR 1.3 (1.2 to 1.5), P < 0.01
- Unknown disease stage: aRR 1.9 (1.6 to 2.2), P < 0.01
- CD4% 10% to 20% (versus > 20%): aRR 0.8 (0.7 to 1.0), P = 0.03
- Severe anemia (hemoglobin < 7 g/dL): aRR 1.4 (1.0 to 1.8), P < 0.01
- Starting ART in 2005-2007 (versus before 2005): aRR 2.5 (2.1 to 2.9), P < 0.01
- Starting ART after 2007 (versus before 2005): aRR 3.6 (3.0 to 4.5), P < 0.01

Care in a private clinic rather than a public clinic lowered the risk of 18-month loss to follow-up (aRR 0.2, 95% CI 0.1 to 0.2, P < 0.01), while care in a nonurban clinic versus an urban clinic doubled the risk (aRR 2.1, 95% CI 1.7 to 2.5, P < 0.01). Being in a larger cohort independently raised the risk of loss to follow-up (500 to 800 children versus under 250, aRR 2.6, 95% CI 2.1 to 3.3, P < 0.01; 800 or more children versus under 250, aRR 1.4, 95% CI 1.1 to 1.8, P < 0.01). Having to pay for lab tests doubled the risk of loss to follow-up (aRR 2.1, 95% CI 1.5 to 3.1, P < 0.01), and having to pay for antiretrovirals raised the risk more than 6 times (aRR 6.2, 95% CI 3.9 to 9.7, P < 0.01). For undetermined reasons, loss to follow-up tracing with phone calls alone rather than phone calls plus home visits independently lowered the risk of loss to follow-up (aRR 0.4, 95% CI 0.3 to 0.5, P < 0.01).

Leroy and colleagues cautioned that missing data for both outcomes and predictors could influence results. They suggested that correlations between loss to follow-up and both recent ART initiation and larger cohort size underline the challenges faced by health facility overload. The investigators concluded that “innovative and feasible approaches to retain children in ART programs are urgently required.”

**Stopping breastfeeding raises morbidity risk in PEPI-Malawi infants**

Infants who stopped breastfeeding during follow-up in the PEPI-Malawi study had a higher risk of morbidity in three follow-up periods and a higher risk of malnutrition. Receiving cotrimoxazole protected infants from both morbidity and malnutrition. Earlier studies correlated breastfeeding cessation with infant morbidity, but the time between cessation and morbidity had not been closely examined until this analysis.

The study involved HIV-exposed but uninfected Malawian infants originally enrolled in the PEPI-Malawi trial of extended infant prophylaxis. All mothers were counseled to breastfeed exclusively and to stop breastfeeding at 6 months. The analysis began when infants were 6 months old and included infants known to be breastfeeding or not breastfeeding over the following 9 months. Researchers excluded women with an inconsistent breastfeeding history. They defined breastfeeding as any reported breastfeeding at the start and end of three study intervals: 6 to 9 months, 9 to 12 months, and 12 to 15 months. No breastfeeding meant lack of breastfeeding at the start of the interval or breastfeeding at the start of the interval but no breastfeeding at the end. The researchers defined morbidity as any illness or hospital admission, and they defined malnutrition as weight-for-age z score below -2 during the 3-month study intervals. Illness could be clinically diagnosed or reported by the mother, so ascertainment bias may affect results.

Morbidity and malnutrition rates were much higher in nonbreastfeeding infants in the first period assessed (6 to 9 months) and remained higher in the two subsequent 3-month periods:

**Morbidity events per 100 person-years**
• 6 to 9 months: breastfeeding versus no breastfeeding: 51.3 versus 139.1
• 9 to 12 months: breastfeeding versus no breastfeeding: 52.8 versus 90.2
• 12 to 15 months: breastfeeding versus no breastfeeding: 30.2 versus 67.0

**Malnutrition events per 100 person-years**
• 6 to 9 months: breastfeeding versus no breastfeeding: 20.5 versus 42.5
• 9 to 12 months: breastfeeding versus no breastfeeding: 29.1 versus 37.8
• 12 to 15 months: breastfeeding versus no breastfeeding: 20.2 versus 44.9

The PEPI-Malawi team conducted a Poisson regression multivariable analysis that included (1) extended infant antiretroviral prophylaxis versus sdNVP plus 1 week of zidovudine, (2) maternal HIV disease stage 3 or 4 versus stage 1 or 2, and (3) infant cotrimoxazole prophylaxis versus no prophylaxis. This model determined that no breastfeeding independently raised the risk of morbidity 70% at 6 to 9 months (adjusted rate ratio [aRR] 1.70, P < 0.0001), 66% at months 9 to 12 (aRR 1.66, P < 0.0001), and 75% at months 12 to 15 (aRR 1.75, P < 0.0008). Cotrimoxazole prophylaxis lowered the risk of morbidity 44% at months 6 to 9 (aRR 0.56, P < 0.0001), 35% at months 9 to 12 (aRR 0.65, P < 0.0001), and 23% at months 12 to 15 (aRR 0.77, P < 0.005). Maternal HIV disease stage 3 or 4 versus stage 1 or 2, and (3) infant cotrimoxazole prophylaxis versus no prophylaxis. Type of infant antiretroviral prophylaxis did not affect morbidity risk in this analysis.

In the same regression model, no breastfeeding raised the risk of malnutrition: aRR 1.37, P = 0.02 at 6 to 9 months; aRR 1.34, P = 0.07 at 9 to 12 months; and aRR 1.91, P = 0.002 at 12 to 15 months. Cotrimoxazole prophylaxis lowered the risk of malnutrition at 6 to 9 months (aRR 0.72, P < 0.005) and 9 to 12 months (aRR 0.71, P = 0.003), but not at 12 to 15 months (aRR 1.11, P = 0.34).

Cumulative mortality did not differ significantly with versus without breastfeeding from 6 months of age through approximately 12 months. From month 12 to 15, however, the mortality curves diverged significantly (P = 0.034); at month 15 cumulative mortality was 6.4% with no breastfeeding versus 3.5% with breastfeeding. A Cox proportional hazards model determined that no breastfeeding independently raised the risk of infant mortality 78% [hazard ratio (HR) 1.78, 95% CI 1.02 to 3.12, P = 0.04]. Maternal HIV stage 3 or 4 versus 1 or 2 also independently raised the risk of infant mortality 78% (HR 1.78, 95% CI 1.16 to 2.67, P = 0.008). Type of infant antiretroviral prophylaxis and cotrimoxazole prophylaxis did not affect mortality in this analysis. Because weaning was associated with acute as well as late serious adverse outcomes in this study, the investigators proposed that prolonged breastfeeding should be encouraged, as recommended in the most recent WHO guidelines (see note39). The PEPI-Malawi team noted that their analysis could not account for reverse causality, that is, switching from no breastfeeding back to breastfeeding because the infant became sick.

**Impact of antiretrovirals on low birth weight and preterm birth**

Review of the Antiretroviral Pregnancy Registry from 1989 through January 2009 revealed higher overall rates of preterm birth (before 37 weeks) and low birth weight (less than 2500 g) in infants exposed to PI-containing combinations than in those exposed to non-PI combinations.40 Rates of very preterm birth (before 32 weeks) among monotherapy-exposed infants were significantly higher than among combination therapy-exposed infants. Very preterm birth rates were no different with PI versus non-PI combinations.

The Antiretroviral Pregnancy Registry is a prospective birth defect registry of pregnant women taking one or more antiretrovirals. Clinicians register women before the outcome of their pregnancy is known. This analysis included 12,451 pregnancies in 66 countries, including 85% from the United States. There were 10,022 singleton live births with evaluable data (80.5%), and this analysis focused on 9513 births with available data from 1989 through 31 January 2009.

Karen Beckerman and colleagues compared infants exposed to a single antiretroviral or exposed to a combination of two or more antiretrovirals including or excluding a PI. Preterm birth rates did not differ significantly between the 1439 infants exposed to a single antiretroviral (15.3%) and the 8503 exposed to any combination (13.0%). Nor did these groups differ significantly in proportions with low birth weight (15.4% versus 16.1%). Preterm births were more frequent in infants exposed to a PI-containing combination than in those exposed to a non-PI combination (14.1% versus 11.8%, P = 0.003), but rates of preterm births before 32 weeks did not differ significantly between these groups (2.3% versus 1.8%, P = 0.16). Proportions of infants with low birth weight were significantly higher with a PI combination than with a non-PI combination (P = 0.001). Birth weights below 1500 g were also more
prevalent with a PI combination (2.5%) than with a non-PI combination (1.6%).

However, in an analysis controlling for race, very low birth weight (less than 1500 g) in each antiretroviral exposure group did not differ from overall population prevalence. And rates of birth weight below 1500 g did not differ significantly between the PI combination group and the single-antiretroviral group.

Beckerman, an obstetrician who works in the Bronx, NY, emphasized that preterm birth and low birth weight are incompletely understood in women with and without HIV. She cautioned that these findings do not address the overall impact of maternal HIV disease stage on complications of pregnancy. Because infection and immunologic factors are thought to be the most common causes of preterm delivery, the investigators called for further research on the impact of immunologic dysfunction on pregnancy outcome.

Coinfections in HIV Infected Children

Coinfection studies presented at the Pediatrics Workshop focused on toxicity rates with combined nevirapine and cotrimoxazole prophylaxis, TB survival with early or delayed antiretroviral initiation, and paclitaxel plus ART for children with Kaposi sarcoma.

No increase in toxicity with extended nevirapine plus cotrimoxazole

Infants who received cotrimoxazole plus extended daily nevirapine prophylaxis while breastfeeding had no more hematologic toxicity or rash than infants who received only cotrimoxazole in a placebo-controlled trial in Uganda and Zimbabwe. The findings are important because the WHO recommends cotrimoxazole for HIV-exposed but uninfected children during breastfeeding. Rash and neutropenia have been reported as side effects of both cotrimoxazole and nevirapine.

HPTN 046 (version 2.0) was a phase 3, randomized, double-blind, placebo-controlled trial that began recruitment in Ugandan and Zimbabwean PMTCT antenatal clinics in January 2007. All infants received single-dose nevirapine with or without 1 week of zidovudine and began cotrimoxazole at 6 weeks of age. Children were randomized to receive daily nevirapine or placebo until 6 months of age or cessation of breastfeeding, whichever came earlier. After release of SWEN study results in 2007,42 the HPTN 046 trial design changed so that infants receiving placebo and younger than 42 days were switched to nevirapine through day 42. All infants enrolled afterwards received the SWEN regimen (6 weeks of extended nevirapine). Of the 350 infants enrolled, 57 enrolled after 10 August 2007 received the SWEN protocol, while 293 enrolled before that date were randomized to 6 months of placebo (n = 145) or nevirapine (n = 148). Forty-one infants randomized to placebo switched to nevirapine during the trial. The placebo and 6-month nevirapine groups differed in proportions taking antimicrobials at 6 weeks (79% versus 90%, $P = 0.02$) and in median baseline hemoglobin (11.4 versus 10.7 g/dL, $P = 0.002$). The two randomization groups did not differ in proportions of females, weight, baseline absolute neutrophil count, baseline skin rash, or mothers on ART.

Through 18 months of follow-up the three study groups did not differ in proportions with at least one episode of (1) neutropenia or anemia, (2) grade 3 or 4 neutropenia or anemia, or (3) grade 2 or worse skin rash (Figure 5). During the first 6 weeks, absolute neutrophil count fell in all treatment groups but then rebounded and did not differ across groups through 18 months. In an analysis adjusted for baseline hemoglobin and antimicrobial use, during the first 6 months children receiving nevirapine did not differ from those receiving placebo in (1) any grade of neutropenia or anemia, (2) grade 3 or 4 neutropenia or anemia, or (3) grade 2 or worse skin rash.

Jim Aizire and colleagues reported that adverse event rates were high during the first 6 weeks of life regardless of treatment group. But based on December 2004 DAIDS Toxicity Tables, rates of new adverse events did not differ for infants who received only single-dose nevirapine, 6 weeks of nevirapine, or 6 months of nevirapine. The investigators concluded that, in HIV-exposed but uninfected infants receiving prolonged prophylaxis with cotrimoxazole, nevirapine did not appear to increase the immediate or long-term risk of neutropenia, anemia, or skin rash.

Simultaneous ART improves survival in Indian children with TB

Children who began ART with anti-TB therapy had approximately a 70% lower risk of death than children who began ART after completing anti-TB therapy, according to results of an observational study in India. The findings add to data from adult trials indicating better survival when starting ART soon after starting anti-TB therapy rather than waiting.44-46

Tripti Pensi (Dr. Ram Manohar Lohia Hospital, New Delhi) and colleagues planned this observational
study to analyze the impact of simultaneous versus delayed ART in HIV-positive children diagnosed with TB at their clinic from 2002 through 2006. Follow-up continued through March 2010. Children in the delayed ART group completed anti-TB therapy before starting antiretrovirals. The analysis excluded children receiving ART for more than 2 months before TB diagnosis.

Of the 298 children in the cohort, 119 (40%) had TB, including 96 who received simultaneous ART (81% of 119) and 23 who did not. The simultaneous and delayed ART groups did not differ significantly in baseline characteristics, except for a lower CD4 count in the simultaneous group. Among the 119 children with TB, 80% had pulmonary TB, 10% miliary TB, 4.2% disseminated TB, 3.4% central nervous system TB, and 2.4% abdominal TB.

A Cox proportional hazards regression model adjusted for age, gender, and CD4 count at TB diagnosis determined that simultaneous ART and year of TB diagnosis independently lowered the risk of death, while other AIDS-defining illnesses tended to raise the risk of death at the following hazard ratios (HR) and 95% CIs:

- Simultaneous ART: HR 0.32 (0.17 to 0.71), P = 0.001
- Year of TB diagnosis: HR 0.77 (0.60 to 0.92), P = 0.028
- Other AIDS-defining diagnosis: HR 1.74 (0.88 to 3.41), P = 0.11

At 3 years of follow-up, the hazard ratio for survival with simultaneous ART was 0.24 (95% CI 0.062 to 0.926, P = 0.01), and at 5 years the HR was 0.33 (95% CI 0.11 to 0.78). A similar survival advantage with simultaneous ART was maintained through the end of follow-up. Kaplan-Meier analysis through 7 years of follow-up confirmed shorter survival in children who started ART after completing anti-TB therapy (P = 0.033).

Pensi and colleagues cautioned that their analysis is partially retrospective and that they do not have data on treatment side effects, drug interactions, or immune reconstitution inflammatory syndrome with ART.

Long-term KS remission with paclitaxel plus ART in children
Six perfusions of paclitaxel induced complete and sustainable remissions of AIDS-related Kaposi sarcoma (KS) in 20 of 28 children who began treatment in Mozambique.47 Four children in this retrospective cohort study died early in the course of treatment, and 4 died after initial KS remission.

Paula Vaz and colleagues at Pediatric Day Hospital in Maputo retrospectively analyzed outcomes in all 32 children with biopsy-proven KS seen at this HIV pediatric reference center from December 2003 through December 2008. HIV prevalence stands at 12% in Mozambique, including more than 100,000 HIV-infected children. Children are eligible for ART in Mozambique if they have WHO stage 3 or 4 disease, if their CD4 percent lies below 20% and they are under 36 months old, or if their CD4 percent lies below 15%
and they are 36 months old or older.

Children diagnosed with KS began a course of paclitaxel (six infusions of 75 mg/m\textsuperscript{2} every 4 weeks) plus prednisolone (1 mg/kg 24 hours before paclitaxel). Chemotherapy began at least 1 month after ART. Four children with KS died before chemotherapy began, and 28 received chemotherapy. Among the 28 treated children, 20 (71\%) were boys, age averaged 8.3 years (3.4 SD), and CD4 percent before chemo averaged 16\% (9.2 SD). All 28 children received stavudine/lamivudine, 22 with nevirapine, 4 with efavirenz, and 2 with lopinavir/ritonavir.

Seventeen children had KS confined to skin or lymph nodes, or minimal oral disease, or all presentations. Eleven children had T cell-associated edema or ulceration and extensive oral, gastrointestinal, or nonnodal visceral lesions. Four children had a CD4 percent at or above 15\%, while 23 had a lower CD4 percent. Fourteen children had no history of opportunistic infection or thrush and no B symptoms, while 13 did.

Of the 28 children who received chemotherapy plus ART, 4 died and 24 survived during treatment. Of these 24, 21 had a complete remission and 3 had relapses. After 4 late deaths through an average 27 months of follow-up, 20 children were alive and well. Children who died did not differ from survivors in age, CD4 percent, or TIS staging; nor did relapsers differ from nonrelapsers in these measures. Mean CD4 percent rose from 16\% before chemotherapy to 29.2\% after chemotherapy ($P = 0.02$).

There were 2 cases of grade 4 anemia, 2 cases of grade 3 or 4 neutropenia, and 1 case of grade 4 thrombocytopenia. Clinical side effects included grade 3 vomiting in 2, grade 3 diarrhea in 1, and grade 4 arrhythmia in 1. There were no cases of immune reconstitution inflammatory syndrome. Despite the expense of paclitaxel, Vaz and colleagues argued that “the feasibility and tolerance of this combined HAART-chemotherapy approach are compatible with use in a limited-resource setting.” Before trying paclitaxel for KS, this group used doxorubicin, bleomycin, and vincristine, with poor results. David Burger (University Medical Center Nijmegen) recommended paclitaxel level monitoring when possible because concentrations of this agent can rise or fall with concomitant ART.
Reference


36. Senou E, Azondekon A, Akpoli R, et al. Siblings testing by HIV-infected (and whose infants are HIV uninfected or of unknown HIV status) should exclusively breastfeed their infants for the first 6 months of life, introducing appropriate complementary foods thereafter, and continue breastfeeding for the first 12 months of life. Breastfeeding should then only stop once a nutritionally adequate and safe diet without breast milk can be provided." World Health Organization. HIV and infant feeding: revised principles and recommendations. Rapid advice. November 2009. http://www.searo.who.int/LinkFiles/HIV-AIDS_Rapid_Advice_Infant_feeding%28web%29.pdf


39. In November 2009, the World Health Organization issued the following rapid advice on breastfeeding by HIV-infected mothers: "Mothers known to be HIV-infected (and whose infants are HIV uninfected or of unknown HIV status) should exclusively breastfeed their infants for the first 6 months of life, introducing appropriate complementary foods thereafter, and continue breastfeeding for the first 12 months of life. Breastfeeding should then only stop once a nutritionally adequate and safe diet without breast milk can be provided." World Health Organization. HIV and infant feeding: revised principles and recommendations. Rapid advice. November 2009. http://www.searo.who.int/LinkFiles/HIV-AIDS_Rapid_Advice_Infant_feeding%28web%29.pdf


van psychische aandoeningen), Stevens Johnson syndroom.

HCV=9 %, HBV+HCV=1 %) en niet eerder behandelde patiënten (N=34/563 of 6 %; HBV=4 %, HCV=2 %,
C) meedoen op voorwaarde dat de leverfunctietests bij baseline niet hoger waren dan 5 keer de normale
van ISENTRESS bij patiënten met een gelijktijdige infectie met hepatitis B-en/of hepatitis C-virus gelijk aan die van ... t.o.v. baseline vertegenwoordigen van het AST, ALT of totaal bilirubine op bij respectievelijk 29 %, 34 % en 13 %

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verhoogd aspartaataminotransferase, verhoogde

ijzergebrek, pijn in lymfeklieren, lymfadenopathie,
stemming, depressie, ernstige depressie,
glossitis, odynofagie, acute pancreatitis, maagzweer, rectale bloeding, hepatitis, hepatische steatose, acné, alopecia, dermatitis acneiforme, droge huid, erytheem, ingevallen gezicht, hyperhidrose,
minder trombocyten, positief op rode bloedcellen in
urine, grotere tailleomtrek, gewichtstoename, minder witte bloedcellen, onbedoelde overdosis.

Niet bekend:
suïcidale gedachten, suïcidaal gedrag
(vooral bij patiënten met een voorgeschiedenis
van hepatitis C die worden behandeld met antiretrovirale combinatietherapie hebben een hogere kans op ernstige en mogelijk fatale psychische aandoeningen). Als gevolg hiervan moeten de patiënten die met ISENTRESS worden behandeld, de volgende symptomen nauwkeurig worden geobserveerd:

- vernauwing van de ogen of de ogen dicht doen, verstoring van het zicht,
- duizeligheid of verzwakking van de ademhaling,
- pijn in de borst of in andere delen van het lichaam,
- onwélzijn, in productie van vocht in het lichaam,
- emoties die niet passen bij de situatie,
- gedachten of gedrag die tegen de norma

P-glycoproteïne gereguleerde transport niet remt.

Op basis van deze gegevens wordt niet verwacht dat ISENTRESS de farmacokinetiek beïnvloedt van geneesmiddelen die substraten zijn van deze enzymen of P-glycoproteïne.

in vivo- onderzoeken wordt en
Op basis van in-vivo-onderzoek is gebleken dat raltegravir geen remmer is van de UDP-glucuronosyltransferases (UGTs) 1A1 en 2B7, doet één klinisch onderzoek op grond van een waargenomen effect op de glucuronidatie van bilirubine vermoeden dat er een toename van de glucuronidatie van bilirubine kan ontstaan bij gebruik van ISENTRESS. Het verband hierbij is echter onbekend.


Dat overeenstemming in door het aantal individuen die de proefvolgorde volgen, de standaardopname wordt
met andere ART's worden aangegeven. Een combinaat van antiretrovirale middelen met enkele ART's kan worden gebruikt bij patiënten met een ernstige overwichtsnelheid.

Er zijn geen bepaalde restricties op het gebruik van raltegravir bij patiënten met een overwichtsnelheid.

raltegravir moet worden voorgeschreven bij patiënten met hepatitis B en hepatitis C-infectie. In een tweede fase van de studie (BENCHMRK 2, Protocols 018 en 019) bij niet eerder met antiretrovirale middelen behandelde en met hiv-1 geïnfecteerde volwassen patiënten.

Gerandomiseerde, dubbelblinde, placebo-gecontroleerde studie (STARCH-01) bij een totale groep van 2181 HIV-1-positieve patiënten die eerder met een antiretrovirale therapie behandeld waren en die behandeling hadden afzegd. De studie werd onderzocht bij patiënten met een door antiretrovirale middelen behandelde, met hiv-1 geïnfecteerde volwassen patiënten.

Centraal-indicaties

Deze indicatie is gebaseerd op gegevens over

gebaseerd op analyses van 96-weeksdata uit
twee lopende, gerandomiseerde, dubbelblinde, placebogecontroleerde studies (BENCHMRK 1 en
BENCHMRK 2, Protocols 018 en 019) bij eerder met
antiretrovirale middelen behandelde, met hiv-1 geïnfecteerde volwassen patiënten. De resultaten
van deze studies zijn eerder gepubliceerd.

Klinisch-effectief

Bijwerkingen waarbij er volgens de onderzoekers een causaal verband was (alleen of in combinatie met andere ART) worden hieronder per frequentie opgesomd.
open een wereld van nieuwe mogelijkheden

ISENTRESS, de eerste integraseinhiber, nu geregistreerd voor zowel behandelingseenoude als eerder behandeld volwassenen met HIV in combinatie met andere antiretrovirale middelen. Dus kies vanaf het eerste begin ISENTRESS!

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Raadpleeg de volledige productinformatie (SPC) inclusief dosering, contra-indicaties en waarschuwingen akkoord ISENTRESS voor te schrijven. Zie elders in dit blad voor de verkorte bijsluiter.

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Meeting reports

5th International Workshop on HIV Transmission – Principles of Intervention
15-16 July 2010, Vienna, Austria

2nd International Workshop on HIV Pediatrics
16-17 July 2010, Vienna, Austria