

# HIV treatment bulletin

## S O U T H

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## htb south

HIV Treatment Bulletin

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## EDITORIAL

Welcome to the July/September 2009 issue of HTB South. In this issue we include our first coverage from the IAS Conference held in Cape Town in July.

Probably the most important findings at this conference come from the Development of AntiRetroviral Therapy in Africa (DART) trial. We summarise the main results and pregnancy outcomes in this issue. We also encourage readers to look at the many presentations that are posted to the study website:

<http://www.ctu.mrc.ac.uk.dart>

Other reports from this conference include maternal health, breastfeeding and prevention of mother to child transmission, and an overview of TB studies.

We also include reports from the Clinical Pharmacology Workshop held earlier in the year, and articles from Richard Jeffery's blog, which has the best coverage of basic science news.

We have had many questions about swine flu (H1N1) in people with HIV so we lead this issue with a summary from the i-Base Q&A, which may be useful to photocopy for patients and healthcare workers.

We also include a summary of key information and recommendations for management of swine flu and HIV from the South African National Institute of Communicable Diseases (NICD).

Our next issue of HIV South will have further reports from IAS including coverage of paediatric research presented at this conference and at the 1st International Workshop on HIV Paediatrics also held in Cape Town.

### Southern African HIV Clinician's Society

Since its inception in 1997, with a membership of approximately 250 members, the Southern African HIV Clinician's Society has grown to a membership of over 15 000 in the Sub Saharan region and internationally - a clear recognition of the services and support provided.

The Southern African HIV Clinician's Society is the largest special interest group within the South African Medical Association (SAMA). It is also the largest HIV interest group in the world.

The Society is thrilled to be part of the HIV Treatment Bulletin South initiative. This is a valuable publication for all Health Care Practitioners. This publication has essential, current and scientific information about research and HIV treatment updates with particular implications for clinical practice.

For more information about the Society or on how to become a member please visit:

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## SWINE FLU

### i-Base Q&A: swine flu and HIV

Caution: The swine flu pandemic is new. Some of the advice in this Q&A is likely to become outdated soon as scientists learn more about this virus.

#### What is swine flu?

Swine flu is a new strain of influenza (flu). The medical name for this strain is H1N1v. It has been called a “pandemic” because of the speed with which it spread to many different countries in a short time.

#### Are HIV-positive people more at risk of catching swine-flu?

No. Generally, as with other strains of flu, having HIV does not increase your risk of catching swine flu.

#### Are HIV-positive people at risk of becoming more ill from swine flu?

It may be more serious especially if you have a low CD4 count (less than 200 cells/mm<sup>3</sup>). If you have flu symptoms and either a low CD4 count or other health complications, you should ask your clinic to treat you with antiviral medicines such as oseltamivir, especially if you have had symptoms for less than 48 hours, when these medicines are most useful. But at this stage of the pandemic, it is unknown how much greater risk for complications swine flu is for people with HIV. It is also possibly more serious in HIV-positive children.

#### Are pregnant women at risk of becoming more ill from swine flu?

Pregnant women are at greater risk of becoming ill with severe symptoms, especially in the third trimester of pregnancy. Complications can develop rapidly. If you are pregnant and develop flu-like symptoms (fever, muscle pain, sore throat, dry cough), you need urgent treatment with oseltamivir (Tamiflu). You must not wait for any laboratory test results,

#### How is swine flu different from regular seasonal flu?

Because this is a new strain of flu virus, people do not have immunity against it. People older than 65 appear to be less affected by it than people aged 10 to 45. Researchers are already working to produce a vaccine, and this may, or may not, be ready in time for the next flu season.

#### Is swine flu very dangerous?

The vast majority of people with swine flu, both HIV-positive and HIV-negative, make a full recovery, even without special treatment. Nevertheless, if you have symptoms of swine flu and you are HIV-positive, especially if you have a low CD4 count or

other health complications, including being pregnant, you should take the steps recommended in this fact sheet.

#### How is swine flu spread?

Swine flu is spread by person-to-person contact, just like regular flu - specifically through not covering your mouth when sneezing and not washing your hands. Washing your hands regularly and trying to avoid unnecessarily touching your nose, mouth and eyes with your hands might help reduce the risk of you spreading or contracting flu. Remember to wash your hands after touching other people's hands.

#### Will flu medicines work in people who are HIV-positive?

Antiviral medications used to treat flu, such as oseltamivir (Tamiflu), will work against swine flu, but do not have a big impact on most people's symptoms. The National Institute of Communicable Diseases (NICD) recommends oseltamivir for people with HIV within the first 48 hours of flu symptoms. After 48 hours it is a choice you will have to make with your doctor.

#### Will flu medicines interact with antiretrovirals?

There is a potential for interactions between oseltamivir, boosted PIs and some nucleoside reverse transcriptase inhibitors (3TC, FTC and tenofovir) but the benefits outweigh this small risk. Your doctor, nurse or pharmacist should advise you on this.

#### What do I do if I think I have symptoms?

If you have HIV or are pregnant or both, you should contact your doctor or clinic (preferably first phone if possible). The NICD considers people with HIV possibly to be at higher risk of more severe illness (though scientists are unsure) and should be prioritised for receiving flu medicines. Testing is not always necessary and oseltamivir should be given on the basis of symptoms and before any swine flu test results are received.

#### When does seasonal flu occur?

Most people get seasonal flu during late autumn and winter, from April through to August. Almost all cases of flu are now swine flu.

#### Should I have the flu vaccine?

Yes, especially if you are HIV-positive or likely to become pregnant. Children and people living in institutions should also get vaccinated. The vaccine is seldom available in the public health system, but most pharmacies and private doctors can vaccinate you. The vaccine reduces the risk of you getting seasonal flu. Currently there is no vaccine that works against swine flu. Vaccination should ideally be given in April each year for seasonal flu. A new vaccine is given each year because the flu virus changes form year to year.

#### Where can I find out more information?

There is a swine flu helpline: 0861-364-232. Also look online at the NICD website: [www.nicd.ac.za](http://www.nicd.ac.za).

## Swine flu pandemic

**Nathan Geffen, TAC**

This is a summary of key information on the H1N1 pandemic, with emphasis on the treatment and management of this disease in HIV-positive people. The main source for this information is the National Institute of Communicable Diseases (NICD) which together with the National Health Laboratory Services (NHLS) produces a health workers' handbook on pandemic influenza A(H1N1) 2009. It is regularly updated and available on their website. [1]

**Table 1: Contact information**

Latest information on the swine flu pandemic in South Africa	www.nicd.ac.za
Department of Health website	www.doh.gov.za/swineflu/swineflu-f.html
World Health Organisation website	www.who.int/csr/disease/swineflu/en/
Centers for Disease Control and Prevention website	www.cdc.gov/h1n1flu/
Daytime NICD Influenza Hotline (8am to 5pm Monday to Friday) FOR HEALTH PROFESSIONALS ONLY	082 477 8026
After hours FOR HEALTH PROFESSIONALS ONLY	082 883 9920
For additional information on VTM and swabs contact the National Influenza Centre (Amelia Buys/ Cardia Fourie) FOR HEALTH PROFESSIONALS ONLY	011 386 6373
Department of Health Communicable Disease Control hotline FOR PATIENTS AND THE GENERAL PUBLIC	0861-DOH-CDC (0861-364-232)

### Current NICD position on swine flu

The new pandemic influenza A(H1N1) 2009 virus appears to have a higher attack rate than seasonal influenza and is spreading fast, particularly among young people from ages 10 to 45. The severity of disease ranges from very mild symptoms to severe illnesses that can result in death. The majority of people who contract the virus experience mild disease and recover without antiviral treatment or medical care. Of the more serious cases, more than half of hospitalised people had underlying health conditions or weak immune systems. Although the majority of people affected experience mild, self-limiting illness, a characteristic of pandemic strains is the ability to cause severe disease and fatalities in otherwise healthy, young people. Therefore it is vital that pandemic influenza be considered in the differential diagnosis of all patients currently being admitted with severe acute respiratory illness. The overall severity of this influenza pandemic has been assessed to be moderate.

### Pregnant women and people with metabolic conditions at higher risk of death

An analysis of 574 swine flu deaths worldwide published by Vaillant and colleagues in Eurosurveillance indicates that there are two important risk factors for mortality: pregnant women

and people with metabolic conditions including obesity. [2] As of 16 July 2009, 16 women (10% of all individually documented female cases who died and 30% of the 20-39 year-old women who died) were pregnant or had delivered at the time of their death. Among these 16 women, at least eight had documented underlying health risks (obesity, heart disease or a respiratory disease such as asthma or tuberculosis). No information was available as to the underlying health status of the eight remaining women who died.

A sub-analysis of swine flu deaths showed that diabetes and obesity were the most frequently identified underlying conditions and were found in fatal cases over the age of 20. In 13 fatal cases with individual detailed data on metabolic conditions, seven had obesity, five had diabetes, and one had both.

The NICD defines the following people to be at high risk of serious complications from swine flu:

1. Adults or children with underlying medical conditions and who are receiving regular medical care for conditions such as chronic pulmonary disease (including asthma) and cardiac disease (excluding hypertension), chronic renal and hepatic diseases, diabetes mellitus and similar metabolic disorders.
2. People who are immunosuppressed (including HIV-positive people, and people on immunosuppressive medications). Currently, there is no good data on the interaction between HIV and swine flu. However, experience with seasonal flu suggests that HIV-positive people may be at higher risk of complications.
3. Adults and children who have any condition (e.g. cognitive dysfunction, spinal cord injuries, seizure disorders, or other neuromuscular disorders) that can compromise respiratory function or the handling of respiratory secretions or that can increase the risk for aspiration.
4. Morbid obesity (BMI > 30) has been identified as a risk factor for complications of influenza as well as pulmonary embolic disease.
5. Children and adolescents who are receiving long-term aspirin therapy and who might be at risk for experiencing Reye's syndrome after influenza virus infection.
6. Residents of nursing homes and other chronic-care facilities.
7. Pregnant women, especially in the third trimester and also in the period after delivery.

### NICD positions on HIV and swine flu

There is currently no information on the effect of swine flu in HIV co-infected persons. Evidence that influenza can be more severe for HIV-infected adults and adolescents comes from US studies of HIV-positive people who had seasonal influenza, but these data are limited. However, several studies have reported higher hospitalisation rates, prolonged illness and increased mortality, especially among people with AIDS.

It is now clear that the pandemic virus has been established throughout South Africa and that sustained community transmission is occurring. Moving forward, a strategy that concentrates on the detection, laboratory confirmation and investigation of all cases, including those with mild illness is extremely resource-intensive, leaving little capacity for the

monitoring and management of severe cases. In addition, this diverts limited resources away from managing other diseases such as HIV and TB. In line with a WHO recommendation, it is therefore prudent to limit routine laboratory testing of all suspected cases of swine flu infections, to focus on people at risk for complications, those with severe illness and to monitor virus characteristics.

### NICD Case definitions

The NICD defines three case definitions of increasing seriousness: (1) Influenza Like Illness (ILI) – mild disease, (2) Severe Acute Respiratory Infection (SARI) – moderate to severe disease and (3) severe illness. High fever, persistent vomiting, and marked prostration with progressive / persistent symptoms may suggest ongoing viral replication and predict progression to more severe illness.

**Table 2: NICD Case definitions**

ILI	An individual with recent onset of an influenza-like illness (ILI), which may include fever $\geq 38^{\circ}\text{C}$  PLUS ONE OR MORE of the following acute respiratory symptoms: sore throat, rhinorrhoea / nasal congestion, cough or other signs part of the respiratory complex, myalgia, diarrhoea  PLUS absence of evidence of lower respiratory tract disease.
SARI: People 2 days to < 3 months old	Any child with diagnosis of suspected sepsis or physician diagnosed lower respiratory tract infection (LRTI) irrespective of signs and symptoms. Patient presenting within 7 days of the onset of illness.
SARI: People $\geq$ 3 months old to < 5 years old	Child has physician-diagnosed acute lower respiratory infection (LRTI) including bronchiolitis, pneumonia, bronchitis and pleural effusion. Patient presenting within 7 days of the onset of illness.
SARI: People $\geq$ 5 years	Any person presenting with: sudden onset of fever ( $>38^{\circ}\text{C}$ ) AND cough or sore throat AND shortness of breath, or difficulty breathing with or without clinical or radiographic findings of pneumonia. Patient presenting within 7 days of the onset of illness.
Severe illness in children	Any child age 2 months up to 5 years with: Cough or difficult breathing AND with any general danger signs (unable to drink or breast-feed, vomits everything, convulsions, lethargy or unconsciousness), OR Chest indrawing or stridor in a calm child.
Severe illness in adults	Severity criteria in adults of any age group include: respiratory distress, dyspnoea, hypotension and / or evidence of hypoxia.

### NICD recommendations on who should be tested

The NICD does not recommend routine testing for ILI patients. Patients with SARI or severe illness should be tested as well as people who are suspected to have died of swine flu.

### NICD treatment guidelines

Patients with HIV who present within 48 hours should receive antiviral therapy.

Patients with specific features of severe immunosuppression should be considered for oseltamivir after 48 hours at the discretion of the attending physician, including HIV-positive people with CD4 counts  $< 200$  cells/mm<sup>3</sup> or WHO Stage 4 (AIDS) or HIV plus active TB on treatment or other pulmonary infection.

People with moderate to severe illness (based on a clinical assessment) that require hospital admission should be managed as follows:

- Where possible people should be isolated in their own room with the door closed for the duration of hospital stay.
- Droplet and contact precautions should be instituted.
- Health workers should wear a surgical mask on entry into a patient's room and a properly fitting N95 mask should be used for aerosol-generating procedures.
- The patient should wear a standard surgical mask whenever he or she is required to leave the isolation room.
- Where separate isolation rooms are not available, suspected cases should be cohorted in a designated ward and the above precautions instituted.
- Oseltamivir or zanamivir should be used for treatment of moderate to severe cases.

Given the widespread current outbreak, infection with swine flu must be considered as part of the differential diagnosis in all patients presenting with community acquired pneumonia, Acute Respiratory Distress Syndrome (ARDS), any severe acute respiratory infection (SARI) and myocarditis. **Strong consideration must be given to urgent treatment with a neurominidase inhibitor such as oseltamivir or zanamivir without waiting for laboratory confirmation.**

### NICD notes on zanamivir and oseltamivir

The virus is currently sensitive to zanamivir and oseltamivir but it is resistant to the M2 proton channel inhibitors, amantadine and rimantadine.

There are sporadic reports of oseltamivir-resistant isolates and recommendations for use of antivirals may change as data on antiviral susceptibilities become available. Oseltamivir is orally administered and is registered for use in individuals aged  $\geq 1$  year of age. Zanamivir is administered through an inhaler and is registered for use in individuals aged  $\geq 12$  years of age.

Antiviral treatment with oseltamivir or zanamivir should be initiated as soon as possible after the onset of symptoms. Although benefit is likely to be greatest when therapy is initiated within 48 hours, some benefit may still be obtained in patients whose therapy is started later in the course of illness. The use of antivirals should be guided by the clinical condition of the patient and the clinical judgment of the treating physician, at whose discretion the decision to treat rests.

Recommended duration of treatment is five days, but there is limited data on the optimal dosage and duration for people with severe illness from the virus.

**Table 3: NICD dosage recommendations for oseltamivir and zanamivir**

Age group	Weight	Oseltamivir dosage	Zanamivir dosage
Adults		75mg twice daily	Two 5mg inhalations twice daily
Children	15kg or less	30mg twice daily	Two 5mg inhalations twice daily (only in children 12 years or older)
	15-23kg	45mg twice daily	
	24-40kg	60mg twice daily	
	>40kg	75mg twice daily	

### ARV interactions

According to the Liverpool Pharmacology Group (LPG), there may be potential interactions between oseltamivir and protease inhibitors. [3]

Oseltamivir can cause neuropsychiatric side-effects that might be made worse with protease inhibitors, although CNS symptoms are more likely caused by influenza itself. The LPG recommends caution when using oseltamivir in renally impaired patients using FTC, tenofovir and lamivudine.

There are no clinically significant interactions expected between zanamivir and any ARVs. The LPG also recommends that zanamivir may be preferable in a patient with renal failure as it is poorly systemically absorbed.

Detailed recommendations on dosage for HIV-positive people are provided at

<http://www.hiv-druginteractions.org>

### References

1. NICD and NHLS. Revised health workers handbook on pandemic influenzaA(H1N1)2009“SwineFlu”version3, updated 19August2009. (Latest version obtained by personal communication, but website is updated regularly: <http://www.nicd.ac.za>)
2. Vaillant et al. Epidemiology of fatal cases associated with pandemic H1N1 influenza 2009. Eurosurveillance, Volume 14, Issue 33, 20 August 2009. <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19309>
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(Thanks to Lucille Blumberg for assistance with this article.)

## CONFERENCE REPORTS

### 5th IAS Conference on HIV Pathogenesis, Treatment and Prevention

19-23 July 2009, Cape Town

### Introduction

There was a large amount of data presented at this conference. Particularly important data was presented from the DART study and infant feeding studies. We summarise DART here but will include detailed reports on it in the next issue. For the first time there was a track on operational research. The main presentations from this track will also be summarised in the next issue.

In this issue we include the following articles:

- Five-year survival rates of 87% without routine CD4 or laboratory monitoring in DART study demonstrate an important model for ARV access programmes
- Reducing HIV transmission during breastfeeding
- PEPI Malawi: transmission rates by maternal CD4 count
- Low transmission rates and favourable pregnancy outcomes reported in DREAM
- Pregnancy rates and outcomes among women in the DART trial
- Pregnancy outcomes in HAART exposed infants in Johannesburg
- Efavirenz conceptions in Soweto
- Impact of regimen and duration of therapy on risk of mother-to-child HIV transmission in Johannesburg
- A cost-effectiveness analysis of the OCTANE trial
- Pharmacokinetics of atazanavir/ritonavir during pregnancy
- The PEARL study
- Presentation with late stage HIV diagnosis in pregnancy
- Overview of TB-related studies at IAS
- Biomarkers associated with mortality: long-term follow up from SMART
- Influenza vaccine effective in HIV-positive adults
- Time from seroconversion to treatment in Europe and Africa
- Results from HSV-2 acyclovir studies

For the first time, web casts of several sessions are available via the conference website together with searchable online abstracts and PDF files of many of the posters or presentations:

<http://www.ias2009.org>

The abstract database from the meeting is online at the same site.

## Five-year survival rates of 87% without routine CD4 or laboratory monitoring in DART study demonstrate an important model for ARV access programmes

Simon Collins, HIV i-Base

The most important study results presented at the 5th IAS conference were from the DART (Development of AntiRetroviral Treatment in Africa) trial. [1]

Sponsored by the UK's Medical Research Council and University College London, DART randomised over 3300 treatment-naive patients in Uganda and Zimbabwe to be managed by either routine three-monthly CD4 count and laboratory monitoring (LCM group), or by clinically driven monitoring (CDM). Laboratory monitoring was also performed for this group, but results were only given to the treating doctor when a grade-4 toxicity was identified. Viral load was not monitored in either arm.

Criteria for switching patients to second-line therapy included a CD4 count less than 100 cells/mm<sup>3</sup> in the LCM group or by symptoms/progression in the CDM group.

Patients received one of three treatments, all with background AZT+3TC: abacavir (9%, n=300), nevirapine (7%, n=247) or tenofovir (74%, n=2469). d4T was not included as a first-line regimen, even though some patients switched to d4T during the study (for example to avoid anaemia). They were then followed for five years (median 4.9 years, IQR 4.5 – 5.3).

The Data and Safety Monitoring Board (DSMB) closed an earlier component of DART looking into treatment interruptions in March 2006.

The rationale behind DART was to determine whether ARVs could be used effectively without routine monitoring, in order to broaden access to treatment in settings where CD4 and laboratory monitoring are either not available or where they are difficult to access.

Enrollment criteria included being treatment-naive with a CD4 count <200 cells/mm<sup>3</sup>. Baseline median CD4 count and mean viral load were 86 cells/mm<sup>3</sup> (IQR 31-139, range 0-199; one-third of patients had a CD4 count <50 cells/mm<sup>3</sup>) and 5.4 log (SD +0.7) copies/mL. WHO stage 2/3/4 was diagnosed in 20%, 56% and 23% patients respectively.

At the conference DART had a separate satellite symposium within the main conference programme and this is one of the sessions that has been web cast. The top line results were both impressive and challenged common assumptions. Both arms showed a remarkable and similar 5-year survival rate - 90% vs 87% in the lab and clinical arms respectively - separated only by a small percentage difference that only occurred after the first two years on study. This compared to an historical 5-year survival rate prior to HAART of only 8%. Clinic attendance was >98% with high reported adherence and only 7% patients lost to follow-up over five years.

The event rates for a new WHO Stage 4 event or death were 6.94 versus 5.24 per 100 person-years in the CDM vs LCM arm [n=459 (28%) vs 356 (22%) HR 1.31 [1.14-1.51], log-rank p=0.0001]. Death rates/100PY were 2.94 in CDM versus 2.18 in LCM (p=0.004).

Differences between strategies occurred from the third year on HAART whereas lower rates of switching to second-line HAART occurred in CDM from the second year. There were no differences between strategies in time to first serious adverse event, grade-4 toxicity or HAART-modifying toxicity (see Table 1).

Table 1: 5-year event results from DART study

	Event rate/100 PY		HR [95%CI]
	[n (%)]		p-value
	CDM	LCM	
New WHO Stage 4 event or death	6.94 [n=459 (28%)]	5.24 [n=356 (22%)]	HR 1.31 [1.14-1.51] log-rank p=0.0001
Death	2.94	2.18	p=0.004
Time to first SAE			HR 1.12 [0.94-1.31] p=0.20
Time to grade-4 toxicity			HR 1.08 [0.97-1.20] p=0.18
Time to HAART-modifying toxicity			HR 1.01 [0.88-1.16] p=0.85

Around 60% of patients in each arm remained on their first line therapy after five years, with 20% modifying one or more drugs for tolerability and 20% of patients in each arm switching to second-line.

The higher mortality in the clinical monitoring arm was explained by patients being switched to second-line treatment at lower CD4 counts than the laboratory monitored group.

In an analysis of the two strategies, 3-monthly routine monitoring was determined to not be cost effective (based on the cost of treatment used in DART and relative to the WHO target for Incremental Cost Effectiveness Ratio (ICER) of 3 x GDP per capita). [2]

Lab unit costs were CD4 (\$8.80), haematology (\$5.30) and biochemistry (\$29.50), with biochemistry carrying the highest cost with the least efficacy benefit. This was used to support the DART main conclusion that treatment should not be withheld while waiting for monitoring and that resources for treatment access programmes should prioritise treatment over monitoring.

Several other presentations at the IAS conference presented a wealth of other aspects of the study including:

- Impact of different WHO 3/4 events on HAART on subsequent survival (Abstract MOPEB003)
- Impact of cotrimoxazole in patients on HAART: showing a 50% reduction in mortality during the first 72 weeks independent of CD4 count (Abstract MOPEB020)
- 5 year follow-up of participants initiating HAART with Combivir plus nevirapine or abacavir (randomised): showing >90% survival and >80% alive and event-free, with clear virological and CD4 advantages for the nevirapine arm (Abstract MOPEB057)
- Assigning clinical endpoints in clinical trials in resource limited settings (Abstract TUPEB098)

- Impact of HAART on incidence of malaria in Uganda (Abstract TUPDB104)
- 5 year follow-up of creatinine and estimated GFR in patients receiving and not receiving TDF first-line: showing overall low incidence of renal impairment on all regimens (2.9% GFR ever <30, 5.9% confirmed <60) with no differences between the LCM and CDM arms (Abstract TUPEB184)
- Pregnancy outcomes in women in DART: showing 378 pregnancies (57% live births, 6% still births and 36% termination/miscarriage), similar rate of congenital abnormalities (~3%) as the ARV pregnancy register, and no HIV-infected babies to date (Abstract WEPEB261) (we cover this on this issue)

These and other presentations are now posted on the MRC website. [3]

See the next issue of HTB for detailed coverage of some of these studies.

#### COMMENT

**These results strongly support expanding access to treatment to wider populations independent of access to routine laboratory monitoring and that delaying treatment access while waiting for laboratory infrastructure to be developed is resulting in extensive mortality and morbidity.**

**While the cost effectiveness analysis was limited to the strategy determined at the study outset, a sensitivity analysis looking at absolute real world costs based on using generic drugs (rather than subsidised Western brands) and using an annual CD4 count from year two would be likely to bring this within the WHO target, and may reverse the slightly higher mortality linked to late CD4 switching seen in the clinical monitoring arm.**

**For example, in DART the annual drug costs for the three first line treatment arms were \$432 (tenofovir), \$444 (nevirapine) and \$698 (abacavir) and \$954 for second-line (lopinavir/r + ddl).**

**The modelling for the DART analysis concluded that CD4 tests would have to drop to \$3.80 or less to be cost-effective, but this was based on providing 4 tests per year. Simply adding an annual test after two years would raise the minimum target cost per CD4 test to \$15.20, which is well over the current costs. This implies that an annual CD4 test is actually cost effective and would detect those patients with CD4 counts <100 cells/mm<sup>3</sup> who are at highest risk of serious events.**

**It is important to note that DART did not recommend excluding laboratory monitoring: the strategy was for treating without routine three-monthly monitoring, and the difference is critical to the study interpretation.**

**These results should also not be used to reduce the research and investment into the development of low cost diagnostics.**

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## Reducing HIV transmission during breastfeeding

### Polly Clayden, HIV i-Base

Three late breaker posters showed data from randomised trials evaluating different maternal and infant ARV regimens, among women not indicated to receive HAART by current guidelines, in order to reduce the risk of mother-to-child transmission, particularly during breastfeeding. [1, 2, 3]

### Mma Bana

In an oral presentation, Roger Shapiro from Harvard University, Boston, presented findings from the Mma Bana Study. Mma Bana is a randomised controlled trial, conducted in Botswana, comparing antiretroviral regimens in pregnant and breastfeeding HIV-positive women. [1]

This study enrolled 730 women from four clinical sites. Women were stratified by CD4 count. Those who did not meet the eligibility criteria for HAART, with CD4  $\geq$ 200 cells/mm<sup>3</sup> were randomised to receive: abacavir (ABC), zidovudine (AZT) and lamivudine (3TC) co-formulated as Trizivir (Arm A), or lopinavir/ritonavir (LPV/r), AZT and 3TC as Kaletra and Combivir (Arm B). Women with CD4 counts <200 cells/mm<sup>3</sup> were enrolled into an observational arm and received nevirapine (NVP) plus AZT and 3TC in accordance with Botswana National Guidelines.

Women with higher CD4 counts (n=560) were randomised between 26-34 weeks of gestation to Arm A or Arm B and they continued treatment until weaning their infants within 6 months. Women in the observational arm (n=170) initiated treatment at 18-34 weeks and continued indefinitely. This group also weaned their infants before 6 months.

All women received supplementary AZT during delivery. Infants received a single dose of NVP and one month AZT post partum. Follow up will continue for two years post partum.

The primary endpoints of the study were viral load <400 copies/mL at delivery and throughout breastfeeding and overall rate of mother-to-child transmission (MTCT).

Dr Shapiro reported low loss to follow up with 95% of mothers and 97% of mothers followed to 6 months or death. The majority of participants met both virologic and transmission endpoints: 99% women had viral load <400 copies/mL at delivery and 99.7% during breastfeeding; and 99.6% infants had birth PCR results (3 died before test) and 95% at 6 months or within one day of testing.

Baseline characteristics of the women were similar in the two randomised arms, their median ages were 26 and 25 years, CD4 393 and 403 cells /mm<sup>3</sup> and viral load 13,300 and 9,100 copies/mL in Arm A (n=285) and Arm B (n=275) respectively. Both randomised arms had a median baseline gestational age of 27 weeks. Women in the observational arm (n=170) were

older, median 29 years, with lower CD4 counts, 147 cells/mm<sup>3</sup> and higher viral loads, 51,700 copies/mL. Women received a median of 11 weeks in the randomised arms and 13 weeks in the observational arm of HAART prior to delivery.

Adherence to breastfeeding and HAART was good, 97% of women initiated breastfeeding and 93% breastfed exclusively until weaning. The majority of women, 71%, breastfed for  $\geq 5$  months and only 1% breastfed beyond 6 months. HAART adherence was similar across all three arms, 6% of women missed  $\geq 3$  days treatment.

At delivery (n=709), 96%, 93% and 94% of women in Arms A, B and the observational arm respectively had viral load  $< 400$  copies/mL (A vs B, 95% CI for difference, -2%, 10%). During breastfeeding (n=669), 92%, 93% and 95% had viral load  $< 400$  copies/mL (A vs B, 95% CI for difference, -8%, 6%). Risk factors for detectable viral load were higher viral load at baseline,  $p < 0.001$  and later gestational age at enrolment,  $p < 0.001$ .

At 6 months the overall transmission rate in this study was 1% (95% CI, 0.5%, 2.0%). Of these, 5 occurred in utero, there were no intrapartum transmissions and two were during breastfeeding (see Table 1: MTCT at 6 months in Mma Bana).

**Table 1: MTCT at 6 months in Mma Bana**

Infections, live born infants	Arm A (n=283) ABC/AZT/3TC	Arm B (n=270) LPV/r/AZT/3TC	Obs Arm (n=156) NVP/AZT/3TC
In utero	3 (1.1%)	1 (0.4%)	1 (0.6%)
Intrapartum	0	0	0
Breastfeeding	2 (0.71%)	0	0
Total	5 (1.8%)	1 (0.41)	1 (0.6%)

There was no statistically significant difference between Arm A vs Arm B,  $p = 0.53$ . Dr Shapiro noted that these results excluded one unconfirmed HIV-infected infant that died in Arm A. When this infant was included in the analysis the difference did not reach statistical significance,  $p = 0.42$ .

Maternal risk factors for transmission at delivery were fewer weeks of HAART, higher baseline viral load and poorer adherence. Higher baseline viral load and poorer adherence were also risk factors for transmission during breastfeeding.

Stillbirth occurred more frequently in the observational arm: 11 (7%) vs 8 (3%) and 5 (2%) in Arms A and B, randomised vs observational,  $p = 0.07$ . Prematurity was more frequent in Arm B vs Arm A, 61 (23%) and 42 (15%) respectively, A vs B,  $p = 0.04$ , and in 16 (10%) in the observational arm. Low birth weight did not differ by HAART regimen, 37 (13%), 45 (11%) and 23 (15%) in Arms A, B and observational respectively. Nor was there a difference in congenital abnormality, which occurred in 5 infants in each arm (2%, 2% and 3% respectively).

There were few maternal deaths, 1 ( $< 1\%$ ) in Arm A and 3 (2%) in the observational arm. Grade 3 or 4 maternal adverse events occurred in 42 (15%), 32 (12%) and 48 (28%) women in Arm A, Arm B and the observational arm respectively. These were treatment-limiting in 7 (2%), 6 (2%), and 18 (11%) women in Arms A, B and observational.

Dr Shapiro concluded that using maternal HAART, among 709 live births, the overall mother-to-child transmission rate was only 1% with only 2 (0.3%) of infections occurring during the 6-month period of breastfeeding. "The lowest MTCT rate ever recorded in a breastfeeding population", he said.

## BAN

In a second oral late breaker, Charles Chasela from the University of Northern California Project, Lilongwe, Malawi showed preliminary, 28 week, results from the Breastfeeding Antiretroviral and Nutrition (BAN) study. [2]

Formula feeding is not recommended in Malawi due to its high cost and greater association with infant mortality frequently observed in resource-limited settings.

BAN is a randomised controlled trial of mother infant pairs. Its aim is to evaluate two antiretroviral interventions over 24 weeks of exclusive breastfeeding followed by a four-week period of weaning, among women with CD4 counts  $> 250$  cells/mm<sup>3</sup> with infants uninfected at birth and  $\geq 2000$  grams.

In this study all mothers and infants received single dose NVP plus one week AZT/3TC "tail" coverage. All women received nutritional supplementation, which the investigators described as, "enhanced standard of care". Mother and infants were randomised to receive maternal HAART or NVP infant prophylaxis or nutritional supplementation alone (control). After cessation of breastfeeding, infants receive plumpy nut weaning food until 48 weeks. The primary endpoint was infant HIV status at 28 weeks.

A total of 2367 mother infant pairs were randomised within one week of birth; 851 received maternal HAART, 848 received infant NVP and 668 were in the control arm. There were no significant differences in the participants across the arms. The women's median ages were 26, 25 and 26 years in the HAART, NVP and control arms respectively,  $p = 0.7$ . Their median CD4 counts were 428, 440 and 442 cells/mm<sup>3</sup>,  $p = 0.16$ .

Dr Chasela noted that during the trial the maternal HAART changed from a NVP-based regimen to nelfinavir in February 2005 and to LPV/r in January 2006. Nucleosides were AZT and 3TC.

There were no significant differences in maternal grade 3/4 toxicities, except for low neutrophil count in women receiving HAART 6.7% vs 2.9 and 2% in the HAART, NVP and control arms,  $p < 0.0001$ . This is known to be associated with AZT.

Among the infants, 16/848 experienced possible NVP hypersensitivity which all resolved when the NVP was discontinued. Additionally symptoms were observed in one infant whose mother was receiving NVP.

The investigators found both the infant NVP and maternal HAART regimens significantly reduced 28 week HIV transmission to the infants compared with the enhanced control arm.

The 28-week transmission in the infant NVP arm was 1.8% vs 6.4% in the control arm,  $p < 0.0001$ . In the maternal HAART arm the transmission rate was 3.0% vs 6.4% in the control arm,  $p = 0.0032$ .

The estimated HIV-transmission or infant death rate was 7.6% in the control arm vs 4.7% in the maternal HAART arm,  $p = 0.031$ . This estimation was 2.9% vs 7.6% in the NVP and control arms,  $p > 0.0001$ .

Dr Chasela concluded that this study shows both maternal and infant ARV prophylaxis during 28 weeks of breastfeeding are safe and effective in reducing postnatal mother-to-child transmission of HIV.

He added, "Although this study was not powered to directly compare the maternal and infant interventions, there was some suggestion that the transmission of HIV was lower in the Infant NVP arm."

Final 28-week visit for this study will be August of this year and 48-week visit will be January 2010.

### Kesho Bora

A late breaker poster authored by Isabelle de Vincenzi and the Kesho Bora Study Group reported preliminary data from a comparison of maternal HAART to short course prophylaxis regimens in women also not currently eligible for treatment.

In this study - conducted in five sites in Burkina Faso, Kenya and South Africa - pregnant women with CD4 counts 200-500 cells/mm<sup>3</sup> were randomised between 28 and 36 weeks gestation to receive either maternal HAART (AZT+3TC+LPV/r to approximately 6.5 months after delivery or breastfeeding cessation if earlier) or short-course AZT plus single-dose NVP in labour. All infants received single-dose NVP post partum. During the course of the study one-week maternal "tail" coverage was added to the short course regimen and one week AZT for all infants.

Participating women received infant feeding counselling recommending either replacement feeding with free formula or exclusive breastfeeding, weaning from 5.5 months over a two-week period.

Women in both study arms were a median age of 27.4 and had a median CD4 count of 335 cells/mm<sup>3</sup> at enrollment.

There were 805 live births, 402 in the HAART and 403 in the short course arms.

The investigators reported 76.4% and 78.2% of infants were ever breastfed in the HAART and short course arms respectively. Of these 47.5% and 45.6% were breastfed exclusively and the median duration was 21.4 weeks.

Kaplan-Meier estimates of the cumulative infant infection rates in the HAART arm were: 1.8% (95% CI, 0.8-3.7) at birth, 3.3% (95% CI, 1.9-5.6) at 6 weeks, 4.9 (95% CI, 3.1-7.5) at 6 months and 5.5 (95% CI, 3.6-8.4) at 12 months.

In the short course arm these rates were: 2.2% (95% CI, 1.2-4.3) at birth, 4.8% (95% CI, 3.1-7.4) at 6 weeks, 8.5 (95% CI, 6.1-11.8) at 6 months and 9.5 (95% CI, 6.9-13.0) at 12 months. This gave a 42% reduction in transmission risk at 12 months,  $p=0.039$ .

Provisional estimate of cumulative death rate at 12 months showed 6.3% (95% CI, 4.3-9.3) in the HAART arm vs 10.0% (95% CI, 7.3-13.6) in the short course arm. This was a risk reduction of 37% but this was not significant,  $p=0.086$ .

And provisional estimate of HIV infection or death at 12 months showed 10.4% (95% CI, 7.7-13.9) in the HAART arm vs 16.3% (95% CI, 12.9-20.5). Giving a 36% risk reduction,  $p=0.022$ .

Subgroup analysis of infants who ever breastfed revealed a cumulative infection rate of 5.0% vs 8.8% at 6 months and 5.9%

vs 10.2% at 12 months, in the HAART vs short course arms. This was not significant,  $p=0.064$ .

Cumulative infection rate for infants whose mothers had a baseline CD4 200-350 cells/mm<sup>3</sup> was 5.5% vs 10.5% at 6 months and 6.1% vs 11.1% at 12 months in the HAART and short course arms,  $p=0.044$ .

Among infants whose mothers had a baseline CD4 350-500 cells/mm<sup>3</sup> the rates were 4.1% vs 5.9% at 6 months and 4.9% vs 7.4% at 12 months, which were not significant,  $p=0.33$ .

The investigators concluded that maternal HAART given to women with CD4 counts 200-500 cells/mm<sup>3</sup> during pregnancy and through breastfeeding reduces risk of HIV transmission and improves HIV-free survival compared to standard short course regimen. They noted that the largest effects were between 6 weeks and 6 months and among infants with mothers with baseline CD4 200-350 cells/mm<sup>3</sup>.

Importantly they found some postnatal HIV transmissions occurred despite maternal HAART and suggest that mothers may not have been able to wean at 6 months, underlining the importance of continuing HAART until complete breastfeeding cessation, or that this may be explained by inadequate adherence.

Final 12 months results from this study will be available in December 2009 and 18 months results in June 2010. These will include data on maternal health.

### COMMENT

**Together these data contribute to what we know and will influence policy and clinical practice. However, they do not yet resolve the question of how best to prevent mother-to-child transmission among women not yet indicated for treatment for their own health during pregnancy and breastfeeding.**

**It is difficult to compare transmission rates between these studies, as there are differences to consider between duration of antepartum treatment, maternal baseline CD4, exclusive vs mixed breastfeeding, and levels of adherence, etc.**

**Mma Bana reported 93% exclusive breastfeeding, over 90% adherence and nearly three months antepartum treatment, all of which combine to give lower transmission rates than the other studies. This does not tell us though whether HAART is better than short course plus infant prophylaxis for women with high CD4 counts. In Kesho Bora, for the subgroup of women with CD4 counts of 350-500 cells/mm<sup>3</sup>, there was no difference with HAART vs short course and no postnatal prophylaxis.**

**Kesho Bora looked at two issues: transmission rates at birth, and postnatal transmission rates. Transmission at birth reflects receipt of HAART vs short course during pregnancy and here transmission rates were almost identical. Postnatal transmission rates after birth reflect a comparison of HAART vs nothing during breastfeeding. HAART was better than nothing. HAART is better than short course with nothing during breastfeeding. We already know infant prophylaxis is better than nothing, so that question is no longer relevant. The important comparison for breastfeeding is: maternal HAART vs infant prophylaxis. The BAN study showed both work and, in this study, if anything, infant prophylaxis had the lower postnatal transmission rate.**

But for maternal HAART to be most effective, starting at birth (as in BAN) is not an ideal intervention, it takes weeks, even months, for someone to be fully suppressed with HAART so starting too late in pregnancy (or at delivery) will put the baby at risk while the virus is detectable (though the threshold viral load for quantifying risk is not clear). In Mma Bana, the investigators found starting HAART >30 weeks gestation led to only 85% suppression by delivery whereas starting <30 weeks was associated with 97% suppression at delivery. This may be associated with early breastfeeding risk as well as in utero/intrapartum risk (previous studies correlate breast milk viral load with transmission risk and plasma and breastmilk viral load are also correlated).

In Kesho Bora there was less exclusive breastfeeding and possible poorer adherence and the rate of viral suppression was not presented (but should be soon). Their median time on HAART before delivery was shorter than Mma Bana so it is likely that more women were unsuppressed at delivery and in early breastfeeding. Concerns about mixed feeding in later breastfeeding are probably less important than those of suppression and adherence. If there is little or no virus in breast milk then that would most likely prevent additional risk from gut issues related to mixed feeding, but the relative contribution of different transmission risks have not yet been studied.

It was interesting to see data for a triple nucleoside regimen in Mma Bana for women with higher CD4 counts, but it is likely that lopinavir/r-based regimens will remain the standard of care for women in this situation particularly as it more available and cheaper in Africa and has more safety data. Importantly both regimens performed very well in this study.

None of these studies looked at breastfeeding beyond six months postpartum, which could contribute to better infant survival but also to more potential risk for failure over longer duration and greater cost.

So we still need data to answer questions concerning the efficacy and optimal duration of maternal HAART vs infant prophylaxis for preventing post-natal mother-to-child transmission. And we need more information about the safety of stopping (or continuing) maternal HAART if used just to prevent mother-to-child transmission in healthier women. PROMISE, a multi-country study beginning soon will look at these questions in 8,000 women who do not need treatment for their own health (<350 cell/mm<sup>3</sup>).

Thanks to several researchers, particularly Roger Shapiro and Lynne Mofenson, for discussion of these studies.

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## PEPI Malawi: transmission rates by maternal CD4 count

Polly Clayden, HIV i-Base

The post exposure prophylaxis of infants (PEPI) Malawi trial previously reported that antiretroviral postnatal prophylaxis significantly reduces mother-to-child transmission during breastfeeding to age 14 weeks.

In this trial breastfeeding HIV-uninfected infants were randomised to three arms:

1. Control – single dose NVP plus 1 week AZT
2. Ext NVP – control plus daily NVP to 14 weeks
3. Ext NVP/AZT – control plus daily NVP and AZT to 14 weeks

At 14 weeks there was a 67% reduction in transmission in the Ext NVP and Ext NVP + AZT arms compared to the control and 50% reduction at 9 months. There was no significant difference in efficacy between the Ext NVP and Ext NVP +AZT arms. We reported results from PEPI in previous issues of HTB. [1, 2]

A poster authored by Lynne Mofenson and coworkers showed a stratification of transmission rates by maternal CD4 count (<= 200, 200-350, >350 cells/mm<sup>3</sup>). [3]

For this evaluation the investigators obtained maternal baseline CD4 count between delivery and 3 days postpartum and Kaplan-Meier curves were calculated stratified by maternal CD4 count. See table 1.

This analysis showed that even with prophylaxis the post natal transmission rates to infants with mother with CD4 <350 cells/mm<sup>3</sup> were substantial. Therefore the investigators recommend that HAART initiation in pregnancy would both benefit maternal health and reduce the risk of MTCT.

For infants with mothers with >350 cells/mm<sup>3</sup> the transmission risk with extended prophylaxis was low (1.4% and 2.3% with Ext NVP and Ext NVP +AZT respectively) and compares with that reported for maternal HAART. So for mothers with higher CD4 counts they suggest that infant prophylaxis may be an option.

#### COMMENT

**These data suggest that infant nevirapine prophylaxis for 14 weeks reduces HIV mother-to child transmission through breast-feeding regardless of maternal CD4 cell count and that the addition of AZT adds little further protection. Obviously the investigators recommend treatment for mothers indicated for their own HIV (CD count <350 cells/mm<sup>3</sup>). These findings add to those from the three studies discussed above.**

**In addition to the cost implications, the avoidance of extended infant AZT is attractive given the associations with anaemia and**

**Table 1: PEPI transmission rates by maternal CD4**

Study arm	CD4 <=200		CD4 200-350		CD4>350	
	MTCT	95%CI RR	MTCT	95%CI RR	MTCT	95%CI RR
Control	17.6 (12.2-25.2)	1.0	9.00 (5.9-13.8)	1.0	5.5 (3.8-7.9) 1.0	1.0
Ext NVP	5.8 (3.0-10.8)	0.33 (0.16-0.68)	3.4 (1.7-6.7)	0.37 (0.17-0.84)	1.4 (0.7-3.0)	0.25 (0.12-0.59)
Ext NVP + AZT	6.1 (3.3-12.4)	0.36 (0.17-0.78)	3.2 (1.3-4.1)	0.32 (0.13-0.78)	2.3 (1.3-4.1)	0.42 (0.22-0.83)

**prolonged neutropenia following in utero exposure. The overall result however remains surprising given the individual efficacy of the agents and their proven additive benefit when given during the peripartum period.**

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## Low transmission rates and favourable pregnancy outcomes reported in DREAM

### Polly Clayden, HIV i-Base

Drug Resource Enhancement and Malnutrition (DREAM) is a large HAART programme with sites in sub-Saharan Africa attended by about 75,000 people. A major part of DREAM is nutritional supplementation and prevention of mother-to-child transmission of HIV.

Women in DREAM receive HAART in pregnancy irrespective of their CD4 counts. Those indicated for treatment with CD4 <350 cells/mm<sup>3</sup> receive NVP-based HAART from 14 weeks gestation which is continued indefinitely. Women with CD4 >350 cells/mm<sup>3</sup> receive HAART from 25 weeks gestation, which is stopped after weaning at 6 months post partum. Women who stop treatment receive 21 AZT/3TC days tail coverage. PCR DNA determines infant infection.

In oral presentations Leonardo Palombi presented data from Mozambique and Malawi describing transmission rates and infant outcomes. Both analyses were retrospective record reviews.

### Rates of transmission and infant mortality

This analysis looked at:

1. HIV free survival at 1 and 6 months
2. Transmission rates by maternal CD4
3. Infant health at 6 months

There were 3148 live births from 3,273 pregnancies between

July 2005 and December 2008. At one month 93 infants were lost to follow up, 7 had died, and 2,994 had test results. Of these 22/2994 (0.7%) were HIV infected.

Transmission was 0.9% (26/2,707) in mothers who received at least one dose of HAART before delivery (VL 3.55 log<sub>10</sub>) and 5.1% (2/39) in women who did not initiate HAART until delivery (VL 4.51 log<sub>10</sub>), p<0.001. Infant HIV free survival at one month was 97.6%.

At 6 months a further 143 infants were lost to follow up and 41 died. Six-month testing found 15/2120 infected infants (5 awaiting confirmation). The cumulative 6-month transmission rate was 1.4-1.9%, mortality rate 2.1% and loss to follow up 7.5%.

The investigators found that duration of antenatal HAART and the combined endpoint of infant infection or death were associated across baseline maternal CD4 counts: 1.3% (16/1231) vs 3.8% (6/157) infants were infected or died who had mothers with CD4 <350 cells/mm<sup>3</sup> who received >=30 days and <=30 days HAART respectively, OR 0.33 (95% CI 0.12-0.86). And 0.7% vs 2% infants were infected or died who had mothers with CD4 >350 cells/mm<sup>3</sup> who received >=30 days and <=30 days HAART respectively, OR 0.33 (95% CI 0.10-1.09).

In multivariate analysis at one month there was an association between antenatal HAART-exposure and transmission or death: 2.4% with 1-30 days, 1.1% with 31-90 and 0.9% with >90 days, AOR 0.57 (95% CI 0.36-0.88), adjusted for maternal baseline viral load, CD4, haemoglobin and BMI.

At six months 3.1% vs 8.8% infants were infected or died who had mothers with CD4 <350 cells/mm<sup>3</sup> who received >=30 days and <=30 days HAART respectively, OR 0.33 (95% CI 0.16-0.7). And 1.8% vs 2.1% infants were infected or died who had mothers with CD4 >350 cells/mm<sup>3</sup> who received >=30 days and <=30 days HAART respectively, OR 0.81 (95% CI 0.24-2.83). That is, at higher maternal CD4 counts the impact of HAART > 30 days vs <30 days was not significant.

In multivariate analysis at 6 months, duration of HAART and maternal viral load were associated with transmission or infant death.

At six months HIV free survival was 90.9% in infants whose mothers had received <30 days pre-delivery HAART and 96.6% for those whose mothers received >30 days pre-delivery HAART.

Dr Palombi noted that the effect of HAART observed in DREAM was found across all maternal CD4 counts and that mothers

with >350 cells/mm<sup>3</sup> comprised 37% of transmissions where they occurred

Overall transmission rate at 6 months was 2% in this cohort.

### Outcomes

In the second analysis the investigators looked at maternal health/mortality, and infant outcomes ie prematurity, spontaneous abortion and stillbirth (defined as foetal death at <or>=32 weeks gestation respectively).

Overall they reported 42 maternal deaths giving a maternal mortality rate (MMR) of 1.2%. The majority of women in DREAM received longer duration of HAART but 68 women received none and 365 women <30 days HAART. Although infrequent, maternal mortality was significantly associated with HAART (7.4% if no HAART vs 0.7% >=90 days antenatal HAART) and CD4 count (3.2% vs 0.7% if >= 200; p< 0.001).

Foetal death included 3.1% stillbirth and 2.1% spontaneous abortion. The prematurity rate was 19.1%.

Duration of antenatal HAART was associated with infant outcomes. The rate of abortion and stillbirth was 5.2% among infants whose mothers received >90 days HAART compared to 26.5% among those whose mothers received no HAART and 7.1% <30 days HAART, p<0.001.

Maternal CD4 was also associated with abortion and stillbirth, with a rate of 16.7% among mother with CD4 <200 cells/mm<sup>3</sup>.

In this cohort, prematurity was associated with shorter duration or no HAART. The investigators reported a 70.8% reduction (Mantel-Haenszel test) overall, OR=0.16 (95% CI, 0.12-0.21) and within each CD4 strata.

In multivariate analysis BMI, OR 0.27 (95% CI, 0.15-0.50) and viral load at delivery (OR 1.44; CL95% 1.22-1.70) were associated with prematurity (see Table 1). Low birth weight was 11.5% and not associated with HAART duration or CD4 count.

**Table 1: Prematurity rates in DREAM**

CD4 count	Antenatal HAART (days)	Premature delivery (n)	%	OR (95%CI)
>500	<30	43/77	55.8	0.16
	>30	121/712	17.0	(0.10-0.26)
	TOTAL	164/789	20.8	
351-500	<30	27/56	48.2	0.18
	>30	94/661	14.2	(0.10-0.31)
	TOTAL	121/717	16.9	
201-350	<30	45/63	71.4	0.08
	>30	124/779	15.9	(0.04-0.13)
	TOTAL	169/842	20.1	
≤200	<30	10/32	31.3	0.45
	>30	85/497	17.1	(0.21-0.99)
	TOTAL	95/529	18.0	

Table reproduced from abstract

The investigators found low incidence of SAEs: 8.6% women had grade 3/4 anaemia; 6.9% d4T-associated peripheral neuropathy; 2.2% grade 3/4 liver toxicity; 1.2% cases of Stevens-Johnson Syndrome and 2.4% grade 3/4 rash.

They also found no resistance in a small sub-study of women (n=26) who had discontinued HAART after weaning.

They wrote: " HAART was strongly associated with improved pregnancy outcomes including reduction in prematurity, regardless of CD4 strata. HAART is beneficial for PMTCT and protects against unfavorable pregnancy outcomes".

### COMMENT

**The data from DREAM seem impressive and it was suggested that they demonstrate that the low rates of transmission now associated with HAART during pregnancy in resource-rich settings can be reproduced in a large roll-out programme in a resource-poor environment.**

**However, several things make these data very difficult to interpret including significant loss to follow-up and that the number of infants evaluated at different time points is different.**

**The data on premature delivery and HAART are curious. The authors conclude that short-duration of HAART is associated with prematurity, and at <30 days with as much as 71.4%, these rates are extremely elevated. But perhaps this finding is not surprising since duration is timed from initiation to delivery, and will inevitably be shorter in those who deliver preterm. Data reported by Karin van der Merwe from Johannesburg (reported on in this issue) looking at HAART vs no HAART and longer vs shorter HAART, found higher rates of preterm delivery with HAART and with longer HAART, completely contradicting the DREAM data.**

**The extensive use of nevirapine-based HAART in this study, with relatively little toxicity reported, despite use at CD4 counts >250 cells/mm<sup>3</sup> is also noteworthy and adds to the extensive literature of uncertainty regarding nevirapine, pregnancy and CD4 cell counts.**

**So these data raise a lot of questions. It was unfortunate that neither the slides nor the webcast from these sessions were available online to check some of the information presented.**

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2. Marazzi MC et al. Favorable pregnancy outcomes with reduction of abortion, stillbirth, and prematurity rates in a large cohort of HIV+ women in Southern Africa receiving highly active antiretroviral therapy (HAART) for prevention of mother-child-transmission (PMTCT) 5th IAS Conference, Cape Town, South Africa.19-22 July 2009. Oral Abstract TUAC102. <http://www.ias2009.org/pag/Abstracts.aspx?AID=1874>

## Pregnancy rates and outcomes among women in the DART trial

Polly Clayden, HIV i-Base

Pregnancy rates and outcomes among women participating in DART were shown in a poster authored by Paula Munderi and coworkers on behalf of the DART trial team. [1]

The investigators had previously shown findings from an evaluation of pregnancy at a median of 2.8-years of follow-up, which we reported previously in HTB. [2]

This further analysis was at a median of 4.6 years follow-up from January 2003 to June 2008, and included information on maternal/infant outcomes and infant feeding. Longer-term infant follow-up and documentation of infant outcomes are ongoing in a separate sub-study.

Of the 2156 women enrolled in DART 1876 (87%) were of childbearing age <45 years. No women were pregnant at enrollment and pregnancy tests were performed six-monthly. Women in DART were given contraceptive advice, including free condoms, counselled if they wished to conceive, and encouraged to disclose any pregnancy.

If they became pregnant on the trial they continued HAART in their study randomisation, received extra diagnostics as required for pregnancy within the trial and were referred for routine antenatal care.

Data on infant follow up to 2 weeks of age were analysed for congenital abnormalities, early infant survival and HIV status if tested.

The investigators reported, that 378 pregnancies occurred in 299 women. The majority (n=235) had one pregnancy but 50, 13 and 1 women/woman had 2, 3 and 4 pregnancies respectively.

Multiple pregnancies occurred in 16% women <45 years and 33% women <30 years and this was similar across sites.

The overall pregnancy rate in women <45 years was 4.83/100 woman years (95% CI 4.36-5.34).

Incidence pregnancy rates peaked at 2-3 years across all age groups and then declined. It was highest in the 18-29 years age group.

The median CD4 count was greater among women who were ever pregnant vs never pregnant 106 (IQR 32-142) vs 87 (IQR 31-141) cells/mm<sup>3</sup> respectively, p=0.01.

The majority of mothers (60%) received TDF+AZT+3TC regimens. Of the remaining, 17% received NVP+AZT+3TC; 7% d4T containing HAART; 6% second line with LPV/r; 5% ABC+AZT+3TC 3% were off HAART and 2% received other first line HAART.

Four mothers died, 2 during pregnancy (1 malaria, 1 septic abortion) and 2 peripartum (1 post partum haemorrhage and 1 puerperal psychosis).

There were 206 live births and 26 stillbirths. Any congenital abnormalities were reported in 7 (3%) infants. These were club-foot (3; 2TDF, 1 NVP); hydrocephalus (1 TDF, died); cardiac anomaly (1 NVP); undescended testes (1 NVP) and skin tag on neck (1 TDF).

Prematurity <37 weeks occurred in 9% live births (16% live and still births). Low birth weight <2500gm occurred in 17% of live births (13% >=37 weeks). The mean weight in infants >37 weeks was 3.0 (SD 0.54) kg.

At two weeks post partum the investigators reported 9 neonatal deaths of which 6 occurred within 24 hours; 5 infants were HIV-DNA PCR antibody negative and 4 were not tested. Causes of death were: foetal distress (2), prematurity (1), intestinal obstruction (1), haemorrhagic disease (1) and 4 were from unknown causes.

Only a small number of children (n=15, 7%) were tested by the DART assessment visit at 2 weeks and none were HIV-infected.

The infant follow up sub-study has enrolled 174/206 infants. Of these 152 are known to be still alive. Of the 137/174 (74%) infants with results available, none are HIV-infected.

At two weeks only a minority of women (30%) chose to breastfeed across all study sites.

The investigators concluded that in this group of women pregnancy rates increased after the first year and declined from the 4th year on HAART. Rates were higher among younger women with less severe HIV disease.

Rates of foetal loss were high and are consistent over time. They suggest that this may reflect improved reporting within a clinical trial but note that increased foetal loss has been reported in other studies.

The low rates of congenital abnormalities are encouraging and similar to those shown elsewhere: 3.0/100 95%CI 2.4-3.7) among HIV-positive women with first trimester HAART exposure in the Antiretroviral Pregnancy Register (APR) and 2.7/100 live births in the CDC birth defects register.

### COMMENT

**At first sight the preterm delivery rate is encouragingly low, much lower than in most other studies (eg 19% in DREAM see above, 17% in Europe, 18% in North America). Contributing factors may include conception on therapy, negating the effect of HIV infection on preterm delivery, and the regimen prescribed. Conversely the stillbirth rate is high and highly associated with preterm delivery. More data on the gestational age are needed to interpret these findings.**

**The low rates of congenital abnormalities are also encouraging and this large dataset from women receiving TDF in pregnancy is very useful. These data have been submitted to the Antiretroviral Pregnancy Registry. [3]**

### References

1. Munderi P et al. Pregnancy rates and outcomes among women on triple-drug antiretroviral therapy (HAART) in the DART trial. 5th IAS Conference, Cape Town, South Africa. 19-22 July 2009. Poster abstract WEPEB261.
2. <http://www.i-base.info/htb/v8/htb8-3-4/Pregnancy.html>
3. <http://www.apregistry.com/>

## Pregnancy outcomes in HAART exposed infants in Johannesburg

Polly Clayden, HIV i-Base

Reports are conflicting concerning the association between preterm birth or low birth weight and HAART.

Karin van der Merwe and coresearchers investigated the impact of HAART exposure on birth weight and gestational age among infants of South African women with advanced HIV disease attending antenatal antiretroviral clinics in Johannesburg. [1]

This review included 1630 women attending clinics between April 2004 and July 2008. All women had CD4 <250 cells/mm<sup>3</sup>.

Gestation and birth weight of infants were compared: maternal HAART exposed vs unexposed; early (<28 weeks gestation) vs late (≥28 weeks) and PI-based vs NVP-based vs EFV-based. Multivariate logistic regression was used and included maternal CD4 and infant HIV status (PCR).

The investigators found the median CD4 counts for mothers of HAART exposed and unexposed infants were 154 (IQR 101-cells/mm<sup>3</sup> and 191(IQR 136-220) cells/mm<sup>3</sup> respectively, p<0.001. The two groups were similar for other risks of adverse infant

**Table 1: Infant outcomes in women exposed and unexposed to HAART and by duration of exposure**

Variables	HAART-unexposed A n=233	HAART-exposed B n=1397	p value (A vs B)	Early HAART-exposed C n=533	Late HAART-exposed D n=427	p-value (C vs D)
Time received HAART Median weeks (IQR)		9.7(5.0-17.6) n=921		18.4 (12.1-42.6) n=412	5.8 (3.3-8.5) n=416	<0.001
Gestation: n (%)	n=147	n=946	0.002	n=389	n=427	<0.001
Extremely preterm	6 (4%)	58 (6%)		40 (10%)	3 (1%)	
Preterm	1 (1%)	80 (8%)		41 (11%)	18 (4%)	
Term/Postdates	140 (95%)	808 (85%)		308 (79%)	406 (95%)	
Birth weight (kg): n	n=224	n=1003	0.008	n=388	n=407	0.39
Mean (SD)	2.8 (0.6)	2.9 (0.6)		2.9 (0.6)	2.9 (0.5)	
0.75-1.49	10 (4%)	16 (2%)		8 (2%)	2 (0%)	
1.5-2.49	50 (22%)	199 (20%)		82 (21%)	74 (18%)	
>2.5	164 (73%)	789 (79%)	0.015	298 (77%)	331 (81%)	0.071

**Table 2. Infant outcomes in women exposed to HAART by regimen**

Variables	Early HAART-exposed			p	Late HAART-exposed			p
	PI-based HAART	NVP-based HAART	EFV-based HAART		PI-based HAART	NVP-based HAART	EFV-based HAART	
Time taking HAART	n=139	n=192	n=81		n=290	n=107	n=19	
Median weeks (IQR)	17.1 (13.7-23.1)	15.6 (10.7-25.8)	62.7 (33.1-86.4)	<0.001	6.1 (3.3-8.7)	5.1 (3.0-7.8)	5.2 (3.9-9.4)	0.38
Gestation: n	n=131	n=167	n=91		n=290	n=116	n=21	
Extremely preterm (%)	13 (10%)	15 (9%)	12 (13%)	0.048	0 (0%)	3 (3%)	0 (0%)	0.024
Preterm	6 (5%)	25 (15%)	10 (11%)		9 (3%)	8 (7%)	1 (5%)	
Term/Postdates	112 (86%)	127 (76%)	69 (76%)		281 (97%)	105 (91%)	20 (95%)	
Birth weight (kg): n	n=135	n=158	n=95		n=284	n=103	n=20	
Mean (SD)	3.0 (0.6)	2.9 (0.5)	2.7 (0.6)	0.002	2.9 (0.5)	2.9 (0.5)	2.8 (0.5)	
0.75-1.49	5 (4%)	0 (0%)	3 (3%)		2 (1%)	0 (0%)	0 (0%)	0.59
1.5-2.49	18 (13%)	31 (20%)	33 (35%)		46 (16%)	23 (22%)	5 (25%)	
>2.5	112 (83%)	127 (80%)	59 (62%)	<0.001	236 (83%)	80 (78%)	15 (75%)	0.50

outcomes: smoking, alcohol, hypertension, diabetes, anaemia, syphilis serology and history of previous miscarriage.

Prematurity rates were 6% (8/143) in HAART-unexposed infants vs 14%(129/949) in HAART-exposed infants (p=0.01). The HAART-exposed infants had mothers with a higher rate of previous preterm infants than the unexposed group, 11% vs 6%, p=0.055.

See tables 1 and 2 for infant outcomes by duration of exposure and HAART regimen.

The investigators concluded that, in this analysis, any HAART exposure was associated with preterm birth between 34-37 weeks gestation. This was strongest when HAART was initiated before 28 weeks gestation. However they did not find an increased risk of extremely preterm birth (<34 weeks gestation).

Overall they found neither low birth weight nor very low birth weight to be associated with HAART exposure. In this cohort HAART unexposed infants were more likely to have low birth weight.

PI exposure was not a risk factor for preterm or low birth weight. But, of the three regimens, early EFV exposure was associated with low birth weight. The investigators suggested that higher levels of TB among this group of women could be confounding, as EFV is frequently used in South Africa in pregnancy in the presence of HIV/TB coinfection. TB is a risk factor for preterm birth and low birth weight.

They added that these findings could help guide PMTCT policies in South Africa.

COMMENT

**As the investigators suggest, the observation that there was an association between early EFV exposure and low birth weight may be subject to confounding.**

**They included two useful tables showing published studies that looked at HAART exposure and preterm delivery or low birth weight. Data from Africa is slowly emerging.**

References

1. van der Merwe K et al. The impact of in-utero antiretroviral therapy (HAART) exposure on infant outcomes in Johannesburg, South Africa. 5th IAS Conference, Cape Town, South Africa.19-22 July 2009. Poster abstract WEPEB262. <http://www.ias2009.org/pag/Abstracts.aspx?AID=2256>
2. Lorenzi P et al. Antiretroviral therapies in pregnancy: maternal, foetal and neonatal effects. Swiss HIV Cohort Study, the Swiss Collaborative and Pregnancy Study, and the Swiss Neonatal HIV Study. AIDS 1998, 12:241-247.
3. Thorne C et al. Increased risk of adverse pregnancy outcomes in HIV-infected women treated with highly active antiretroviral therapy in Europe. AIDS 2004, 18:2337-2339.
4. Cotter A et al. Is antiretroviral therapy during pregnancy associated with an increased risk of preterm delivery, low birth weight, or stillbirth? J Infect Dis 2006. 193:1195-1201.
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**Table 3: Major studies showing a link between preterm birth or low birth weight and HAART exposure**

Study	Year of publication	Population	Findings
European Collaborative and Swiss Mother and child HIV cohort study[2]	1998	3920 mother-child pairs in Europe	Protease inhibitor exposure linked with preterm birth
European Collaborative study[3]	2004	4372 live births in Europe	HAART-initiation pre-pregnancy associated with preterm birth especially extreme preterm birth
Miami study, Cotter et al[4]	2006	1337 women in Miami	Protease inhibitor exposure associated with preterm birth
National Population-based surveillance study, Townsend et al[5]	2007	4445 pregnancies in UK and Ireland	HAART exposure associated with preterm birth especially extreme preterm birth

**Table 4: Major studies showing no link between preterm birth or low birth weight and HAART exposure:**

Study	Year of publication	Population	Findings
US combined cohort[6]	2002	3266 women in the US	No association between LBW or preterm birth and HAART exposure
WITS[7]	2005	2543 women in the US	Preterm birth decreased in association with HAART exposure
Szyld et al[8]	2006	681 women in Latin America and the Caribbean	PI-exposure not significantly associated with LBW or preterm birth compared to NNRTI exposure
Paediatric Spectrum of Diseases[9]	2007	11 321 infants in the US	HAART not significantly associated with preterm birth

## Efavirenz conceptions in Soweto

Polly Clayden, HIV i-Base

A poster from the Perinatal Research Unit in Soweto, South Africa showed findings on the rate of miscarriages and still births from a retrospective review of women receiving efavirenz (EFV) in pregnancy. [1]

Although EFV is FDA category D, increasingly women are conceiving while already receiving this antiretroviral.

In this review the investigators looked at records of 886 women receiving HAART between August 2004 and March 2008. Among this group 117 pregnancies were recorded and 83 women (70.9%) had conceptions that were EFV-exposed for a mean duration of 97.05 days (range 12-343 days); of these 3/83 (2.6%) miscarried, 1/83 (1.2%) were stillbirths, and 28/83 (33.7%) were electively terminated.

The remainder of HAART exposed conceptions, 34/117 (29.1%), were EFV-unexposed; of these 5/34 (14.7%) miscarried, 1/34 (2.9%) were stillbirths, and 2/34 (5.9%) were electively terminated.

The investigators found that compared to live births, elective termination of pregnancy (TOP) rates were significantly higher among EFV-exposed than non-EFV exposed,  $p=0.00418$ .

They also note that South African public sector surveillance reports miscarriage rate of 6.3% (2001), stillbirth rate of 2.4% (2006-07), and elective termination of pregnancy rate of 13.6% (2001).

They suggest that this high rate of TOP in women receiving EFV-containing HAART may reflect provider teratogenicity counselling. Additionally they suggest that it reflects provider choice to initiate EFV-containing regimens for women not expressing the desire to have children. They note that they did not find an increase in miscarriage or stillbirths in women receiving EFV compared with the general population.

### COMMENT

**The investigators explained that this evaluation was performed in response to findings from Brazil showing a higher rate of miscarriage among EFV-exposed pregnancies. In our comments concerning the Brazilian data we wrote: [2,3]**

**“These data should be treated with extreme caution. The miscarriage rate reported from this notes review is extremely low (1.38%). It is generally thought that approximately 30% of conceptions are miscarried. The potential for biased reporting is very high and this is more likely to be seen with efavirenz than other antiretroviral drugs.**

**Although the congenital anomaly rate is reported to be more than twice the background for Rio de Janeiro (2.2% v 0.8%) the later figure is only about one third of the normally cited of congenital malformations and 2.2% is a more realistic figure.”**

**This South African review did not find a higher rate of miscarriage or stillbirth among women receiving EFV at conception.**

**However, the high rate of termination of pregnancy among women taking efavirenz is striking and the investigators**

**suggest that this may reflect provider teratogenicity counselling. FDA category D states that there is evidence that the drug is associated with teratogenicity in humans. This is a relative contraindication and this FDA classification also states that the benefits may outweigh the potential risk (category X states that the drug should not be prescribed in pregnancy because of the risk). Despite this categorisation, the Antiretroviral Pregnancy Registry, which now has sufficient numbers of first trimester efavirenz exposures to detect at least a two-fold increase in risk of overall birth defects, report no such increase to date, 14/477, 2.9% (95% CI, 1.6-4.9%). [4]**

**The data from DART above show that even in a clinical trial with pregnancy counselling 4.8% of women conceived per annum. In this cohort from Soweto 13% of the women on HAART conceived with the majority (71%) taking an efavirenz-based regimen.**

**So, as the investigators suggest, consideration needs to be given both to provider antiretroviral choice and to provider counseling with women of child-bearing age who may or do become pregnant.**

### References

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## Impact of regimen and duration of therapy on risk of mother-to-child HIV transmission in Johannesburg

Polly Clayden, HIV i-Base

There are limited data describing the effects of HAART on mother-to-child transmission among HIV-positive women in Africa with CD4 counts <250 cells/mm<sup>3</sup>.

A poster authored by Risa Hoffman and coworkers showed an analysis of the impact of HAART regimen and duration of treatment on risk of transmission in women attending antenatal clinics in Johannesburg. [1]

This group had looked at transmission among women initiating HAART in pregnancy in an earlier analysis from this cohort. They found that for each additional week of HAART, the odds of transmission were reduced by 27%. We reported this study previously in HTB. [2]

In this more recent analysis they included both women who became pregnant while receiving HAART and those initiating HAART during pregnancy. The authors used chi square tests and logistic regression to evaluate the effects of regimen and duration on transmission. An infected infant was defined as having a positive DNA PCR at 6 weeks.

A group of 1,115 women were followed from April 2004 until July 2008. At baseline the women were a mean age of 31 years and their mean CD4 count was 159 cells/mm<sup>3</sup>. Most women (97.3%) received a nucleoside backbone of d4T/3TC. Similar proportions received LPV/r (448; 40.2%) and EFV (469; 42.1%); and a smaller group of women received EFV (198; 17.8%).

Data for initiation of therapy were available for 874 women. Of these, 16.0% became pregnant while already receiving HAART. For those already on HAART the mean duration was 93.4 weeks. For those initiating HAART in pregnancy the mean duration was 10.7 weeks of therapy.

The investigators reported an overall transmission rate for women with known date of initiation of 4.7% (43/874). They found no significant differences between HAART regimens. This finding remained with or without adjustment for prior single-dose NVP.

Women who became pregnant on HAART had significantly lower transmission rates than women who initiated HAART during pregnancy, 0.7% vs 5.7%,  $p=0.01$ .

Women initiating HAART during pregnancy ( $n=553$ ) had higher transmission rates with shorter duration of therapy: 9.3%, <4 weeks; 5.5%, 4-16 weeks and 3.5%, 17-32 weeks. There were no transmissions among women receiving >32 weeks of HAART. Each additional week of HAART reduced the odds of transmission by 8%, OR 0.92,  $p=0.02$ , CI 0.87-0.99.

“To improve rates of MTCT, strategies are needed to facilitate earlier identification of HIV-infected pregnant women”, they wrote.

## COMMENT

**Data continues to accumulate to support early identification of HIV-positive women in pregnancy and timely initiation of treatment.**

## References

- Hoffman R et al. Impact of antiretroviral therapy regimen and duration of therapy on risk of mother-to-child HIV transmission in Johannesburg, South Africa. 5th IAS Conference, Cape Town, South Africa. 19-22 July 2009. Poster abstract WEPEB260. <http://www.ias2009.org/pag/Abstracts.aspx?AID=1127>
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## A cost-effectiveness analysis of the OCTANE trial

### Polly Clayden, HIV i-Base

The Optimum Combination Therapy After Nevirapine Exposure (OCTANE)/ACTG5208 trial found superior outcomes among women exposed to single dose nevirapine (NVP) receiving lopinavir/ritonavir (LPV/r)-based HAART compared to those receiving NVP-based regimens. [1,2]

In OCTANE, women with CD4 <200 cells/mm<sup>3</sup> exposed to NVP as PMTCT prophylaxis a median of 17 months prior to HAART initiation were randomised to receive either LPV/r or NVP-based HAART. Over a median of 73 weeks follow up, women in the LPV/r arm had significantly lower risks of virological failure or death compared to those in the NVP arm. We covered these findings in previous issues of HTB.

However, the difference in cost between the two drugs is quite considerable: LPV/r is 12 times more expensive than NVP.

Andrea Ciaranello presented an analysis that examined the cost-effectiveness of first line LPV/r based HAART, compared to first line NVP-based HAART, for women with prior single dose NVP exposure in South Africa. [3]

This analysis utilised the Cost-effectiveness of Preventing AIDS Complications (or CEPAC) computer simulation model. The model included incidence, prophylaxis, and treatment of opportunistic infections in South Africa, CD4 count, viral load and HAART efficacy. The investigators used data for the modeling from OCTANE and from published South African cohorts, including natural history data from the Cape Town AIDS Cohort.

They projected two year and lifetime outcomes associated with first line HAART strategies for women with prior single dose NVP exposure. These projections included risk of opportunistic infections, deaths, and per-person HIV-related costs.

Dr Ciaranello explained that cost-effectiveness analysis a method of comparing alternative health care strategies. This method uses an incremental cost-effectiveness ratio (ICER). To calculate the ICER, the number of additional health care resources needed for one strategy compared to another is determined. This is divided by the additional health benefits gained by this strategy compared to the other.

Currency per year of life saved (YLS) is the most common unit for an ICER. A lower ratio, when less money is needed to produce a health benefit, shows a more cost-effective intervention.

To determine whether an intervention is cost effective, WHO compare ICER to per-capita GDP. ICER <1xGDP/YLS is considered to be “very cost effective” and ICER <3x GDP/YLS is “cost effective”. South African GDP (2006) is \$5400.

Three strategies were evaluated in this analysis:

- No HAART (comparator)
- First line NVP/TDF/FTC (Second-line LPV/r/ddI/AZT)
- First line LPV/r/TDF/FTC (Second-line NVP/ddI/AZT).

Following second line failure, women were assumed to receive a maintenance regimen of LPV/r and 3TC, which was common practice at OCTANE sites.

Baseline data for the women were used from the OCTANE cohort and included median: 31 years of age; CD4 139 cells/mm<sup>3</sup>; viral load, 5.15 copies/mL and 17 months since exposure to single dose NVP.

The efficacy of each regimen was modeled on that observed in the OCTANE trial. Efficacy was defined as achieving viral load suppression <400 copies/mL at 24 weeks after initiation of HAART.

With a median time from single dose NVP exposure of 17 months, efficacy of the first line NVP regimen was 84.6%, and the efficacy of the first line LPV/r regimen was 96.7%.

The investigators noted that these data, are slightly different from those previously presented as a composite endpoint of virologic failure or death was shown.

There are very few data on the efficacy of NNRTI-based second-line regimens. For the model data were extrapolated from two studies to give an estimate of 43% virologic suppression at 24

weeks for second-line NVP. Data for second-line PI-based HAART are more common and, using multiple sources, an estimate of 72% suppression was used.

The investigators assumed that routine viral load tests were not available and that HAART was switched for severe opportunistic infection, 50% CD4 decline or toxicity.

They used HIV-related healthcare costs derived from the South African Health Systems Trust. These included the cost of a day in hospital of \$221, the cost of an outpatient clinic visit of \$11 and the cost of a CD4 test of \$9.

For drug costs, they used prices from the Clinton Foundation HIV/AIDS Initiative. Annual drug costs were \$38 for NVP and \$444 for LPV/r. For the nucleosides, the figures were \$142 for TDF/FTC and \$238 for ddI/AZT.

Projecting outcomes for the entire cohort at two years revealed that with no HAART 41.7% of women would survive at a per-person cost of \$2650. Using a NVP-based first line regimen survival was 96.1% at a per-person cost of \$2450, and providing LPV/r-based HAART first line, survival was 97.1% at a per-person cost of \$2780. Compared to first line NVP, LPV/r gave a 26% risk reduction in mortality at an additional per-person cost of \$330.

Projecting long-term outcomes showed life expectancy of 1.8 years at a per-person cost of \$3540 if the cohort were untreated. With NVP-based first line survival increased by 13.6 years to 15.4 years at a per-person cost of \$14,040 for an ICER of \$770/YLS. Using LPV/r –based first line gained a further 1.1 years survival (16.5 years) at a per-person cost of \$16,180 for an ICER of \$1970/YLS. So using LPV/r first line would be “very cost effective” compared to NVP according to WHO criteria for South Africa.

Additionally, the investigators then conducted a sensitivity analysis. They evaluated many model inputs parameters and assumptions. Dr Ciaranello presented estimates for parameters of particular importance.

Importantly they evaluated the efficacy of second line NVP, for which there are few data (in the range of 16-45%). The investigators looked at 0-100% efficacy and found that LPV/r remains “very cost effective” by WHO criteria unless the efficacy of second line NVP is less than 15%.

They also looked at the influence of NNRTI resistance at the time of initiation of HAART. Among the OCTANE cohort, 86% of women had no detectable NNRTI resistance using standard genotype assay at the time of starting treatment. For this group of women the efficacy of first line NVP was greater than for the cohort overall, 89% vs 85% (97% for LPV/r first line). For women with no resistance the ICER of first line LPV/r compared to NVP was \$10,990/YLS, and no longer a “very cost-effective” intervention in South Africa.

OCTANE also included stratification by time from NVP exposure to initiation of HAART. Looking at cost effectiveness according to these strata, the investigators found LPV/r was “very cost effective” with 6-24 months between NVP exposure and treatment, at an ICER of \$2000/YLS. However >24 months the ICER reached the WHO threshold for South Africa at \$5400. They noted that LPV/r first line became less cost effective as time from NVP exposure increased.

Dr Ciaranello acknowledged that the limitations to these estimates include some input data from cohorts of both men and women;

costs and cost effectiveness thresholds that are specific to South Africa and results are sensitive to data that are not yet available from the OCTANE trial.

She stressed that reducing the impact of NNRTI resistance related to single dose NVP is particularly important in order for women to benefit from available classes of antiretrovirals in the treatment of their own HIV.

She also emphasised the importance of HIV and CD4 testing of pregnant women in order to initiate HAART in eligible women before delivery, both improving maternal health and reducing mother-to-child transmission.

However she added: “Despite these efforts, many of the single dose NVP-exposed women living in resource-limited settings are likely to need to initiate HAART soon. The choice of optimal first-line HAART for these women will require important data about long-term outcomes, particularly outcomes of second-line HAART. Such outcomes can only be observed after the conclusion of most clinical trials. OCTANE, cohort studies, and HAART programme monitoring and evaluation efforts will be crucial sources of these data”.

#### COMMENT

**This cost-effectiveness analysis is useful to demonstrate that for NVP-exposed women, particularly those with an interval since exposure of <24 months, using LPV/r based HAART first line is very cost-effective.**

**However, wouldn't it be better to avoid risk of resistance in the first place with early identification and treatment for women indicated for their own health <350 cells/mm3 and more complex PMTCT regimens for healthier women (short course PI based HAART? AZT plus single dose NVP, plus tail coverage?)**

**As the investigators suggest it is not clear what future options NVP-exposed women in resource limited settings will have for second line treatment.**

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## Pharmacokinetics of atazanavir/ritonavir during pregnancy

### Polly Clayden, HIV i-Base

Previous reports have shown plasma concentrations of some PIs are reduced in pregnant women.

A late breaker poster authored by Francesca Conradie and coworkers from the A1424182 study showed PK data from women receiving atazanavir/ritonavir (ATV/r) once daily during pregnancy.

This was a multicentre, open label, single arm phase one study with sites in South Africa, Puerto Rico and the USA. Enrolled women were between 12 and 32 weeks gestation with CD4  $\geq$ 200 cells/mm<sup>3</sup>.

This study determined multiple clinical and PK parameters during the second and third trimesters and post-partum.

ATV was dosed ATV/r 300/100 mg (n=20) or ATV/r 400/100 mg (n=21) in combination with AZT/3TC 300/150 mg twice daily during the third trimester. Second trimester and post partum dosing was ATV/r 300/100mg.

Third trimester exposures were compared to historical ATV 300/100mg exposures in non-pregnant adults. Foetal:maternal ratio was determined using cord blood samples. Infants were followed up for 6 months.

The investigators reported all mothers had fully suppressed viral load (<50 copies/mL) before or at delivery. At the time of analysis all infants (n=40) were HIV DNA negative. There were no infant deaths.

Maternal drug-related serious adverse events (SAEs) were: hyperbilirubinemia (n=1) and anaemia (n=4). Grades 3-4 hyperbilirubinemia occurred in 6/20 and 13/21 mothers in the 300/100mg and 400/100mg groups, respectively.

Three infants had drug-related SAEs. Infant bilirubins were within normal limits to day 14; 7 had Grade 3 hyperbilirubinemia after day 14 (maximum 8.5 mg/dL at day 15). One infant received 3 days phototherapy from day 3. This infant had other risk factors (low birth weight and prematurity).

For the 300/100mg group, they reported that C<sub>max</sub> and AUC during the third trimester were 27% and 21% lower and C<sub>24</sub> was similar to historical data in non-pregnant HIV-positive patients taking ATV/r 300/100 mg once daily.

For the 400/100mg group they found AUC and C<sub>max</sub> similar to, and C<sub>24</sub> 39% higher than historical; post partum exposures were higher than historical. The investigators noted that elevated levels have been observed with other PIs in the post partum period. Levels of ATV appeared to normalise by 16 weeks post partum.

The ATV foetal:maternal ratio was 0.19 and 0.12 for 300/100 and 400/100, respectively. This ratio indicates that ATV, like other PIs, does not cross the placenta well.

The investigators concluded that this phase 1 study suggests that no dose modification of ATV 300/100mg once daily is necessary in the third trimester of pregnancy. Clinical outcomes indicate that this dose suppressed HIV viral load effectively in the participating women, and prevented vertical transmission of HIV to their infants, when used in combination with AZT/3TC twice daily. Treatment with ATV/r in the mothers appeared to be well tolerated.

Reference

Conradie F et al. The safety, efficacy, and steady state pharmacokinetics of atazanavir/ritonavir (ATV/r) once daily given in combination with twice daily AZT/3TC during pregnancy: results of study A1424182. 5th IAS Conference, Cape Town, South Africa. 19-22 July 2009. Poster abstract LBPEB06. <http://www.ias2009.org/pag/Abstracts.aspx?AID=3732>

**The PEARL study**

**Polly Clayden, HIV i-Base**

David Coetzee from the University of Cape Town presented data from the PEARL study. This study was a four-country evaluation of the effectiveness of PMTCT programmes in Africa. [1]

PEARL evaluated 43 sites in Zambia, Cote D'Ivoire, South Africa and Cameroon. The primary outcome was coverage ie the proportion of mother-child pairs with HIV antibody-positive cord blood with confirmed receipt of maternal (detectable NVP in cord blood) and infant (documented) NVP. The study also evaluated AZT and 3TC where appropriate.

All PMTCT sites used a minimum intervention of single dose NVP. Some also used short course AZT plus single dose NVP and/or HAART.

Cord blood was collected anonymously from every delivery between April 2007 and October 2008 and tested for HIV (there was an excellent collection rate of about 98% of consecutive deliveries). If the result was positive, the presence of NVP (AZT and 3TC if applicable) was determined by high performance liquid chromatography.

The investigators also used documented information collected anonymously from patients' notes including infant ingestion of NVP.

The investigators collected 28,060 cord blood samples of which 3250 were HIV-positive.

Evaluating both sets of information together they described the "coverage cascade" (table 1).

**Table 1: Coverage cascade**

Intervention	%
Positive cord blood	100
Information in folder	92
Offered HIV test	84
HIV tested	81
Result in folder	74
Maternal receipt NVP	71
NVP in cord blood	57
Coverage	50

Coverage = maternal and infant ingestion of NVP

They reported variation in coverage across sites (17%-69%).

Reasons for failed coverage included: no offer of HIV test; HIV test declined; testing declined; test result not given; NVP not dispensed; mother did not adhere and infant not dosed.

The risk of failed coverage increased with younger age:  $\leq$ 20 years adjusted OR 1.58 (1.23-2.02) vs >30 years. The association also increased with fewer antenatal visits: 0-1 visits AOR 2.92 (2.22-3.84) vs >6 visits.

Risk of poor maternal adherence was significantly higher in the 26-30 years age group, adjusted AOR 1.42 (1.04-1.93) vs >30 years; with greater gravidity, AOR 1.62 (1.12-2.34), 4 vs 1; fewer antenatal clinic visits AOR 2.98 (2.07-4.48), 0-1 vs >6 visits; vaginal delivery AOR 1.51(1.11-2.05) vaginal vs caesarean; and AZT plus NVP prophylaxis, AOR 1.42(1.04-1.93) vs NVP alone

(HAART was not significantly associated with poorer maternal adherence).

The study included an analysis of PMTCT in the Western Cape, where guidelines have recommended HAART for women with CD4 <200 cells/mm<sup>3</sup> and short course AZT plus single dose NVP with CD4 >200 cells/mm<sup>3</sup> since 2007.

In this province 12% women received HAART (the investigators used detectable 3TC as a surrogate for HAART) and 47% AZT plus NVP, so overall 59% received standard of care. However, 6% received only NVP, so 65% received "at least NVP"; 8% received AZT alone and 27% of pregnant women received nothing at all. The investigators noted that CD4 data was not collected in this study (so they would not be able to estimate how many women should have received HAART).

The investigators concluded that failures in the "cascade" of interventions occur at every step of the way, giving only 50% coverage overall across sites.

Even in settings with two-drug prophylaxis and HAART over a quarter of women are not covered by PMTCT prophylaxis (so also not receiving HAART for their own HIV if indicated).

"Interventions must systematically target better performance at each step to maximise their benefits", they wrote.

#### COMMENTS

**These data provided an important reality check and show that despite interventions that benefit maternal health and/or prevent transmission to their infants, inadequate roll out and coverage is the norm.**

**The PEARL Study's primary outcome variable is uptake of PMTCT interventions. As such, PEARL measures programme coverage, but does not assess programme effectiveness at preventing or treating HIV infection. During the conference, reports on national roll out of PMTCT protocols were notable for the absence of infrastructure to detect infant HIV infection, and therefore the effectiveness of PMTCT interventions in practice. Early detection of paediatric HIV is belatedly but rapidly becoming a priority in many countries. We will report on research presented on infant diagnostics in the next issue of HTB South.**

**WHO PMTCT consultations have also used PMTCT cascades and Lynne Mofenson showed estimations from these in her plenary at the paediatric meeting preceding the IAS conference. [2] In a "typical" scenario they estimate that of 1000 mothers, 90% (900) will attend ANC; of these 70% (630) will be counselled and tested for HIV and 50% of this group (315) receive ARVs. If 685 women receive no ARVs, that will mean 25% (172) of their infants will be infected.**

**In this estimation, overall transmission rates, if all women with CD4 <200 cells/mm<sup>3</sup> receive HAART and the remainder receive either single dose NVP, short course AZT plus single dose NVP or HAART, would be 19.7%, 18.1% and 17.6% respectively.**

**With improved uptake, if 96% (960) mothers attended ANC, 99% (950) were counselled and tested and 98% (931) received ARVs, that would mean 69 mothers receive no ARVs and 17 infants would be infected. In this estimation the overall transmission rates would**

**be 9.1%, 4.5% and 3.1% for single dose NVP, AZT plus single dose NVP and HAART respectively.**

**Cocoka Mnyani and coworkers showed data from January to December 2008 from the Soweto programme on which the improved figures were based. [3] In March 2008 short course AZT was introduced in addition to single dose NVP for women with CD4 >200 cells/mm<sup>3</sup>. During this period, 30180 women attended ANC of which 99% (29968) accepted HIV testing and 29% (8774) tested HIV-positive. Of these 5704 mothers and 6641 infants received AZT plus single dose NVP and 96% (8137/8514) elected to formula feed. A total of 324/5572 infants were HIV-infected giving a transmission rate of 5.8%. The transmission rate was 7.2% during the period when single dose NVP was used alone and 4.4% using AZT plus NVP, OR 1.67 (95%CI, 1.24-2.3), p=0.0004.**

**As Lynne Mofenson explained, "Programme efficacy is as much related to the PMTCT cascade as the specific regimen."**

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## Presentation with late stage HIV diagnosis in pregnancy

### Polly Clayden, HIV i-Base

HIV-positive women are frequently unaware of their status prior to antenatal testing.

Late diagnosis of HIV results in some advanced management considerations. The extent to which this occurs in pregnancy in Europe has not previously been quantified, nor have the implications for maternal and child health.

Claire Townsend from the European Collaborative Study presented data from an analysis performed to quantify this occurrence within the cohort, describe management strategies and the impact of late diagnosis on MTCT, prematurity and low birth weight.

The investigators defined late diagnosis as women diagnosed antenatally with CD4 count <200 cells/mm<sup>3</sup>. The analyses used logistic regression and linear mixed effects models.

Date of first positive HIV test was available for 3605 women, of which 1256 had CD4 data available.

Overall 654 (53%) were white, 499 (41%) black and 73 (6%) were of other ethnicities. Of these 15% (185/1256) had late stage

diagnoses. This proportion has increased over time with 12% between 1985-89 and 19% between 2005-2008,  $p=0.24$ .

The median baseline CD4 count was 140 cells/mm<sup>3</sup> (IQR 90-147) among the late diagnosis women vs 460 cells/mm<sup>3</sup> (IQR 333-650) among non-late diagnosis women. Of the late diagnosis group, 11% ( $n=20$ ) had an AIDS-defining illness in pregnancy.

In logistic regression analysis limited to 613 women enrolled after 1996, the investigators found late diagnosis was associated with black African ethnicity, AOR 2.02 (95%CI 1.17-3.48,  $p=0.01$  vs white ethnicity; and older maternal age, AOR 2.17 (95% CI 1.10-4.25),  $p=0.02$  for women aged 30-34 years vs <25 years.

More women with late diagnosis received antenatal HAART than other women 85% (94/110) vs 67% (388/580),  $p<0.001$ . The median duration of HAART was 16.9 (IQR 11.6-20.7) weeks vs 13 (IQR 11.6-20.7) for women with late stage and non-late stage diagnosis respectively.

Adjusting for time of measurement and type and duration of regimen, late stage diagnosis was associated with a significantly higher viral load throughout pregnancy,  $+0.29\log_{10}$  copies/mL vs non late-stage diagnosis women,  $p<0.001$ . The estimated mean viral loads at time of delivery were  $2.94\log_{10}$  copies/mL and  $2.65\log_{10}$  copies/mL for late diagnosis and non-late diagnosis women respectively.

More infants born to women with late diagnosis were premature, 24.0% (44/183) vs 13.7% (145/1062),  $p<0.001$  and of low birth weight, 27.5% (46/167) vs 16.1% (165/1022),  $p<0.001$  than other infants.

In 2000-08, MTCT rates were similar, 3.0% (95%CI 0.37-10.5) and 1.5% (95%CI 0.5-3.51),  $p=0.4$  in the late diagnosis and non-late diagnosis groups respectively.

This analysis found that an increasing minority of HIV-positive women in Europe, newly diagnosed through antenatal testing, already has advanced disease. Although these women are more likely to initiate HAART, and to do so earlier, they still have worse pregnancy outcomes than women with better functioning immune systems.

"Barriers preventing timely access of women to HIV testing are important to address, both for the health of the mother and her infant". Dr Thorne concluded.

#### COMMENT

**This detailed analysis of women diagnosed through antenatal testing from Europe has implications for Southern Africa.**

**Most importantly that "late stage" is defined as CD4 <200 cells/mm<sup>3</sup> ie the threshold at which most national guidelines in the region recommend initiation of HAART, so the overwhelming majority of women who do receive HAART in pregnancy will be in this category.**

**In this study advanced disease was associated with significantly higher viral load in pregnancy and at delivery, and with preterm deliveries and infants of low birth weight. This has implications for maternal and child health and "results in some advanced management considerations."**

**It is important that national guidelines are revised if necessary and implemented.**

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Thorne CN et al. Presentation with late stage HIV disease at diagnosis of HIV infection in pregnancy. 5th IAS Conference, Cape Town, South Africa. 19-22 July 2009. Poster abstract TUAC103. <http://www.ias2009.org/pag/Abstracts.aspx?AID=1155>

## Overview of TB-related studies at IAS

### Nathan Geffen, TAC

Some useful TB research has been published since the last HTB. Approximately 120 TB-related abstracts were reported at IAS2009. Some of these are summarised here.

### Epidemiology

The most interesting TB research presented at the IAS conference dealt with the epidemiology of TB/HIV co-infection.

A presentation by Keren Middelkoop and colleagues analysed the association between the introduction of HAART into a community and active TB rates. [1]

They collected TB notification data, prior to HAART (1998 to 2004) and after HAART was introduced (2004 to 2008). In Masiphumelele township in Cape Town. HIV prevalence was estimated to be 23% in the township. Pre-HAART adult TB notifications increased by an average of 212 cases per 100,000 people per year ( $p$  for trend = 0.005). Post-HAART, adult cases decreased by 116 per 100,000 people per year ( $p$  for trend = 0.16).

TB rates in HIV-uninfected people did not change substantially over the period and averaged 697 cases per 100,000 per year. TB rates in HIV-positive people increased by an average of 826 cases per 100,000 per year over the same period ( $p$  value for trend 0.08), but after the introduction of HAART, declined by 600 cases per 100,000 per year ( $p=0.16$ , non-significant for trend). For HIV-positive people not on HAART, the average decline in the post-HAART era was 421 cases per 100,000. For people on HAART, it declined by 1,394 cases per 100,000 and this trend was significant ( $p=0.05$ ). The authors concluded that wide-scale HAART implementation and the community's well-functioning TB programme were associated with modest TB declines.

Masiphumelele's population is only 20 to 30,000 which might explain a lack of power to detect statistically significant trends.

But another study in Masiphumelele also conducted by Middelkoop and colleagues makes the argument for the effect of HAART on a declining TB rate more compelling. [2]

In 2008, they repeated a randomly sampled cross-sectional survey that was first performed in 2005. About 30% of HIV-positive adults were estimated to be on treatment in 2008. Two sputum samples were obtained from each participant. Participants also filled in questionnaires on TB history. Those who were not being treated for TB and had two TB-positive sputum or culture tests were considered to have undiagnosed TB. The survey measured a significant decline in the TB rate in HIV-positive people since 2005 (all testing was anonymous), including a significant decline in undiagnosed TB in this group. No such decline was seen in HIV-negative people. The study also found that after adjusting for age, sex and HIV status, the overall 2008 TB prevalence was

significantly lower than the overall 2005 prevalence ( $p=0.02$ ). See Table 1.

**Table 1: Results of survey of 2005 and 2008 TB and HIV surveys in Cape Town township**

	HIV-positive			HIV-negative		
	2005	2008	p	2005	2008	p
	n=174	n=310		n=584	n=949	
On treatment TB (%)	4.00%	1.30%	0.19	0.70%	1.20%	1.0
Undiagnosed TB (%)	5.20%	1.60%	0.01	0.50%	0.80%	0.7
TOTAL	9.20%	2.90%	0.01	1.20%	2.00%	0.81

Shortly after the conference, members of this research group published an article in AIDS that examined the association between current CD4 count and risk of TB. [3]

They analysed TB incidence in 1,480 patients receiving HAART for up to 4.5 years. Updated CD4 cell counts were measured 4-monthly. During 2,785 person-years of observation, 203 cases of TB were diagnosed, giving an overall incidence of 7.3 cases/100 person-years. TB incidence by CD4 count strata is described in table 2 ( $p<0.001$ ).

**Table 2: TB incidence by CD4 count strata (from Lawn et al.)**

CD4 count (cells/mm <sup>3</sup> )	Unadjusted TB incidence (per 100py)
0-100	16.8
101-200	9.3
201-300	5.5
301-400	4.6
401-500	4.2
>500	1.5

The authors found that updated CD4 cell counts were the only patient characteristic independently associated with long-term TB risk. They concluded that updated CD4 cell counts were the dominant predictor of TB risk during HAART in this low-resource setting. They also found that among those with baseline CD4 cell counts less than 200 cells/mm<sup>3</sup>, the excess adjusted risk of TB during the first four months of HAART was consistent with unmasking of disease missed at baseline screening. They noted that TB incidence at CD4 cell counts of 200-500 cells/mm<sup>3</sup> remained high and concluded that TB prevention would be improved by HAART policies that minimized the time patients spend with CD4 cell counts below 500 cells/mm<sup>3</sup>.

These studies provide evidence that widespread HAART implementation can reverse the growth in active TB rates in Southern Africa.

A study by Van Rie and colleagues analysed risk factors for TB in patients who had been on HAART for greater than six months in a prospective cohort from the Themba Lethu clinic in Johannesburg. [4] Their study provides quantitative data on TB incidence and its relationship to patients failing treatment. Of 5,934 adults, 217 (4%) developed TB after six months. Median time to TB was 418 days (IQR: 276-672, incidence of 2.3 cases per

100py). The incidence was four times lower than in the first six months of HAART. Significant risk factors associated with active TB were BMI < 18.5 (HR: 6.52, 95%CI: 3.60-11.80 compared to BMI  $\geq 25$ ), history of TB treatment (HR: 1.50, 95%CI: 1.09-2.50), current viral load >10,000 copies/mL (HR: 2.44, 95%CI: 1.57-3.79) and CD4 count  $\leq 50$  (HR=0.84, 0.45, and 0.34 for CD4 51-100, 101-200, and 201-350 respectively).

In a study from Khayelitsha, Pepper and colleagues examined patients in their cohort who had clinical deterioration. [5]

They enrolled 298 people with who had initiated TB treatment from June to August 2008 and then followed them for six months. In this group 209 (71%) were HIV-positive, with a median CD4 count of 129 cells/mm<sup>3</sup> (IQR: 62-277). At TB diagnosis, 35 (17%) HIV-positive patients were receiving HAART. This rose to 112 (54%) on HAART six months later.

Within 6 months, 117 patients (39%) experienced 208 episodes of clinical deterioration. Of these, patients, 71% were HIV-positive. There was an escalating risk of clinical deterioration in HIV-positive patients as CD4 counts decreased (CD4>350: RR:1.4, 95%CI=0.7-2.9; CD4 200-350: RR:2.0, 95%CI: 1.1-3.6; CD4<200: RR=3.0, 95%CI=1.9-4.8). AIDS-defining illnesses (n=30), TB-IRIS (n=22) and MDR-TB (n=10) were important causes for clinical deterioration. The number of deaths was 17, of whom 15 were HIV-positive with a CD4 count < 200 cells/mm<sup>3</sup> at TB diagnosis. The authors also noted that health-care use was significantly higher in HIV-positive patients with low CD4 counts. They pointed out that starting HAART initiation at higher CD4 counts is likely to reduce this clinical burden.

De Bruyn and colleagues presented data from Soweto that showed an association between HAART and neutrophil count. [6]

They explain that neutrophil granules contain antimicrobial peptides that kill *M. tuberculosis*. In HIV-negative people exposed to TB, neutrophil count is inversely associated with risk of latent TB infection and positively associated with ability to contain mycobacterial growth in vitro. However, neutrophil functional defects occur in HIV-positive patients. The authors therefore examined the association of incident TB with neutrophil count.

They followed a prospective cohort of almost 2,700 HIV-positive adults for over 5,500 person-years. Median age was 32. Women comprised 79% of the cohort. Median CD4 count was 282 cells/mm<sup>3</sup>. The median neutrophil count was 2.46 copies/nL. TB incidence was 5.2/100py (95%CI: 4.6-5.8). HAART was associated with a reduced risk of TB (HR: 0.26;  $p<0.001$ ), as was increasing CD4 count. Increasing neutrophil count was also associated with increased risk of TB (HR: 1.18;  $p=0.02$ ). For patients on HAART, there was a trend showing that risk of TB was reduced by 75% per nL increase in neutrophil count (HR: 0.25,  $p=0.08$ ).

The authors conclude that the association between neutrophil count and risk of tuberculosis in HIV-positive adults varies according to whether HAART is administered. Their results suggest that HAART critically influences the relationship between neutrophils and the risk of TB.

### Drug resistance

Research on drug-resistant TB continued to be a concern.

A study by Max O'Donnell and colleagues who have produced excellent data on the drug-resistant TB epidemic, found that health

care workers in Kwazulu-Natal had a much higher incidence of TB than the general population. [7]

Based on data from King George V Hospital, MDR-TB incidence was 58.9 per 100,000 people for the province's health workers and 10.7/100,000 for the province's general population (OR: 5.53; 95%CI: 4.70-6.50). XDR-TB incidence was 4.0/100,000 among health workers and 1.0/100,000 in the general population (OR: 3.89 95%CI: 2.02-7.11). There were 235 cases of health workers with drug-resistant TB and 3,391 cases for non health-workers at the hospital. About half of drug-resistant patients were HIV-positive and this did not differ between health workers and the general population. The high incidence amongst health workers is clearly related to occupational exposure. The researchers therefore conclude that screening and controlling occupational exposure among health workers is critical to limit nosocomial spread of drug-resistant TB.

In another Khayelitsha study, Helen Cox et al. reported on drug-resistant TB prevalence. [8]

Her team conducted a representative cross-sectional survey of clients attending two clinics suspected of having pulmonary TB between May and November 2008. Of 1,850 TB suspects surveyed, 536 (30%) were culture-positive. HIV status was known for 427 (80%) cases with 261 HIV-positive (61%). Rifampicin resistance was found in 4% of new cases and 10% of previously treated cases (p=0.003), and in 8.0% of HIV-positive and 4.8% of HIV-negative cases (p=0.18). They estimated rifampicin resistance in Khayelitsha to be 50 to 72/100,000 people per year. They concluded that there is extremely high prevalence of drug-resistant TB in the township. Moreover, it is high amongst HIV-negative people too.

PACTG 1041 was a double-blind placebo controlled trial to test isoniazid preventative therapy (IPT) in perinatally HIV-exposed infants. The primary endpoint was TB disease, infection or death. The DSMB stopped the trial because of futility. Partial results were reported at ICAAC 2008 (covered in HTB Nov/Dec 2008) and CROI 2009. At IAS2009 a poster by Anneke Hesseling and colleagues reported the results of an analysis of the 22 culture-confirmed cases of TB in the trial. [9]

Of these, 18 were sent for drug-susceptibility testing. Five were drug-resistant (one to INH, four MDR-TB). The authors conclude that the high rate of drug-resistant TB in the trial is consistent with the growth of the adult drug-resistant TB epidemic and has potential consequences for the programmatic implementation of IPT.

### Isoniazid preventative therapy (IPT)

One concern about community-wide IPT is that it will result in higher isoniazid resistance. A study by Halsema and colleagues provides promising data that will help allay, albeit not completely, this fear. [10]

The Thibela TB study is a large cluster-randomised trial to study IPT strategies in South African gold mines. Individual mine shafts are randomised to receive either standard TB control (IPT to miners with silicosis or HIV infection) or the intervention (IPT to everyone in the mine, from miners to executives).

In this case-controlled study, drug susceptibility data from TB cases among people who received IPT in the Thibela TB intervention clusters were compared to two groups: (1) TB cases in the control clusters and (2) a subset of patients from

a laboratory substudy confined to the first TB episodes in the control clusters. The comparison cases were restricted to the same calendar period as the intervention cases. The Thibela TB intervention began in July 2006 and the study included all TB cases in the intervention clusters up to mid-February 2009.

The intervention group included all participants receiving IPT who attended at least one follow-up visit and were subsequently treated for TB, unless they did not have positive TB cultures. Of the 126 individuals who met the inclusion criteria in the intervention arm all but one were male. The median age was 43. Of 103 with known HIV status, 89 (86.4%) were HIV-positive. Median CD4 cell count was 196 cells/mm<sup>3</sup> (n=51). The median time from starting IPT to TB treatment was 316 days (IQR: 174-491). For 94 (74.6%) people this was their first TB episode (32, or 25.4%, were retreated). TB was pulmonary for 87 (69.0%), extra-pulmonary for 22 (17.5%) and disseminated for 17 (13.5%).

Amongst the intervention cases, 96 outcomes had been documented at the time of the analysis: 39 (41%) were cured, a further 23 (24%) completed treatment, 8 (8.3%) died, there was one treatment interruption, one treatment failure, 11 (11.5%) transferred out or were lost-to-follow-up and for 13 (13.5%) cases the outcomes were unknown.

The authors concluded that TB disease after IPT may be largely due to re-infection in this high HIV prevalence group.

Data on drug susceptibility was presented for 58 people in the intervention group, 182 in the control clusters and 32 in the laboratory substudy. Table 3 presents the results of their analysis.

**Table 3: Drug-resistance in three groups from Thibela TB study**

	TB after IPT group (n=58)	Control clusters (n=182)	Laboratory substudy (n=270)
Isoniazid resistance	7 (12.1%)	12 (6.6%)	32 (11.8%)
MDR	1 (1.7%)	6 (3.3%)	21/269 (7.8%)

There were no significant differences between the groups in any of these outcomes. However, the authors noted the very wide confidence intervals (depicted graphically on their poster). In their discussion, they explained that if IPT is more effective at treating isoniazid susceptible latent TB, then an increased proportion, but not absolute numbers, of isoniazid resistant TB cases would be expected among those who have taken IPT. They concluded however that the proportion of TB episodes in the intervention groups do not differ from the controls and that these data do not support concerns about IPT induced resistance.

### TB treatment

The standard model for the management of TB patients that is promoted by the WHO involves directly observed treatment (DOT). In this model, patients come daily to their health facilities to take their pills under the supervision of a health worker.

Atkins et al. described an alternative model piloted from April 2007 to March 2008 in five health facilities in Cape Town. [11]

In this intervention, adult TB patients received adherence counselling. They also selected a treatment buddy. Lay health workers supported patients' self-supervised treatment.

Using information stored in a routine electronic TB register, TB data from these five clinics was compared against another five clinics that use DOT. Across the five intervention clinics 75% of new patients were treated using the new model. In these clinics, treatment success was 72.4% (95%CI: 67.4-77.4) and in the control clinics it was 75.9% (95%CI: 70.8 to 80.9), a non-significant difference.

In the previous issue of HTB South, we reported on the results of the CAPRISA trial that compared immediate versus deferred HAART in patients coinfecting with TB. It showed significant reduced mortality in the immediate arm. We commented on the importance of integrating TB and HIV services to make this intervention easier to implement. Further evidence of the benefits of integrating TB and HIV treatment comes from a Malawian study.

Malawian TB case notifications have risen dramatically over the last two decades. Chan and colleagues described what happened when the Zomba Central Hospital HAART Clinic, which opened in 2004, began integrating TB services. [12]

Zomba district has a population of 670,000 (80% rural). HIV prevalence amongst 15 to 49 year-olds is estimated to be 16.5%. Routine national TB programme data shows that 69% of TB patients are HIV-positive.

Integration of services began in September 2007 through monthly HIV/TB integration days. By April 2008, services were fully integrated with a daily TB/HIV Integration clinic where all patients registered for TB were also tested for HIV and referred for HIV care in the same physical area. Ministry of Health TB patient records and HAART records from September 2007 to December 2008 were reviewed to assess uptake of HAART. Following integration, HAART uptake increased dramatically from 4% to 33% in HIV monoinfected patients and from 25% to 50% in patients with HIV/TB coinfection. See Table 4.

The CARINEMO-ANRS 12146 Trial is a randomised, open-label non-inferiority study comparing 48 weeks virological suppression and safety of nevirapine (400mg daily without leading dose) vs efavirenz (600mg daily) co-administered with rifampicin. The other ARVs in the study regimen were d4T and 3TC. HAART was started four to six weeks after TB treatment. Preliminary safety data covering the period

November 2007 to December 2008 was presented by Bhatt and colleagues. [13]

By the end of this period, 236 patients with CD4 counts <250 cells/mm<sup>3</sup> and who were co-infected with active TB had been randomised. Of these, 11 (4.7%) discontinued the study (6 due to death, 3 withdrew consent, 1 lost-to-follow-up and 1 other).

Follow-up included weekly clinical assessments for the first eight weeks of HAART and monthly assessments thereafter. Patients also had alanine aminotransferase (ALT) measurements every two weeks during the first eight weeks followed by monthly measurements. 204/236 patients (86.4%) presented at least one adverse event. There were 26 serious adverse events, of which six resulted in death. None of the deaths were drug-related.

Skin-related adverse events were reported in 47 patients (19.9%), but none were severe. Also, 11 (4.7%) patients had an ALT increase ( $\geq$  grade 3). Five patients (2.1%) interrupted treatment due to hepatitis. However, there were no cases of severe rash. The researchers concluded that this plus the relatively low number of cases of severe hepatitis and treatment interruptions due to adverse events were reassuring but needed to be confirmed. Final results are expected at the end of 2010. A study by Kamateeka and colleagues of children taking rifampicin and nevirapine is also reviewed in this issue of HTB.

A study from a Uganda found that efavirenz is associated with a greater decline in TB incidence than nevirapine. Hermans and colleagues reported data from their large cohort of HAART patients (n=7,648). [14]

Between May 2002 and January 2009 they identified TB events in patients who had been on HAART for two years or less.

At baseline, median CD4 was 111 cells/mm<sup>3</sup> (IQR: 38-179) in the cohort and 85 cells/mm<sup>3</sup> (IQR: 30-149) in patients with TB coinfection. For the whole cohort, 30.6% were in WHO stage I or II, 39.8% in stage III and 29.3% in stage IV (the TB patients had similar proportions).

In the first two years of HAART (almost 13,600 PYFU), there were 360 (4.7%) new TB events (2.65 per 100PY; 95%CI: 2.39-2.94). Incidence rates declined with time on HAART. For 0-3, 3-6, 6-12 and 12-24 months they were 9.91 (95%CI: 8.51-11.55), 5.14 (95%CI: 4.11-6.44), 2.16 (95%CI: 1.66-2.82) and 0.82 (95%CI: 0.64-1.05), respectively.

**Table 4: Numbers of patients (%) in HAART uptake following integration of TB and HIV clinics at Zomba Central Hospital, Malawi**

	New TB	TB/HIV co-infected	Already on HAART n (%)	Need to start HAART	Started HAART	% uptake new HAART	% uptake all co-infected
Q3 2007	464	307	67 (22%)	240	9	4%	25%
Q4 2007	482	312	60 (19%)	252	25	10%	27%
Q1 2008	518	325	48 (15%)	277	28	10%	23%
Q2 2008	593	384	75 (20%)	309	84	27%	41%
Q3 2008	650	334	81 (24%)	253	77	30%	47%
Q4 2008	556	212	68 (32%)	144	47	33%	50%

In a multivariate analysis baseline CD4 count <50 cells/mm<sup>3</sup> (HR 1.58; 95%CI: 1.10-2.27; p=0.01) and male sex (HR 1.43; 95%CI: 1.15-1.77; p=0.001) were significantly associated with increased risk for TB.

A key finding of the study is that 100 patients out of 2,842 receiving AZT, 3TC and efavirenz versus 227 out of 3,974 using d4T, 3TC and nevirapine (832 used other regimens) developed TB. Compared to the d4T/3TC/nevirapine regimen, the HR for the AZT/3TC/efavirenz was 0.7 (95%CI: 0.53-0.89; p=0.003).

This difference could not be explained by differences in baseline CD4, calendar year starting HAART or immune restoration status after 14 months of HAART. In a multivariate analysis, the HR was 0.67 (95%CI: 0.53-0.86; p=0.002). The researchers further point out that this association occurred despite clinician bias towards prescribing efavirenz to patients with any TB symptoms to avoid subsequent switching due to interactions between nevirapine and rifampicin. This has not been previously described.

Is therapeutic drug monitoring needed in people with MDR-TB taking ofloxacin? This was a question that arose in a small proof of technology study reported by Mugabo and colleagues at Brooklyn Chest Hospital in Cape Town. [15]

Previously, PK values for ofloxacin have been obtained primarily using high performance liquid chromatography (HPLC) from cohorts in rich country. This study tested liquid chromatography coupled with mass spectrometry and found it to be simple, specific, accurate, sensitive and reproducible.

The inclusion criteria for their study included adult patients (18-65 yrs old) on ofloxacin therapy for at least two weeks who had TB that was resistant to isoniazid and rifampicin but sensitive to second line anti-TB drugs (i.e. strict definition of MDR-TB). Pregnant or breastfeeding women, patients intolerant of ofloxacin or patients on any drugs, other than ARVs, known to interact with ofloxacin PK were excluded.

They researchers found that the PK values of their eight patients with MDR-TB on ofloxacin differed from previous studies, with reduced AUC and C<sub>max</sub>, and prolonged T<sub>1/2</sub> and T<sub>max</sub>.

Five patients were HIV-positive (one was female and four male). The woman and two men were on HAART (d4T, 3TC and efavirenz). All eight patients received kanamycin, ethambutol, ethionamide and pyrazinamide. None were on capreomycin, aminosalicylic acid and terizidone.

Obviously this is a very small study, but the results are concerning because they suggest MDR-TB patients are receiving sub-optimal doses of ofloxacin. The authors therefore recommend ofloxacin plasma monitoring in order to maintain therapeutic plasma levels. Larger studies of patients with MDR-TB taking ofloxacin are also needed to ensure that optimal dosages and timing are determined, taking into account the effects of HIV, liver and kidney dysfunction.

Bhaijee and colleagues reported on a drug-induced life-threatening condition related to the commonly prescribed anticoagulant warfarin. [16]

The incidence of warfarin induced skin necrosis is low (estimated 0.01-0.1%), and by 2000, only 300 cases had been reported. Most of these were in patients receiving treatment for venous thromboembolism. This study was a retrospective review of six cases that occurred in GF Jooste Hospital in Cape Town from April 2005 to July 2008. This is a high concentration at one

facility for such a rare condition. All patients were HIV-positive women (aged 27 to 42) with venous thrombosis and with active TB coinfection. Four died, likely from systemic sepsis when resistant bacteria infected their wounds and one of the survivors underwent bilateral mastectomies and extensive skin grafting at a specialist centre. Median time from skin necrosis to death was 43 days (range 23-45).

No common pattern was detected: three were on HAART, two had TB-IRIS, two had previous TB. While five had low nadir CD4 counts (range 10-56), one of these (on HAART) has a CD4 count of 396 cells/mm<sup>3</sup> at the time of the necrosis. The site of skin necrosis included breasts, buttocks, and thighs.

The authors made four recommendations: (a) active prevention and appropriate management of venous thromboses, (b) parallel heparin therapy for at least the first four days of warfarinisation in patients with venous thrombosis (which they suggest may limit the occurrence of skin necrosis), (c) effective infection control measures, and (d) expedited referral to specialist centres for surgical review for patients who develop this warfarin induced skin necrosis.

Wilkinson and colleagues prospectively analysed their cohort to find immunological differences in drug-sensitive and drug-resistant patients with TB IRIS. [17]

They compared 12 rifampicin-resistant cases (nine had MDR-TB) to 27 case controls. They found no significant differences in the median duration of IRIS, days of HAART to development of IRIS, baseline CD4 count or days of TB treatment prior to HAART between drug-resistant and drug-sensitive groups. They also found no difference between the IFN-gamma spot forming cells/ million PBMCs in response to several M. Tuberculosis antigens (ESAT-6, Acr1, Acr2, 38kDa, PPD and heat killed H37Rv). C reactive protein was elevated in both groups, but without significant difference from each other. The authors concluded that both drug-sensitive and drug-resistant TB-IRIS, are clinically and immunologically indistinguishable, and that the occurrence of TB-IRIS is an opportunity to screen for previously undetected drug resistance.

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#### COMMENT

**While news from the TB treatment front is hardly breathtaking, some of the studies described above are important and merit further comment.**

**The potential role of earlier HAART in reducing the epidemiological impact on TB is encouraging. This is yet another reason why many Southern African governments need to change their guidelines urgently to allow treatment to commence at CD4 counts < 350 cells/mm<sup>3</sup> (as opposed to 200), preferably with non-stavudine containing regimens and lopinavir/ritonavir instead of nevirapine for women with CD4 counts above 250.**

**The importance of the START trial is underlined by these findings. If the immediate treatment arm of the START trial, especially in Southern African sites, has an impact on TB in the trial's cohort, then the case for earlier HAART initiation to stem the TB epidemic will be unanswerable.**

**The growing evidence of a rising drug-resistant TB epidemic that might undermine the benefits of IPT, particularly in infants, is concerning. So too are the high rates of drug-resistance in HIV-**

negative people beyond nosocomial infection in Khayelitsha. The South African government is still not demonstrating adequate commitment to co-ordinating and prioritising infection control measures and contact tracing. For example, it would be useful to have a project to revamp clinic waiting rooms (along similar lines to the waiting room at the Ubuntu clinic in Khayelitsha, which has heaters and a roof, but no walls allowing a continuous flow of air). A public information campaign to keep windows open in public places (e.g. buses and taxis) is also important.

The model of care described by Atkins, based on the HAART model, demonstrated that there are workable, more affordable and more convenient alternatives to DOT that give patients greater autonomy. It deserves further study, and ideally a randomised trial.

The increased uptake of HAART following TB/HIV integration at Zomba Central Hospital offers further evidence of the importance of integration. Although the data from this study can be used for advocacy, one caution should be noted: increased uptake could also have been linked to the general improvements in the facility over time.

The Hermans data is important. One limitation of the study is that a complex and potentially error-prone method was used to merge two separate databases containing patient data to determine the number of TB events. Nevertheless, this data offers evidence that efavirenz and AZT reduced the risk of TB compared to d4T and nevirapine. Their findings are worth testing in clinical trials and perhaps the CARINEMO-ANRS 12146 trial will provide more insight, at least regarding nevirapine versus efavirenz. Furthermore, if d4T-including regimens offer less protection against TB, it is another reason to limit their use in southern African countries.

Diagnostics data at IAS were more disappointing. Data on several methods were presented, including (but not limited to) acid-fast stain, urine lipoarabinomannan and the quantiFERON-TB Gold In-Tube assay. In the last of these, one study found an association between indeterminate results and increased risk of disease progression, but these patients also had lower median current and nadir CD4 counts, which are both, probably, better predictors. [18]

However, no studies at IAS showed algorithms with a combination of high speed, sensitivity and specificity.

The problem is global. One Cambodian study analysed sensitivity and specificity of smear and culture of urine, stool and lymph node aspirate as well as blood culture. It found they added little additional value. The authors aptly concluded, "In HIV settings, there is an urgent need for simple methods for mycobacterial cultures to detect earlier smear-negative tuberculosis." [19]

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## Biomarkers associated with mortality: long-term follow up from SMART

Nathan Geffen, TAC

A poster at IAS2009 by Nick Paton and the INSIGHT SMART Study Group presented long-term follow-up data from the SMART study on biomarkers associated with mortality. [1] This analysis extended an earlier nested case-controlled study of the association between biomarkers and mortality.

The earlier study identified all 85 patients who had died up to 11 January 2006, ie the date that enrollment into SMART was stopped. Each death was matched to two controls by country, age, gender and randomisation date. The study evaluated four inflammatory markers, hsCRP (C-reactive protein measured using the highly sensitive test), interleukin-6 (IL-6), serum amyloid A and serum amyloid P. It also examined three coagulation markers, D-dimer, PA1-1 and prothombin fragment 1+2 (F1.2). Three markers, hs-CRP, IL-6 and D-dimer, were found to have a statistically significant association with mortality on both adjusted and unadjusted odd ratios.

**Table 1: Case-controlled odd ratios by baseline biomarker levels**

Marker	Unadj. OR (4th/1st quartile)	P-value	Adj. OR (4th/1st quartile)	P-value
CRP	2.0	0.05	2.8	0.03
IL-6	8.3	<0.0001	11.8	<0.0001
D-dimer	12.4	<0.0001	26.5	<0.0001

The extended analysis reported at IAS2009 included all deaths up to 11 July 2007 in order to determine whether the association between these biomarkers and mortality persists. There were 167 deaths in the SMART cohort up to that point, 85 before the protocol modification (to offer all patients continuous treatment) and 82 post-modification. For this analysis, the deaths were however divided into early (<=2 years after randomisation, n = 95) or late (>2 years, n = 71). Two cases were matched to each death as in the baseline study. The baseline values of two of the three biomarkers (IL-6 and D-dimer) continued to be statistically significant predictors of late deaths and there was a trend for CRP to be a predictor of late deaths (see Table 2).

**Table 2: Baseline biomarker levels and risk of death**

Marker	Early (0-2yrs) vs. Late (>2yrs)	Deaths		Controls		Adj. OR*	p-value
		No	Median	No	Median		
Hs-CRP (ug/ml)	Early	96	3.13	188	2.08	2.8	0.009
	Late	71	3.09	137	1.93	2.8	0.08
IL-6 (pg/ml)	Early	92	3.58	184	2.14	5.9	<0.0001
	Late	67	3.72	133	2.33	6.4	0.004
D-dimer (ug/ml)	Early	94	0.45	188	0.24	7.3	<0.0001
	Late	69	0.31	138	0.24	8.3	0.002

\*4th/1st quartile

## Greater predictors of risk than other factors

Table 3 shows some of the other risk factors for deaths that have been found in SMART (note that where p-values show non-significance, the factor can still be significant when the early and late groups are counted together).

**Table 3: Risk factors associated with mortality in SMART**

Factor		Deaths (%)	Controls (%)	p
Hepatitis B or C	Early	38.9	20.7	0.002
	Late	46.5	19.3	0.0001
Current smoker	Early	50.5	34.0	0.006
	Late	64.8	38.6	0.005
Diabetes	Early	18.9	10.6	0.07
	Late	22.5	13.6	0.08
Blood pressure drugs	Early	37.9	25.0	0.02
	Late	38.0	23.6	0.02
Prior CVD	Early	10.5	5.9	0.01
	Late	15.5	2.1	0.002
Total/HDL cholesterol	Early	4.4	4.7	0.06
	Late	4.8	4.8	0.99
Treatment group (% on structured breaks)	Early	63.2	52.1	0.09
	Late	59.1	50.7	0.27

The authors noted that IL-6, hs-CRP and D-dimer are associated with greater risk of mortality than smoking and diabetes and about an equivalent risk of prior cardiovascular disease. They conclude that interventions to decrease inflammatory and coagulation pathway activation may be of long-term benefit for people with HIV.

### COMMENT

**The association between mortality and hs-CRP, IL-6 and D-dimer is significant, even after long-term follow-up and the termination of the structured treatment interruption arm. This highlights the importance of further research on whether anti-inflammatory medicines will have an additional role in HIV management of high-risk patients.**

**It would be interesting to know the association between these biomarkers and mortality is in the uninfected population. If they are similarly prognostic, then the question is how much of the associations seen in SMART are HIV-specific.**

**The role of early HAART in mitigating the association with mortality also needs to be determined.**

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## Influenza vaccine effective in HIV-positive adults

Nathan Geffen, TAC

A poster at IAS 2009 by Madhi and colleagues reported the results of a double-blind, randomised, placebo-controlled trial to examine the efficacy of influenza vaccines in HIV-positive adults. Specifically, the study tested the seasonal trivalent subunit vaccine, which protects against three H1N1 and H3N2 influenza strains, two of which were isolated in 2006 and one in 2007. [1]

This was the first community-based randomised controlled trial of the trivalent subunit influenza vaccination in HIV-positive adults. A previous randomised controlled trial in the US on HIV-positive out-patients at a military health facility found that 10/47 patients who received placebo acquired laboratory confirmed influenza versus 0/55 patients who received this type of vaccine ( $p < 0.001$ ). [2]

A Cochrane Review of the vaccine in healthy adults has found this type of vaccine to be 30% effective (95%CI 17%-41%) against influenza-like illness, and 80% (95% CI 56% to 91%) effective against influenza when the vaccine matched the circulating strain and circulation was high, but decreased to 50% (95%CI 27%-65%) when it did not match the circulating strains. [3]

Participants were vaccinated prior to the 2008 influenza season in South Africa. Oropharyngeal swabs were taken from patients with influenza-like symptoms or respiratory illness. Culture and PCR tests were used to identify influenza strains. Only events 14 days post-vaccination were compared.

The number of HIV-positive people enrolled was 506. Of these, 101 were HAART-naive (52 received vaccine, 49 received placebo) and 405 were on HAART (203 received vaccine, 202 received placebo). Median age was 36. Female to male ratio was 5 to 1 in the vaccine arm and 6 to 1 in the placebo one. Median CD4 was 372 (IQR: 254-489) and 363 (IQR: 252-517) in the two arms respectively. Nine women were pregnant in the vaccine arm and four in the placebo one.

Over 90% of patients on HAART were virally suppressed in both arms. The median time on HAART at the time of randomisation was 23 months.

The percentage of people who developed influenza on the placebo arm was 5.3% using a passive surveillance method. [4] The rate of influenza illness was 0.06 per 100 person weeks in the vaccine arm and 0.25 per 100 person weeks in the placebo one. The vaccine efficacy was 75.4% (95%CI 14-93). The protective effect against the seasonal H1N1 strain was 73.5% (95%CI 4-93). There was one case of influenza B and no cases of H3N2 or untyped A.

The authors concluded that their findings support the use of the trivalent subunit influenza vaccine in HIV-positive adults.

## COMMENT

**This study supports vaccinating HIV-positive adults against seasonal influenza, as is routinely recommended in the UK. Because the vaccine is developed seasonally, its efficacy will change from year to year. The seasonal vaccine does not provide protection against the current H1N1 strain of swine flu.**

**At present there is no data on the effect influenza vaccines would have on reducing mortality in HIV-positive people as the contribution of HIV to influenza mortality is not well understood. Nevertheless, it is plausible that the reduced influenza cases conferred by the vaccine will reduce mortality as well.**

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## Time from seroconversion to treatment in Europe and Africa

Simon Collins, HIV i-Base

People who are newly diagnosed with HIV commonly expect a period of 5-8 years after infection until they need to start treatment. However, the UK Register of Seroconverters provided an indication that at least a quarter of patients may need to start treatment within two years of infection. In a review of this cohort published in *AIDS* in January 2008, the median time from seroconversion to HAART initiation was 5.0 years but the IQR was 2.1 to > 10 years. The 25th percentile of time to starting HAART was 2.0, 2.0, 2.0 and 1.4 years in 1998-1999, 2000-2001, 2002-2003 and 2004-2006, respectively. [1]

This was also a conservative analysis as it excluded patients who started treatment within six months of infection due to complications during seroconversion. The most recent data related to a period when UK guidelines recommended starting treatment at a CD4 count of around 200 cells/mm<sup>3</sup>.

At the IAS meeting, two studies from the CASCADE cohort of European seroconverters (which includes the UK data) provided further information on time to progression.

A European analysis, presented by Sara Lodi from the UK's MRC, looked at time to CD4 counts dropping to below 500 cells/mm<sup>3</sup> (in order to inform policy should guidelines broaden to this higher threshold). [2]

Of over 11,700 adults (age >15 years) who seroconverted after 1992, over half (57%) reached CD4  $\leq$  500 cells/mm<sup>3</sup> during a median of 20 months (95%CI:19.6, 20.5), with 29% censored

at initiation of antiretroviral therapy. The proportion of patients with CD4 counts over 500 cells/mm<sup>3</sup> at 6, 12, 24 and 36 months after seroconversion was approximately 92%, 72%, 43% and 30%, respectively.

From these results the authors concluded that 50% of patients would require treatment within 20 months of seroconversion if guidelines change the CD4 initiation threshold to 500 cells/mm<sup>3</sup>.

Increasing age at seroconversion was associated with faster progression (HR, 95%CI= 1.06, 1.03-1.09 per 10-year increment). For example, 50% of the patients aged 15-20 still had counts >500 cells/mm<sup>3</sup> after two years compared to only 35% of patients who were older than 40 at diagnosis. Unadjusted median times for those aged < 20, 20-29, 30-39, and 40+ years were 25.5, 21.9, 19.8 and 17.6 months, respectively.

No association was found with gender, transmission group and acute infection. Although numbers of patients with sub-type A, C and D were very low, there was an indication that progression may have been faster compared with sub-type B.

A second study from the CASCADE group, presented by Andrea de Luca, reinforced the finding that **older age is associated with a shorter time to starting treatment**, but also that older age was associated with better virological response (<50 copies/mL viral load). [3]

Of over 7100 patients who seroconverted after 1993 that were included in the analysis, just under half (48%) initiated antiretroviral treatment. Median time to starting treatment was 3.32, 3.15, 2.64 and 2.08 years for patients aged 15-29, 30-39, 40-49 and 50+ years respectively.

Later calendar period and seroconversion illness, but not age, were found to be independent predictors of CD4 count at ARV initiation. Increasing age was associated with better viral response (HR (95%CI)= 1.17 (1.06, 1.29); 1.30 (1.15, 1.47); and 1.25 (1.07, 1.47) for 30-39, 40-49 and 50+, respectively, compared to 15-29 year olds at seroconversion).

Data on progression rates in an African cohort were presented from the French ANRS 1220 Primo-CI cohort 1997-2008, in patients from Abidjan, Côte d'Ivoire. [4]

This study had a similar design, though it was a much smaller cohort (of 254 adults enrolled, 112 had baseline CD4 >500 cells/mm<sup>3</sup>). Baseline characteristics of these 112 patients followed included 65% men, median age was 28 years (IQR 25-34), median time from estimated seroconversion was 7 months and median CD4 cell count was 677 cells/mm<sup>3</sup> (IQR 591-800). Median duration of follow-up was 7.1 years (IQR 4.2-9.3; 790 person-years).

The probability of reaching CD4 <500/mm<sup>3</sup> (the guideline for starting PCP prophylaxis) was 0.58, 0.70, and 0.78, at 2, 4 and 5 years, respectively. Probability of reaching CD4 <350 cells/mm<sup>3</sup> was 0.22, 0.47, and 0.49, at 2, 4 and 5 years, respectively. Baseline CD4 count and haemoglobin were associated with CD4 decrease below 500.

The study concluded that, in this cohort, half of patients reached CD4 <350 within five years of infection. They also reported higher morbidity and mortality at CD4 counts between 350 and 500 (compared to higher CD4 counts). Mortality was 0.9 per 100 patient years and incidence of WHO stage III/IV events was 0.5.

## COMMENT

**Highlighting the significant interpatient variability in time to starting treatment would give newly diagnosed patients a more realistic understanding of the chance that the optimal time may well be within two years. The probability is likely to be over 25% for any setting where the recommended CD4 threshold is now 350 rather than 200.**

**The results suggest that a significant percentage of patients are already likely to benefit from ARV treatment within two years of infection.**

**The association with older age at infection support the BHIVA guidelines recommendation to consider earlier treatment in older patients.**

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## Results from HSV-2 acyclovir studies

Nathan Geffen, TAC

### Acyclovir studies at IAS 2009

Connie Celum, the principal investigator on the HPTN 039 trial, together with Jai Lingappa, the medical director, and other members of the Partners in Prevention HSV-HIV Transmission Team presented findings at IAS2009 on a counterpart trial to HPTN 039. This study, in HIV serodiscordant couples, looked at whether suppressing HSV-2 in people dually infected with HSV-2 and HIV infections could reduce HIV transmissions to their HIV-negative partners. [1]

HPTN 039 was a double-blind placebo controlled trial of standard doses of acyclovir for HSV-2 suppression (400 mg twice daily) to prevent HIV acquisition among over 3,200 HIV-negative, HSV-2 positive African women and MSM in Peru and the United States. HPTN 039 found that acyclovir suppression did not reduce HIV incidence compared to placebo. Results were reported at CROI2008 and subsequently in the Lancet. [2]

Celum presented the main results of this new double-blind placebo controlled trial, conducted at 14 sites in sub-Saharan Africa, including South Africa, Kenya, Zambia, Botswana, Tanzania, Rwanda and Uganda.

Of 6,544 heterosexual HIV discordant couples screened, 3,408 were enrolled. The inclusion criteria for the coinfecting partner included a CD4 cell count  $\geq 250/\text{mm}^3$ . The other partner could be either HSV-2 positive or negative, but had to be HIV-negative. HIV-positive participants could not be eligible for HAART at trial entry according to their country guidelines.

The HIV-positive partners were randomised to receive either 400mg acyclovir twice daily or placebo twice daily. Couples were followed for a maximum of 24 months. Participants were provided with HAART if they became eligible for it according to country guidelines during the trial.

The primary endpoint was HIV infection in the HIV-negative partners. The secondary endpoints were plasma and genital HIV viral load in the HIV-positive partners, and HIV disease progression. The trial was set up so that if 88 'linked' HIV transmissions (i.e., the virus transmitted from the enrolled partner to the seroconverting partner was determined by molecular sequencing to be linked) were observed, the trial would have high statistical power (90%) to see a 50% reduction in HIV transmissions in the acyclovir arm.

Baseline characteristics of the group were as follows: 67% of HIV-positive partners were female; 65% of volunteers were  $\leq 35$  years old; average partnership duration was five years; 90% were cohabiting; a median of five sex acts were reported in the month prior to baseline measurements and 29% reported unprotected sex; 22% of the HIV-positive partners reported genital ulcer disease (GUD) in the prior three months; 4% of HIV-positive and 7% of HIV-negative partners reported outside partners respectively; median CD4 count was 460 and median plasma HIV viral load was 4.2 log.

Monthly follow-up visits included medication provision, pill count and adherence support and individual and couple HIV risk reduction counselling. Every three months, HIV-positive partners were examined for GUD and plasma viral load and HIV-negative partners were tested for HIV and given risk reduction counselling. CD4 cell counts were taken every six months.

Retention was high. At 24 months, 92% of HIV-positive and 84% of HIV-negative participants were still in follow-up. Adherence measured by pill count was also high: 88% of all bottles were dispensed and 97% of dispensed bottle doses were taken.

### No significant differences in incidence

There were 136 sero-conversions at a rate of 2.8/100py (95% CI: 2.3-3.3), one after an incorrect drug kit was dispensed. Of the remaining 135, 68 occurred on the acyclovir arm and 67 on the placebo arm (HR: 0.92; 95%CI 0.60-1.41;  $p=0.70$ ).

In a modified intention to treat analysis, 43 transmissions were linked by viral sequencing technology to partners on the acyclovir arm and 47 were linked to partners on the placebo arm. However, two in the acyclovir arm and four in the placebo arm were excluded from analysis because the study drug was withheld during pregnancy. Here too, there were no significant differences between the two arms. The sequencing methodology for the study was explained by Mary Campbell in a late breaker poster. [3]

### Benefits of acyclovir

There were fewer GUD events in the acyclovir arm (217 vs 550; RR: 0.39; 95% CI: 0.32-0.48;  $p<0.001$ ). HSV-2-positive GUD as determined by DNA PCR was also lower in the acyclovir arm (92 vs 336; RR: 0.27; 95%CI 0.2-0.36;  $p<0.001$ ).

The acyclovir arm also had a 0.25 log reduction in plasma viral load (95%CI: 0.22-0.29).

A novel component of this study was evaluation of herpes suppression on HIV disease progression, an important secondary endpoint of the Partners in Prevention trial. In a separate analysis presented by Jairam Lingappa, 3,381 of the HIV-positive participants were followed up until a composite endpoint of first of CD4 cell count  $< 200/\text{mm}^3$ , HAART initiation, or death from non-trauma causes. [4]

In the acyclovir arm, 284 participants reached this endpoint versus 325 in the placebo arm (HR 0.83; 95%CI: 0.71-0.90;  $p=0.03$ ). Similar reductions were found for each component of the composite endpoint analysed separately. However, Lingappa's team further calculated that for every 43 people treated with the trial dose of acyclovir for a year, only one person would be prevented from attaining the composite endpoint. (We have previously reported findings demonstrating acyclovir and its pro-drug, valacyclovir's effect on HIV plasma RNA levels, in the October 2006 and July/August 2008 issues of HIV Treatment Bulletin, but this is the first report documenting impact of herpes suppression on HIV disease progression.)

Among participants with CD4 counts  $\geq 350$  cells/ $\text{mm}^3$  at enrollment, acyclovir delayed the time to CD4  $< 350$  cells/ $\text{mm}^3$  (HR 0.81; 95%CI 0.71-0.93;  $p=0.002$ ). Here, 20 people would need to be treated to prevent one person from progressing to a CD4 count  $< 350$  cells/ $\text{mm}^3$ .

### Acyclovir effect on genital viral load

A late breaker poster by Jared Baeten et al presented the results of a substudy that examined genital HIV RNA concentrations as a surrogate marker for HIV infectivity. [5]

Endocervical and semen samples were collected from 2,521 (1,805 women and 716 men) of 3,408 HIV-positive participants. For 1,797 of these, plasma was concurrently taken. For the remainder a plasma viral load within six months was available. Since the genital samples were taken only once during the study, the genital viral load was analysed as a time-independent variable.

HIV was detected in 60% of endocervical swab samples and 57% of semen samples. The median endocervical HIV concentration was 3.2 log (IQR 2.08-3.87) overall. Genital HIV-1 concentrations were significantly lower among those randomised to acyclovir (median 2.98 vs 3.29 for endocervical swabs;  $p<0.001$  and 2.38 vs 2.76 for semen;  $p=0.008$ ). The key finding of the study was that genital HIV concentrations were higher among HIV transmitting couples, where transmission was genetically linked to the partner (3.44 vs 2.49 log copies/mL for semen,  $p<0.001$  and 3.91 vs 3.18 log copies/swab for endocervical swabs,  $p<0.001$ ). Each log increase in genital HIV-1 RNA concentration was associated with 1.85-fold increased odds of HIV transmission for semen ( $p<0.001$ ) and 2.03-fold increased odds of transmission for endocervical swabs ( $p<0.001$ ). The study found no significant difference in genital HIV concentration for participants whose partners acquired HIV from outside sexual partners versus those who did not transmit HIV.

However, despite a 73% reduction in GUD and 0.25 log decline in plasma HIV levels and an approximately 0.3 log decline in genital HIV levels, acyclovir conferred no reduction in HIV transmission. The authors interpret the overall results of the trial to indicate that the plasma and genital tract HIV viral load reduction from herpes suppression with standard doses of acyclovir is too small to confer a protective effect against HIV transmission.

### Future acyclovir trials

Nevertheless, given the promising effect of acyclovir on HIV viral load, Steve Reynolds described an ongoing double-blind placebo controlled trial in Rakai, Uganda. [6]

The purpose of the trial is to evaluate the effect of suppressive HSV-2 therapy among HIV-1/HSV-2 co-infected individuals on progression to AIDS, defined as CD4 count < 250 cells/mm<sup>3</sup> or WHO stage IV disease. Volunteers with CD4 counts between 300 and 400 cells/mm<sup>3</sup>, not on HAART, without WHO III/IV symptoms and no history of opportunistic infections, other than mucocutaneous Kaposi Sarcoma, candida or treated TB were eligible for inclusion. Enrollment was completed in November 2008. The trial assumes that 40% of individuals in the placebo arm will progress to CD4 counts <250 cells/mm<sup>3</sup> or AIDS over 24 months and is powered to detect at least a 20% reduction in HIV disease progression in the intervention arm.

#### COMMENT

**These studies show that a standard dose of acyclovir for HSV-2 suppression does not reduce HIV transmission. These are disappointing findings for an HIV prevention strategy that is already available.**

**A mechanism for the lack of protection has been suggested by Laurence Corey and colleagues in a recent paper in Nature Medicine. [7]**

**By analysing regular skin biopsies taken during acute lesions and over 20 weeks follow-up, they identified a massive localised infiltration of CD4 and CD8 cells, thereby increasing the targets for HIV infection. Eight weeks after lesions healed these levels were still 8-fold higher (655 and 618 cells/mm<sup>2</sup> of skin, respectively, compared to 68 and 55 cells/mm<sup>2</sup> in unaffected skin samples).**

**This paper is reported in detail in the Basic Science section of this issue of HTB. [8]**

**It has been conventional wisdom that wider availability of acyclovir for patients with genital herpes outbreaks would reduce HIV transmissions. We now know this is incorrect, at least with the doses of acyclovir (400 mg twice daily) used in these trials. However, efforts to make acyclovir widely accessible should continue because herpes is a debilitating, unpleasant disease which acyclovir effectively treats and because HSV-2 is widely prevalent in both HIV-negative and HIV-positive people. One of the barriers to its accessibility remains its high price in many developing countries.**

**Despite the negative findings, this trial and its substudies have set a high standard for the testing of future HIV prevention interventions, a field that is frequently characterised by ideological prejudices and too little sound science. Furthermore, modeling studies using**

**the data from this trial provide a potential threshold of HIV plasma viral load reduction in HIV-infected persons that will be needed to impact HIV transmission.**

**We now need to know whether a therapeutic dose of acyclovir could delay the time until initiation of HIV treatment, and whether this would be cost effective. The trial in Rakai described by Steve Reynolds using 400 mg twice-daily dose will provide complimentary information to the Partners in Prevention trial.**

**Studies with higher doses of valacyclovir will evaluate whether greater reduction in plasma HIV levels is feasible compared to acyclovir 400 mg twice daily. However, this research could be overtaken by new developments in HAART management, if guidelines recommend earlier treatment.**

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## CONFERENCE REPORTS

### 10th International Workshop on Clinical Pharmacology of HIV Therapy

15-17 April 2009, Amsterdam

#### Introduction

The following reports from this meeting have been largely compiled from the abstracts of these studies.

It is disappointing that the abstracts from the virology-education meetings are not published online, and that only a selection of presentations from the meeting are available at:

<http://www.HIVpresentation.com>

A useful summary report of the drug-interaction studies presented at the meeting is available on the Liverpool University website (in the April 2009 news archive):

<http://www.hiv-druginteractions.org/new/Content.asp?ID=431&TDM=>

Reports in this issue are:

- Interactions between ARVs and the antimalarials atovaquone and proguanil
- Efavirenz-related studies: genetics, smoking and TDM
- Atazanavir: a suitable case for TDM?
- Raltegravir PK in blood plasma and the genital tract
- A CYP2B6 haplotype influences nevirapine plasma concentrations following a single dose to reduce mother-to-child transmission
- Population pharmacokinetic model of nevirapine maternal to infant transfer through breastfeeding
- Phenotypic and genotypic inhibitory quotients and virologic response in treatment experienced children
- Tenofovir pharmacokinetics in three tenofovir-containing regimens in children and adolescents
- Bioavailability of Thai generic lopinavir/ritonavir

### Interactions between ARVs and antimalarials atovaquone and proguanil

#### Simon Collins, HIV i-Base

Van Luin and colleagues from Nijmegen presented results showing significantly lower levels of the common antimalarials atovaquone and proguanil, commonly used together as prophylaxis, in HIV-positive patients on HAART compared to HIV-negative controls.

Seven-day PK results from HIV-positive patients already on established HAART including efavirenz (n=19), lopinavir/r (n=19) or atazanavir/r (n=19) were compared to levels in 20 HIV-

negative volunteers following single-dose atovaquone/proguanil (250/150mg), administered with a fat standardised breakfast. Patients who were negative for CYP2C19 defective \*2 and \*3 alleles, the key enzyme for proguanil metabolism, were excluded from the proguanil comparisons.

PK parameters were significantly lower in HIV-positive patients (all  $p < 0.05$ ), and are detailed in Table 1. Efavirenz or lopinavir/r resulted in considerably reduced levels suggesting increased dosing may be required. Atazanavir/r considerably lowered proguanil compared to the HIV-negative group, but more modestly lowered atovaquone suggesting that this interaction may be managed with perfect adherence.

**Table 1. Mean [range] atovaquone and proguanil level**

	HIV-neg	efavirenz	lopinavir/r	atazanavir/r
<b>Atovaquone</b>				
AUC 0-t (h*mg/L)	112.9 [43.3-250.1]	35.3 [12.5-91.8]	39.1 [6.3-137.0]	75.3 [22.6-146.6]
Cmax (mg/L)	2.0 [0.44-4.0]	1.2 [0.39-2.8]	1.3 [0.40-3.0]	1.1 [0.54-2.2]
<b>Proguanil (in patients without CYP2C19* or -*3)</b>				
AUC 0-t (h*mg/L)	1.3 [0.40-10.3]	0.55 [0.12-1.8]	0.42 [0.12-1.8]	0.34 [0.10-0.63]

All comparisons to HIV-negative values:  $p < 0.05$ .

#### COMMENT

**An important problem with this study is the use of HIV-negative controls compared to HIV-positive patients, so the differences may have been due to differences in PK of antimalarials in HIV-positive people. These are interesting data, but the results need confirmation and cannot be regarded as definitive.**

#### Reference

Van Luin M et al. Drug interactions between atovaquone/proguanil and antiretroviral agents. 10th International Workshop on Clinical Pharmacology of HIV Therapy, 15-17 April 2009, Amsterdam. Oral poster O-19.

### Efavirenz-related studies: genetics, smoking and TDM

#### Simon Collins, HIV i-Base

Several studies presented interesting results on the PK of efavirenz.

In an oral presentation reporting higher rates of antiretroviral switching by patients with pharmacogenetic markers (notably genotype changes in CYP2B6 G516T) associated with an increased risk of side effects, Colombo and colleagues from the Swiss HIV Cohort reported that patients were more likely to switch from efavirenz if they carried these alleles (42% vs 27%). [1]

Bensemmane and colleagues from a multicentre French study reported on the routine use of therapeutic drug monitoring (TDM) to manage individual patients on efavirenz from 2002-2008. [2]

The target level, based on historical estimates was 1000-4000 ng/mL. Of the 2545 patients (33% women) prescribed efavirenz at 600mg once-daily, with at least one TDM result for Cmin, approximately 5% had levels below the limit of detection for the test (<10ng/mL) suggesting non-adherence. 12% patients had levels below the minimum target, 61% were in the target range and 22% had Cmin levels >4000 ng/mL.

Of the 549 patients with high levels, 41% (n=188) adjusted the efavirenz dose to one of four once-daily doses: 400mg, 300mg, 200mg or 100mg (groups 1 to 4, respectively).

**Table 1. Median efavirenz levels in patients with Cmin >4000 ng/mL**

	N	Baseline Cmin (ng/mL)	Cmin after adjustment (ng/mL)
Group 1	129	5547	2701
Group 2	2	5194	1664
Group 3	54	9263	2480
Group 4	3	11028	2245

Although the poster abstract provided minimal details on the relationship between drug levels and toxicity, it reported that approximately that only 22% of patients making a dose adjustment continued to experience persistent side effects. As the study was unblinded patients who knew they had reduced their dose may have reported side effects differently.

A second poster by Fayet and colleagues from the Swiss Cohort Study reported results from a small prospective study using TDM to individualise efavirenz dosing in 15 patients on stable EFV-based HAART, with levels in the highest quartile. [3]

At baseline, median efavirenz Cmin was 8,409 ng/mL (IQR 6610-10,370). The five patients with levels between 75-95 percentile reduced the efavirenz dose to 400mg QD and then ten patients above the 95th percentile reduced to 200mg QD.

Following dose reductions, ten patients with results achieved the target of 25-75th percentile range (median 2,856, IQR 2192-3157 ng/mL). Three months after the dose adjustment, all patients remained above the minimum 1000ng/mL lower target level and maintained viral load <40 copies/mL.

Cortes and colleagues presented results from a prospective 215 patients (13 women) in Chile, looking at both drug levels and genetics (CYP2B6: 516G>T and 983 T>C; and constitutive androstane receptor (CAR) rs2307424 polymorphisms). [4]

In the group as a whole, mean ( $\pm$ SD) levels were 3100 ng/mL ( $\pm$ 1600), in samples taken a mean 11.9 hours ( $\pm$ 1.6) post-dose. Eleven patients (5%) had levels <1000 and 45 (21%) had levels >4000ng/mL. Alleles at CAR, 516G>T and 983 T>C were present in 49%, 35% and 0% respectively, and were related to drug concentrations in multivariate analysis (see Table 1).

As reported in other studies, c516 polymorphisms were related to efavirenz exposure.

This is the first report of the impact of the associations with the constitutive androstane receptor and the group also reported statistically significant lower levels in smokers compared to non-smokers (2.81 vs 3.32 mg/L, p=0.02).

**Table 2. Efavirenz drug exposure (mg/L) in relation to genetic polymorphisms**

	GG (n=90)	GT (n=87)	TT (n=30)	p
CYP2B6 516	2.21	3.13	5.23	P<0.0001

	CC (n=50)	CT (n=109)	TT (n=48)	p
CAR rs2307424	2.97	3.28	2.53	P=0.008

COMMENT

**These posters show the potential for individualising dosing in patients who metabolise efavirenz more slowly (whether due to genetics, hepatitis coinfection etc) and who end up with levels higher than the upper limit of the recommended target range.**

**The association with smoking status has not previously been reported.**

**It is unclear if the study adjusted for weight differences and whether this would make a difference to the results. If there is an association with weight, then the association with smoking would be in the opposite direction (ie. smokers tend to weigh less, so levels may be higher rather than lower).**

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**Atazanavir: a suitable case for TDM?**

**Simon Collins, HIV i-Base**

Numerous studies at this workshop explored the potential for individualised dosing with atazanavir, relating to individual patient absorption, the use of ritonavir boosting and interactions with HIV and TB medications.

Atazanavir is widely used because it is generally well tolerated, has a low pill count and only requires once-daily dosing. Ritonavir boosting is routinely recommended to maximise drug exposure, and to reduce the risk of low trough levels and interpatient variability. However, higher atazanavir exposure is related to risk of hyperbilirubinaemia and the ritonavir boosting negatively impacts on lipid profiles.

As with efavirenz, the study from Columbo and colleagues that identified genetic polymorphisms associated with absorption showed a higher rate of discontinuation of atazanavir due to side effects in patients with compared to those without these markers (52% vs 20%, p=0.008). [1]

Taburet and colleagues reported results from a substudy (n=15) of the INDUMA trial where treatment naive patients were prescribed atazanavir/ritonavir (300mg/100mg QD) plus 2 nukes (not including tenofovir) and then randomised to continue on the same regimen or switch to unboosted atazanavir (400mg), maintaining the nukes. [2]

Atazanavir levels (adjusted geometric mean ratios) for C<sub>min</sub> and AUC dropped by 10% (5.8-19%) and 34% (26-46%) from the switch (week 0) and after 4 weeks on the reduced regimen. As expected, all PK parameters reduced without boosting and interpatient variability increased (see Table 1).

**Table 1. Atazanavir levels: geometric mean (CV%) with and without ritonavir boosting**

	week 0 ATZ/r 300/100	week 4 ATZ 400
C <sub>max</sub> (ng/mL)	3317 (39%)	1895 (69%)
C <sub>trough</sub> (ng/mL)	543 (92%)	64 (125%)
AUC 0-24 (ng*h/mL)	35617 (52%)	12197 (81%)

However, despite the reduced atazanavir levels, 14/15 patients maintained viral suppression <50 copies/mL at 48 weeks, with only one patient experiencing a blip (to 113 copies/mL).

Regazzi and colleagues presented results from using therapeutic drug monitoring in treatment-experienced patients using atazanavir, with and without ritonavir. [3]

The target range for atazanavir is 150-850 ng/mL.

The group analysed samples from 170 patients (with and without HCV coinfection) using various dosing regimens including ATZ/r 300/100 QD (n=79), ATZ 400 QD (n=57), ATZ 300 QD (n=11), ATZ/r 400/100 (n=5), ATZ 400 BID (n=8) and ATZ/r 200/100 QD (n=10). The main comparison between ATZ/r 300/100 QD and ATZ 400 QD showed lower atazanavir exposure and wider interpatient variability (CV%) without ritonavir (see Table 2).

Although both regimens showed similar levels of viral suppression (~84%), significant differences were seen between patients with or without HCV coinfection.

With the 400mg QD regimen, monoinfected patients experienced significantly lower trough levels (240 [100-400] vs 600 [400-950] ng/mL, p<0.001). Conversely, coinfecting patients using 400mg QD achieved similar trough levels to both mono- and coinfecting patients using the 300/100mg dosing.

The authors do not comment on why this may be the case and it is unclear whether this was a real effect directly relating to coinfection or possible confounding with drug use or adherence (if IDUs were less adherent, they would tend to have lower trough levels).

Guillemi and colleagues from British Columbia looked at using TDM to identify patients with high atazanavir trough levels (>900 ng/mL using ritonavir boosting) and then to confirm unboosted levels were >150 ng/mL prior using 400mg unboosted as a maintenance dose. [4]

They identified 20 patients (14 using tenofovir/FTC and 6 using abacavir/3TC) with baseline median [IQR] trough level of 1369 [1090-1620] ng/mL. Median trough level after 7-10 days on 400mg ATZ (unboosted) was 173 [96-301] ng/mL. CD4 was unchanged and no patient experienced viral rebound, although total bilirubin levels significantly declined (from 52 [28-64] to 18 [12-24] umol/L, p<0.001).

**Table 2. Atazanavir exposure with and without ritonavir**

	ATZ/r 300/100mg QD	ATZ 400mg QD
C <sub>trough</sub> ng/mL, mean	720	340
[IQR] (CV%)	[430-1200] (79%)	[130-600] (147%)
% <150	3.8%	30%
% 150-850	57%	54%
% >850	39.2%	15.8%
% viral load <50 copies/mL	84.8 %	84.2%

The 9/20 patients with C<sub>trough</sub> <150 ng/mL (4 on abacavir, 5 on tenofovir) were switched back to the 300/100 boosting regimen while 11/20 continued on 400mg QD unboosted atazanavir.

A second study from the same group looking at the relationship between tenofovir use and unboosted atazanavir levels and showed that some patients maintained undetectable viral load despite trough level <150 ng/mL. [5]

The median atazanavir trough level in 43 patients was 242 (range 106-1100) ng/mL. Four patients with low trough levels (107-131 ng/mL) increased their dose to 600mg QD, resulting in trough increases to 222-294 ng/mL).

Of 31 patients with undetectable viral load at baseline, 30/31 remained <50 copies/mL.

### Atazanavir and raltegravir

Two studies looking at the interaction between raltegravir and atazanavir suggest that individual monitoring is likely to be important when considering using these drugs in the same combination.

Molto and colleagues presented results from a study in 15 HIV-positive patients (4 women) who added raltegravir 800mg once-daily for 10 days, to the regimens of patients already using 400mg atazanavir once-daily for at least the previous two weeks. Use of tenofovir or proton pump inhibitors was not permitted. Both drugs were given with a light meal.

Previous studies have shown that atazanavir inhibits raltegravir metabolism by UGT1A1, boosting raltegravir exposure.

The geometric mean ration (95%CI) for C<sub>max</sub> and AUC 0-24 were compared with historical data on 20 HIV-negative individuals receiving raltegravir with a high fat meal.

Mean (IQR) raltegravir values for C<sub>max</sub>, T<sub>max</sub>, AUC0-24h and C<sub>trough</sub> were 5.36 (3.22-8.91) uM/mL, 2.95 (2.09-4.18) hours, 29.04 (20.46-41.22) uM\*h/mL and 69.53 (39.58-122.16) nM/mL respectively. Raltegravir C<sub>trough</sub> was <33nM in four patients.

Compared to historical controls using a single 400mg dose of raltegravir GMR (95%CI) for C<sub>max</sub>, AUC 0-24h and C<sub>trough</sub> were 2.81 (1.43-5.50) p=0.004; 1.18 (0.74-1.88) p=0.465 NS and 0.15 (0.07-0.32) p<0.001 respectively. The comparisons were not normalised for comparing the 400mg and 800mg dose, so the practical use of information about the almost 3-fold higher C<sub>max</sub> and 85% reduction in C<sub>trough</sub> are unclear. [6]

Ripamonti and colleagues presented what was perhaps a more pharmacologically useful study. This group switched 21 HIV-positive patients to twice-daily atazanavir (200mg without ritonavir-boosted) plus raltegravir (400mg twice-daily), due to either drug resistance or tolerability on their current regimen. [7]

PK results after at least 2 weeks on the new combination showed wide interpatient variability for parameters of both drugs. The geometric mean (95%CI) for atazanavir AUC<sub>0-12h</sub>, C<sub>max</sub> and C<sub>min</sub> were 6257 (4334-8172) ng\*h/mL, 1062 ng/mL (676-1448) ng/mL and 227 (122-332) ng/mL respectively. The geometric mean (95%CI) for raltegravir AUC<sub>0-12h</sub>, C<sub>max</sub> and C<sub>min</sub> were 9085 (6317-11,854) ng\*h/mL, 2402 ng/mL (1496-3308) ng/mL and 132 (1-263) ng/mL respectively. Five patients had atazanavir levels below the minimum target of 150 ng/mL. About 60% of patients entered the study with undetectable viral load, which was achieved by all patients two weeks after the switch, though these results need to show durability before and comment can be made about efficacy of the combination.

Of concern, the investigators concluded that this combination 'may' provide adequate plasma concentrations for 'some' patients. Clearly the only reliable way to identify those patients is through using TDM on an individual basis.

Drug interaction studies with atazanavir and non-HV drugs included an antimalarial study (see article above). [8]

## COMMENT

**While ritonavir boosting clearly improves atazanavir levels, the results from these studies indicate that for some patients, when supported by TDM, there may be an option to maintain viral suppression on an unboosted regimen.**

**The wide interpatient variability appears to protect some patients even when drug interactions are known to reduce therapeutic levels.**

**For other combinations, notably with raltegravir, and especially when using novel dosing, TDM seems essential.**

## References

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3. Regazzi M et al. Therapeutic monitoring and variability of atazanavir in experienced HIV-infected patients receiving boosted or unboosted regimens. Poster abstract P-35.
4. Guillemi S et al. A short trial of unboosted atazanavir in patients receiving atazanavir/ritonavir with high trough levels to select candidates for unboosted atazanavir maintenance therapy. Poster abstract P-46.

5. Harris M et al. Atazanavir trough levels in patients receiving unboosted atazanavir and tenofovir. Poster abstract P-21.
6. Molto J et al. Pharmacokinetics and safety of once-daily raltegravir (800mg) plus atazanavir (400mg) in HIV-infected patients. Oral abstract O-13.
7. Ripamonti D et al. Steady-state pharmacokinetics of atazanavir (200mg BID) when combined with raltegravir (400mg BID) in HIV-infected adults. Oral abstract O-14.
8. Van Luin M et al. Drug interactions between atovaquone/proguanil and antiretroviral agents. Oral poster O-19.

## A CYP2B6 haplotype influences nevirapine plasma concentrations following a single dose to reduce mother-to-child transmission

Polly Clayden, HIV i-Base

Tim Cressey from the Program for HIV Prevention and Treatment (PHPT), Chaing Mai, Thailand and Harvard School of Public Health, Boston, USA presented data from an evaluation of the association between single nucleotide polymorphisms (SNP) and haplotypes within CYP2B6, CYP3A4 and ABCB1, and nevirapine (NVP) plasma concentrations in Thai women following single dose NVP as part of HIV mother-to-child transmission prophylaxis. [1]

Currently, pregnant HIV-positive women that do not reach eligibility criteria for antiretroviral treatment in Thailand receive AZT from 28 weeks gestation and intrapartum single dose NVP to reduce mother-to-child transmission. Persistence of NVP in plasma following a single dose has been demonstrated to select for NNRTI mutations, which, in turn, can compromise subsequent NNRTI containing HAART.

In this study, investigators from Thailand and the USA, used plasma and DNA samples from 330 women who had received single dose NVP in the PHPT-2 trial. [2] Nine SNPs within CYP2B6, CYP3A4 and ABCB1 were genotyped using real time PCR. Data from 640 plasma samples taken between delivery and 21 days post partum were available.

Nevirapine plasma concentrations were determined by high-performance liquid chromatography and used in a population pharmacokinetic analysis.

For the CYP2B6 516G>T polymorphism, the investigators found, 43.0% (n=142), 46.7% (n=154) and 10.3% (n=34) of women had G/G, G/T and T/T genotypes, respectively. Nevirapine exposure was higher in women carrying the CYP2B6 516G>T polymorphism but this was not statistically significant, p=0.054.

Two tag-SNPs in CYP2B6: g.18492T>C and g.21563C>T, were significantly associated with NVP AUC, p=0.041 p=0.019 respectively.

The mean (SD) NVP AUC was 154.7 (33.7), 160.9 (33.3) and 17.7 (34.4) mcg.hr/mL in women with g.21563C/C C/T and T/T genotypes, respectively, p=0.27.

When they performed a haplotype analysis of CYP2B6 at 5 loci they found that the TGATC haplotype (g.3003T>C, 516G>T, 785A>G, g.18492T.C and g.21563C>T) was significantly associated with NVP AUC, p=0.00061.

The mean (SD) NVP AUC was 164.5 (33.8, n=197), 152.7 (33.9, n=114) and 146.1 (23.9, n=19) mcg.hr/mL for women with non-TGATC, TGATC-heterozygous and TGATC-homozygous genotypes respectively,  $p=0.0029$ .

The median time for NVP concentrations to reach 10 ng/mL postpartum was 18 (IQR 14-21) days, 16 (IQR 13-20) days and 14 (IQR 14-19) days for women with non-TGATC, TGATC-heterozygous and TGATC-homozygous genotypes respectively,  $p=0.02$ .

No other genetic polymorphisms evaluated in this analysis were significantly associated with NVP AUC.

CYP2B6 516G>T has previously been shown to affect NVP oral clearance during chronic treatment. The investigators observed that CYP2B6 516G>T seems to have a more modest impact on single dose NVP than on NVP used in chronic treatment. They suggest that the physiological changes experienced during pregnancy and/or the minimal autoinduction of CYP3A4 and 2B6 enzymes following a single dose compared to steady state (1.5 to 2-fold increase in NVP CL/F during first two weeks of treatment) may explain this observation.

They concluded that CYP2B6 polymorphisms following single dose NVP may account for some of the interpatient variability observed in post partum NVP concentrations but that the clinical significance of this finding may be relatively small.

#### Reference

Chantarangsu S et al. ACYP2B6 haplotype influences nevirapine plasma concentrations postpartum following a single intrapartum dose for the prevention of mother-to-child transmission of HIV in Thai women. 10th International Workshop on Clinical Pharmacology of HIV Therapy, 15-17 April 2009, Amsterdam. Abstract O\_02.

## Population pharmacokinetic model of nevirapine maternal to infant transfer through breastfeeding

### Polly Clayden, HIV i-Base

Edmund Capparelli from the University of California, San Diego, USA, presented a population pharmacokinetic (PK) model of nevirapine (NVP) concentrations in maternal plasma, breast milk and infant dried blood spots (DBS) to better characterise infant NVP exposure via breast milk.

This analysis used data collected in a previously published substudy of the Kisumu breastfeeding study in Kenya (in which pregnant women received NVP-containing HAART to prevent mother-to-child transmission via breastmilk), the substudy measured antiretroviral concentrations in maternal plasma, breastmilk and infant DBS in 67 mother and infant pairs. There were 153 paired plasma and breast milk samples and 191 DBS samples.

The investigators performed PK modelling using the NONMEM programme. They developed a semi-physiologic population model to describe maternal plasma and breast milk concentrations simultaneously. These were linked with infant feeding times in order to estimate breast milk NVP concentrations at the time of feeding. In turn these breast milk concentrations were used to estimate NVP doses for the infant PK model of DBS concentrations.

Liquid chromatography mass spectrometry was used to measure NVP concentrations in the samples. The limits of quantification of the assay were 17ng/mL for plasma and breast milk, 40ng/mL for DBS and 43ng/mL for NVP.

The analysis found maternal plasma NVP PK parameters were stable during the study period. Breast milk and plasma NVP concentrations reached equilibrium rapidly, relative to elimination, providing relatively stable breast milk: plasma ratio with breast milk concentrations above the IC50 throughout the dosing interval.

The investigators reported an overall population NVP breast milk: plasma of 0.74. The typical estimated PK parameters for infants were: CL/F 0.0265 (+/-0.003)L/h/kg and V/F 0.97 (+/-0.125) L/h/kg. CL/F among infants increased with age giving lower median DBS concentrations at 14 weeks (717ng/mL) compared to 2-6 weeks of age (1005ng/mL).

They concluded that infant NVP exposure via breastfeeding achieves prophylactic concentrations as seen in the first weeks of age with PMTCT dose (2mg/kg). They noted that, "NVP breast milk concentrations rapidly equilibrate with maternal system concentrations and while slightly lower than plasma were well in excess of therapeutic NVP concentrations". Also that the variability in both maternal and infant NVP elimination contributes more to infant exposure than NVP the breast milk: plasma ratio variability.

#### Reference

Capparelli et al. Population pharmacokinetic model of nevirapine (NVP) maternal to infant transfer through breastfeeding. 10th International Workshop on Clinical Pharmacology of HIV Therapy, 15-17 April 2009, Amsterdam. Abstract O\_18.

## Phenotypic and genotypic inhibitory quotients and virologic response in treatment experienced children

### Polly Clayden, HIV i-Base

Natella Rakhmanina from the children's National Medical Center, Infectious Diseases, Special Immunology and Pharmacology, Washington, showed findings from a study to investigate whether the lopinavir (LPV) phenotypic inhibitory quotient (PIQ) and genotypic inhibitory quotient (GIQ) in treatment experienced children correlate with treatment response, when receiving LPV containing HAART, as observed in treatment experienced adults.

In this study the investigators collected 52 weeks prospective data from children and adolescents aged 4-15 years receiving LPV/r as single PI within antiretroviral regimens. 12-hour pharmacokinetic (PK) samples were collected and LPV susceptibility measured within 3 months of enrollment. Treatment histories, including resistance information, were obtained from medical records. Viral load and self reported adherence were measured 3 monthly.

IQ was calculated as the rate of plasma 12-hour trough concentration ( $C_{min}$ ) after observed dose divided by the protein-adjusted IC50 for PIQ and the number of LPV-associated mutations for GIQ.

In this analysis, 45 PI experienced children and adolescents were followed for 52 weeks. Their median age was 11 (5.3-17.8) years; 24 were girls and the majority (n=41) were African American. Of the group 40 (89%) received background regimens of 2 NRTIs, 2 received 3 NRTIs and 3 received NRTI plus NNRTI.

The median length of PI experience was 5.2 (0.7-9.2) years and of previous LPV exposure was 2.2 (0.5-5.0) years. Self reported adherence was a mean of 88% (41-100%). About half, 24/45(53%), of the patients achieved viral load, 400 copies/mL, at least once during the study. The median LPV Cmin was 6.2 (0.1- 16.7) mg/L.

Median PIQ (n=36) was 12.6 (0.03-231.1). The investigators noted that a baseline PIQ cutoff of 15 (as in adults) did not distinguish those achieving a viral load of <400 copies/mL from those that did not, p=0.09.

In multivariate analysis, only baseline PIQ >25 was significantly associated with viral load <400 copies/mL: 11/16 (69%) patients with PIQ >25 achieved viral load <400 copies/mL vs 5/20 (20%) with PIQ <25, p=0.01.

The geometric mean PIQ in those patients achieving viral load <400 copies/mL was 16.7 vs 2.4 in those who did not, p=0.09.

The investigators found for every increase in baseline PIQ of 10, the probability of achieving viral load <400 copies/ml, when adjusted for prior duration of LPV treatment, increased 9.6-fold (95% CI 9.2-9.9), p=0.02.

They reported a median GIQ (n=22) of 1.0 (0.03-6.5) and a median of 6 (1-13) LPV mutations per patient. The geometric mean GIQ in those achieving viral load <400 copies/mL was 1.0 vs 0.7 in those who did not, p=0.56.

The investigators concluded that LPV PIQ was associated with viral load <400 copies in PI experienced HIV-positive children and adolescents but GIQ was not. They suggest that a cutoff of LPV PIQ >25 may be a target for maximising efficacy.

#### Reference

Rakhmanina N et al. The phenotypic and genotypic susceptibility lopinavir scores and virologic response in treatment experienced children with HIV. 10th International Workshop on Clinical Pharmacology of HIV Therapy, 15-17 April 2009, Amsterdam. Abstract 0\_17.

## Tenofovir pharmacokinetics in three tenofovir-containing regimens in children and adolescents

### Polly Clayden, HIV i-Base

J King and coworkers reported pharmacokinetic (PK) parameters of tenofovir (TDF) tablets received in combination with efavirenz (EFV) or duranavir/ritonavir (DRV/r) or atazanavir/ritonavir (ATV/r) in a group of children and adolescents age 8-18 years.

This study enrolled patients receiving  $\geq 2$  weeks TDF 300mg in combination with:

Arm 1: EFV 300mg or 600mg once-daily

Arm 2: DRV/ritonavir dosed at 300/100 or 600/100 twice-daily

Arm 3: ATV/ritonavir dosed at 200-400mg/100mg twice-daily

Plasma samples were taken at 0, 1, 2, 4, 6, 8, 12 and 24 hours post dose and plasma concentrations of TDF were measured.

The investigators performed statistical tests to evaluate whether the 90% confidence intervals of the geometric mean (GM) for the PK parameters of TDF were within the target range ie 0.5-2.0 fold of adult values: 2.8 (2.3-3.6) mgxh/L and 0.06 (0.05-0.08) mg/L AUC and Cmin respectively.

They found that among patients receiving EFV (n=15) the TDF GM (90% CI) AUC and Cmin were 2.9 (2.4-3.4) mg.h/L and 0.07 (0.05-0.09) mg/L respectively. AUC and Cmin were 3.1 (2.4-4.0) mg.h/L and 0.07 (0.05-0.09) mg/L in patients receiving DRV/r (n=10) and 3.6 (3.0-4.5) mg.h/L and 0.07 (0.06-0.10) mg/L respectively in patients receiving ATZ/r (n=17).

They noted that the TDF Cmin 90% CI included values above the target upper limit of 0.08 mg/L in all three arms and the AUC 90% CI included values above the upper target limit of 3.6 mg.h/L in patients receiving DRV/r or ATV/r.

The investigators concluded: "These data suggest that DRV/r and ATV/r may increase TDF exposure in HIV-infected children and adolescents."

#### Reference

King J et al. A comparison of tenofovir pharmacokinetics across three tenofovir-based antiretroviral regimens in HIV-infected children and adolescents. 10th International Workshop on Clinical Pharmacology of HIV Therapy, 15-17 April 2009, Amsterdam. Abstract P\_53

## Bioavailability of Thai generic lopinavir/ritonavir

### Polly Clayden, HIV i-Base

A poster authored by J van der Lugt and coworkers from Thailand and the Netherlands described pharmacokinetic (PK) data and short-term safety of a generic lopinavir/ritonavir (LPV/r) 200/50mg formulation tablet. [1]

In this study, patients receiving PI based therapy with viral load <50 copies/mL were switched to generic LPV/r 400/100mg twice daily. Trough concentrations (Cmin) were measured prior to the switch in 16 patients receiving Kaletra and 4 weeks after the switch in all patients.

Plasma levels of LPV and RTV were measured using high performance liquid chromatography with a lower limit of quantification of 0.1mg/L for LPV and 0.045mg/L for RTV.

A group of 37 patients were evaluated in this study; their mean (SD) weight was 60.3 (11.8) kg and 18 were women. Two patients discontinued the study medications due to intolerance.

The investigators reported the mean (SD) Cmin of LPV was 7.3 (1.8) mg/mL. None of the patients evaluated had subtherapeutic levels. They found no difference in LPV Cmin in patients receiving Kaletra before switching to the generic formulation of LPV/r, p=0.21. However, the Cmin of the generic RTV was higher than that reported for Kaletra, p=0.019. They found the coefficient of variation was 25% for the tablet formulation and 54% for the Kaletra. They noted that these values did not appear to be affected by food intake.

They concluded: "These data support the efforts in scaling

10th Intl PK, Amsterdam

up access to generic second line treatment in middle and low income countries.”

COMMENT

There are currently limited protease inhibitors available for second line treatment in low and middle-income countries. Although originator LPV/r (Kaletra) is the most common protease inhibitor in industrialised countries, generic LPV/r is not widely used in resource limited settings as there have been concerns about the quality (including studies by the originator company) and limited data. [2]

These data are reassuring, as is the FDA tentative approval in March this year of Indian generic versions of LPV/r manufactured by Aurobindo and Matrix Laboratories. [3]

Studies of a paediatric “sprinkle” formulation from Cipla are underway.

References

1. Van der Lugt J et al. Bioavailability of generic lopinavir/ritonavir in HIV-1 infected individuals. 10th International Workshop on Clinical Pharmacology of HIV Therapy, 15-17 April 2009, Amsterdam. Abstract P\_41.
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<http://www.fda.gov/InternationalPrograms/FDABeyondOurBordersForeignOffices/AsiaandAfrica/ucm119231.htm>

TREATMENT ACCESS

TMC207 open label trial for MDR-TB

Nathan Geffen, TAC

Tibotec are recruiting for an open label trial called TMC207-TiDP13-C209. Its purpose is to evaluate the safety, tolerability, and efficacy of TMC207 as part of an individualised treatment regimen in patients with sputum smear-positive pulmonary MDR-TB.

Several groups have met with Tibotec to ask the company to facilitate greater access to TMC207 for drug-resistant TB patients with limited options while clinical trial testing of the drug continues (see HTB-South April-June 2009). Tibotec has presented this trial as a first step towards this. The company says it wants to closely monitor safety and tolerability while the compound is still under phase 2 evaluation.

Three sites have opened in South Africa (see Table 1 for details).

Table 1: TMC207-TiDP13-C209 contact details

Site	Lead investigators	Contact Details
King George V Hospital, Durban	Dr Alex Pym Ms Nonkqubela Bantubani  Unit for Clinical and Biomedical TB Research, MRC, Durban	0765249773 0763720870
Sizwe Hospital, Johannesburg	Prof Martin P. Grobusch  Infectious Diseases Unit National Health Laboratory Service	011 489 8537
Brooklyn Chest Hospital, Cape Town	Prof Andreas Diacon Dr Ramonde Patietia Dr Zoja Novelijc  Task Applied Science Head office Karl Bremer Hospital and Trial Unit at Brooklyn Chest Hospital	021 949 7751 021 508 7409

The trial currently provides for 225 patients to be enrolled in sites in Brazil, Estonia, Hong Kong, Kenya, South Korea, Latvia, Peru, Philippines, Thailand, Turkey and Ukraine and South Africa. XDR TB patients can participate in this trial. Currently the criteria exclude patients on HAART (because of potential interactions) and sputum-negative patients. Negotiations are proceeding with Tibotec to review these limitations and to increase the size of the trial.

Patients who participate in the trial will have to attend many obligatory trial visits at the same study centre at one of these locations over a two-year period. Strict adherence to the drug regimen is a requirement.

Reference

Tibotec. TMC207-TiDP13-C209 trial to evaluate the safety, tolerability, and efficacy of TMC207 as part of an individualized multi-drug resistant tuberculosis (MDR-TB) treatment regimen in patients with sputum smear-positive pulmonary MDR-TB.  
<http://clinicaltrials.gov/ct2/show/NCT00910871>

(Thanks to Helen Cox, Andreas Diacon, Alex Pym and Martin Grobusch for additional information.)

**FDA approval of generic ARVs**

Since the last issue of HTB, the US Food and Drug Administration (FDA) has granted tentative approval for the following new generic ARV products.

Drug and formulation	Manufacturer, Country	Approval date
AZT/3TC tablets 300 mg/150 mg tablets	Macleods, India	29 May 2009
3TC 150 mg and 300mg	Matrix, India	22 June 2009
Fixed dose AZT/3TC/ abacavir 300 mg/150 mg/300 mg, tablets	Matrix, India	15 July 2009
Fixed dose d4T/3TC/ nevirapine tablets: 40mg/150mg/200mg & 30mg/150mg/200mg	Emcure, India	16 July 2009
3TC/AZT, 30mg/60mg scored tablets, for pediatric use	Aurobindo, India	23 July 2009
AZT 60 mg scored tablets for pediatric use	Aurobindo, India	23 July 2009
Efavirenz 200mg capsules	Cipla, India	3 August 2009
Lamivudine 150mg/ 300mg tablets	Strides, India	6 August 2009
Efavirenz / Emtricitabine / Tenofovir Disoproxil Fumarate 600mg/200mg/ 300mg	Matrix, India	12 August 2009
Efavirenz, Lamivudine, Tenofovir Disoproxil Fumarate tablets 600mg/ 300mg/300mg	Matrix	3 September 2009

“Tentative Approval” means that FDA has concluded that a drug product has met all required quality, safety and efficacy standards, but because of existing patents and/or exclusivity rights, it cannot yet be marketed in the United States. Tentative approval does, however make the product eligible for consideration for purchase under the PEPFAR programme for use outside the United States.

Effective patent dates are listed in the agency’s publication titled Approved Drug Products with Therapeutic Equivalence Evaluations, also known as the Orange Book:

<http://www.accessdata.fda.gov/scripts/cder/ob/default.cfm>

COMMENT

**This brings the total of FDA approved generic drugs and formulations to 101 since the programme started.**

Since the last HTB South, the FDA has approved two new 1-pill-a-day generic products, both from Matrix. These are tenofovir/FTC/efavirenz (equivalent to Atripla) and tenofovir/3TC/efavirenz. The Lesotho treatment programme already uses one of these. According to the Clinton Foundation HIV/AIDS Initiative, Matrix will sell tenofovir/FTC/efavirenz at \$19.92 per patient per month and tenofovir/3TC/efavirenz at \$17.50 per patient per month.

Before these products can be used in the South African public health system, they need to be registered by the Medicines Control Council and provided for in the public sector tender. These are not insurmountable obstacles and the Department of Health should prioritise making them available.

References

1. FDA. What’s new at FDA in HIV/AIDS.  
<http://www.fda.gov/ForConsumers/ByAudience/ForPatientAdvocates/HIVandAIDSActivities/ucm122951.htm>
2. FDA. President’s Emergency Plan for AIDS Relief: Approved and tentatively approved antiretrovirals in association with the President’s Emergency Plan.  
<http://www.fda.gov/InternationalPrograms/FDABeyondOurBordersForeignOffices/AsiaandAfrica/ucm119231.htm>
3. William J Clinton Foundation. Q&A on new pricing agreements.  
<http://www.clintonfoundation.org/news/news-media/q-a-on-new-pricing-agreements>

**AIDS medicine prices in the South African public and private sectors**

There were minor errors in the antiretroviral pricing data for South Africa in the previous issue, which we have corrected this time. If there are any errors in, or concerns about, the data provided here please let us know.

**Private sector**

The TAC updates its website weekly with the latest private sector prices, supplied by Medprax, of a range of AIDS drugs. See:

<http://www.tac.org.za/community/privatesectorprices>

The lowest prices for certain drugs and drug combinations as of 31 August 2009 are shown in Table 1.

\* BMS sells 400mg enteric-coated didanosine pills branded as Videx. The 400mg version costs R240.20 for 30 pills.

These medicines are particularly expensive:

- A 500mg vial of ganciclovir costs R2558.25 (manufacturer Roche)
- 60 raltegravir 400MG tablets costs R2396.44 (manufacturer MSD)
- 120 darunavir 300MG tablets cost R1034.21 (manufacturer/distributor Tibotec and Aspen). Please note that darunavir is still awaiting registration in South Africa and is only available via Section 21 authorisation (in the previous issue, we erroneously said that darunavir was registered).

Table 1: Lowest private sector prices for ARVs and combinations (Aug 09)

Medicines (based on one month for average adult unless otherwise stated)	Lowest price brands (manufacturer)	Price (incl. VAT but excl. dispensing fee)
<b>1st line DOH guidelines with nevirapine</b>		
Stavudine 30mg BID	Vari-Stavudine (Lebasi)	R31.27
Lamivudine 150mg BID	Sonke-Lamivudine (Sonke)	R44.40
Nevirapine 200mg BID	Sonke-Nevirapine (Sonke)	R171
<b>TOTAL</b>		<b>R246.67</b>
<b>1st line DOH guidelines with efavirenz</b>		
Stavudine 30mg BID	Vari-Stavudine (Lebasi)	R31.27
Lamivudine 150mg BID	Sonke-Lamivudine (Sonke)	R44.40
Efavirenz 600mg QD	Sonke-Efavirenz (Sonke)	R134.52
<b>TOTAL</b>		<b>R210.19</b>
<b>1st line DOH guidelines combination</b>		
Lamivudine/stavudine/nevirapine BID	Sonke Laminevstav (Sonke)	R231.18
Lamivudine/stavudine/nevirapine BID	Triomune 30 (Cipla)	R284.49
<b>1st line US recommendation</b>		
Tenofovir 245mg FTC 200mg QD	Truvada (Gilead)	R397.71
Efavirenz 600mg QD	Sonke-Efavirenz (Sonke)	R134.52
<b>TOTAL</b>		<b>R532.23</b>
<b>2nd Line DOH guidelines</b>		
AZT 300mg BID	Sonke-Zidovudine (Sonke)	R228
* Didanosine 100mg x 2 BID	Sonke-Didanosine (Sonke)	R220.62
Lopinavir 200mg ritonavir 50mg x2 BID	Kaletra (Abbott)	R406.80
<b>TOTAL</b>		<b>R855.42</b>
Fluconazole 200mg x 30 capsules	Apex Fluconazole (Camox)	R256.94
Atorvastatin 40mg x 30 capsules	Aspavor (Pharmacia)	R169.08
Pravastatin 40mg (or 80mg) x 30	Colite (Zydus)	R190.17

## Public Sector

Antiretrovirals are purchased by the state sector on tender. The tender is developed and finalised by the National Department of Health. The drugs are paid for out of the provincial conditional grants for AIDS. Each province is responsible for ordering and payment to the distributors. Table 2 summarises the current tender for the period 1 June 2008 to 31 May 2010.

For each product, the dose, company, volume, price per unit, percentage of the tender that particular brand has and the total cost over the two-year period are given. The last column is a rough estimate of the number of people per month catered for by the tender. The tender documents are available at:

<http://www.treasury.gov.za/divisions/sf/ostb/contracts/m/RT71-2008MF.zip>

The tender volumes are Department of Health estimates. The volumes that have actually been ordered for several drugs are lower than they should be at this point in the tender period.

Furthermore, ordering has been erratic. This has likely had and will continue to have negative consequences, with patients kept longer on waiting lists, threats of moratoriums, stock-outs etc.

Tenofovir and paediatric abacavir are provided for in the tender but they are not provided for by the 2004 guidelines, which are the current ones. New guidelines have long since been drafted and apparently include both tenofovir and abacavir.

Several experts and organisations concerned about the financing of the public sector HAART and PMTCT rollouts met in Johannesburg on 21 August to form the Budget and Expenditure Monitoring Forum. The meeting was organised by the AIDS Law Project. One of the Forum's objectives is to get the provincial and national health departments and treasuries to ensure that procurement is managed properly. A report from this meeting is/will be available at:

[http://www.alp.org.za/index.php?option=com\\_content&task=view&id=88&Itemid=1](http://www.alp.org.za/index.php?option=com_content&task=view&id=88&Itemid=1)

(Thanks to Andy Gray for additional information.)

Table 2: Current tender for the period 1 June 2008 to 31 May 2010.

Item	Dose	Company	Volume	Price per unit	% of tender	Total Cost	No. People
ABC	240ml	GSK	1,747,000	134	100%	234,849,210	
ABC	300mgx60	GSK	43,000	320	100%	13,765,590	
ddl	25mgx60	Sonke	37,000	54	100%	2,003,550	
ddl	50mgx60	Sonke	26,000	56	100%	1,464,580	
ddl	100mgx60	Sonke	683,000	68	100%	46,327,890	14,229
EFV	200mgx90	MSD	1,104,000	297	100%	328,130,880	
EFV	600mgx30	Adcock	7,000,000	108	70%	756,210,000	291,667
EFV	600mgx30	Aspen	3,000,000	116	30%	347,880,000	125,000
3TC	240ml	Aspen	3,138,000	21	100%	67,184,580	
3TC	150mgx60	Aspen	9,735,200	30	80%	290,887,776	405,633
3TC	150mgx60	Sonke	2,433,800	30	20%	72,794,958	101,408
PEP Starter	3TCx6,AZTx18	GSK	15,000	57	100%	855,000	
3TC/AZT	150mg+300mgx60	Aspen	20,000	92	100%	1,835,800	
3TC	300mgx30	Cipla	1,601,000	43	100%	68,042,500	66,708
Lopinavir/Rit	5x60ml bottle	Abbott	1,066,000	319	100%	340,128,620	
Lop/Rit	133.3mg&33.3mgx2x90	Abbott	256,000	319	100%	81,681,920	
Lop/Rit	200mg&50mgx120 HS	Abbott	617,000	319	100%	196,823,000	25,708
NVP	20ml	Cipla	10,000	13	100%	128,000	
NVP	240ml	Aspen	45,000	36	100%	1,633,500	
NVP	200mg	Aspen	8,801,000	32	100%	282,600,110	366,708
Ritonavir	90ml	Abbott	50,000	64	100%	3,186,500	
Ritonavir	100mgx84	Abbott	200,000	74	100%	14,888,000	
d4T	15mgx60	Aspen	489,000	17	100%	8,288,550	
d4T	20mgx60	Aspen	770,000	17	100%	13,051,500	
d4T	30mgx60	Aspen	8,728,000	17	80%	147,939,600	363,667
d4T	30mgx60	Sonke	2,182,000	18	20%	38,512,300	90,917
TDF	300mgx30	Aspen	3,687,000	159	100%	588,039,630	153,625
AZT	20ml	Aspen	731,000	13	100%	9,393,350	
AZT	200ml	Aspen	200,000	23	100%	4,530,000	
AZT	100mgx100	Aspen	70,000	71	100%	4,957,400	
AZT	300mgx60	Aspen	4,000,000	71	100%	284,360,000	166,667
Total						4,252,374,294	

## BASIC SCIENCE

Recent basic science updates from Richard Jefferys excellent web log.

### Immune surveillance below the radar: study offers explanation for acyclovir's failure to reduce HIV risk

Richard Jeffereys, TAG

In a recent post on the Merck vaccine trial, I mentioned a new study from Larry Corey's research group addressing the relationship between HSV-2 infection and enhanced susceptibility to HIV. The data were presented by Dr. Corey at the Keystone conference in March and were published online by Nature Medicine yesterday. The background to the work is that HSV-2 infection has been consistently associated with a 2 to 3-fold increased risk of acquiring HIV; a meta-analysis published in 2006 reported a relative risk of 3.1, 2.7 and 1.7 for women, heterosexual men and men who have sex with men, respectively. Recently analysed data from the Merck vaccine trial are consistent with these findings in that HSV-2-infected participants were found to have approximately double the risk of acquiring HIV infection during the study.

The mechanism by which HSV-2 infection increases HIV acquisition risk is not so clear, however, and has been the subject of debate. The general view is that local inflammation and damage to the integrity of the genital mucosa are plausible ways that HSV-2 infection may increase the chances of HIV transmission. This view led to the logical proposition that suppressing HSV-2 with chronic acyclovir treatment might be a means to also reduce the risk of HIV acquisition. Several large trials have now explored this hypothesis and, while acyclovir treatment was effective at reducing symptomatic HSV-2 reactivations, it did not reduce the incidence of HIV infection.

The new study from Larry Corey's laboratory set out to try and shed light on these trial results. The same group of researchers has previously shown that immune surveillance of HSV-2 is a far more active process than many had surmised. In a 2007 paper in the Journal of Experimental Medicine, they demonstrated that HSV-2-specific CD8+ T cells gather at sites of subclinical HSV-2 reactivation in the genital skin and persist for at least several months after HSV-2 DNA is no longer detectable. A year later in the Journal of Infectious Diseases, they published results of an intensive study in which participants took oral and anogenital swabs four times a day for 60 days. Analysing the swabs for HSV-2 DNA, the researchers found that subclinical reactivations were frequent and typically lasted less than 12 hours, showing that there is an ongoing and dynamic effort on the part of the immune system to keep HSV-2 suppressed.

These findings led the researchers to suspect that perhaps elevated levels of activated HSV-2-specific CD4 T cells would be present in the genital mucosa even during chronic acyclovir treatment, and this is exactly what they report in the Nature Medicine paper. The study initially took biopsies of genital skin during an acute, clinically symptomatic lesion and 2, 4 and 8 weeks after healing. Four participants subsequently initiated

chronic suppressive acyclovir treatment at the start of their next symptomatic episode and had biopsies taken at 2, 4, 8, 16 & 20 weeks after healing. In all cases, control biopsies were obtained from an unaffected area of genital skin at each timepoint.

During an acute HSV-2 lesion, the researchers found a "massive localized infiltration" of cells. Mean numbers of CD4 and CD8 T cells per mm<sup>2</sup> of skin were 655 and 618, respectively, compared to 68 and 55 per mm<sup>2</sup> of the unaffected skin sample. Although follow-up biopsies documented gradual clearance of HSV-2 and a decline in inflammation, elevated numbers of CD4 and CD8 T cells remained present locally for months. Eight weeks after healing, a median of 8-fold more CD4 T cells were present at the affected versus the unaffected site. Furthermore, even after 20 weeks of acyclovir treatment, the number of CD4 T cells remained significantly elevated. Additional analyses illustrated that the majority of these CD4 T cells expressed CCR5 and their presence was also associated with a significant increase in the numbers of DC-SIGN-expressing dendritic cells. The enrichment of CCR5-expressing CD4 T cells and DC-SIGN-expressing dendritic cells at the former lesion site was not significantly altered by chronic acyclovir treatment.

The researchers acknowledge in the conclusion to the paper that the number of participants was small, but nevertheless state: "we feel that our central finding – that HSV reactivation leaves a residual inflammatory response not appreciated clinically – is typical of HSV-2 genital lesions." They also note that "the wide anatomical distribution of HSV-2 in the male and female genital tract underscores the importance that these localized reservoirs of inflammatory cells are likely to have in HIV acquisition."

Source: TAG basic science project (3 Aug 2009).

<http://tagbasicscienceproject.typepad.com/>

### Maximum suppression: ART intensification does not reduce residual viral load

Richard Jefferys, TAG

One of the most controversial questions in the field of HIV research is whether current antiretroviral therapy (ART) combinations maximally suppress viral replication. New technologies have allowed researchers to detect down to just 1 copy of HIV RNA per mL of blood and, even when the viral load is undetectable on commercially available tests (which typically can only detect 50 copies or more), most people on ART have a few copies of HIV RNA detectable in their samples. These HIV RNA copies could either be the product of ongoing rounds of viral replication (in which infected cells release new viruses that go on to infect other cells), or alternatively they could be produced by long-lived chronically infected cells. In the latter scenario, ART would prevent the newly-produced virus from being able to infect any other cells, but the drugs would not be able to eliminate the chronically infected cell.

Over the past few years, scientists have debated – often quite heatedly – which of these possibilities is true. Recent evidence has generally favored the view that, in most cases, ART is maximally suppressing HIV replication; for example, a study of viral evolution in people undergoing intermittent ART interruption found no evidence of ongoing viral evolution during the periods when participants were on therapy.

A study just published in PNAS tackles the question another way, by investigating whether intensifying ART by adding new drugs has an effect on residual viral load. [1]

Out of 15 total participants, only 9 consistently showed HIV RNA levels above 1 copy/mL prior to ART intensification (median 3 copies/mL). The highest level detected was around 30 copies/mL. The researchers found that ART intensification had no effect on these residual viral levels, indicating a lack of ongoing HIV replication. The results add to the evidence that low-level HIV RNA detectable in people on ART does not derive from ongoing viral replication, but rather a stable reservoir of infected cells. The major implication is that, in order to cure HIV infection, new strategies are needed to identify and eliminate this reservoir. PNAS has designated the paper "Open Access," click on the title link for the PDF.

References:

1. Dinoso JB et al. Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. PNAS. Published online 22 May 2009, doi: 10.1073/pnas.0903107106.  
<http://www.pnas.org/content/early/2009/05/22/0903107106.full.pdf+html>

## The role of Ad5-specific CD4 T-cells in enhancing risk of HIV acquisition in the Merck vaccine trial

**Richard Jefferys, TAG**

In September 2007 it was announced that immunizations were being halted in the STEP study, a phase IIb efficacy trial of Merck's HIV vaccine candidate. A review of the interim results by the Data Safety Monitoring Board found that there was no possibility of the vaccine showing efficacy for preventing HIV infection, or reducing post-infection viral load levels in vaccine recipients who became infected. These events and the subsequent fallout were covered in grisly detail on this blog.

The most surprising and disturbing finding from STEP was that receipt of the vaccine was associated with a significantly increased risk of HIV acquisition in a subset of trial participants: uncircumcised men with pre-existing antibodies against adenovirus serotype 5 (Ad5). The Merck vaccine construct used an attenuated Ad5 virus vector as a delivery vehicle for the HIV antigens Gag, Pol & Nef. In its natural form, Ad5 causes severe colds and many people are exposed during childhood and hence have anti-Ad5 immune responses.

When the data from STEP showing enhanced risk of acquisition became known, there was understandably much discussion of what the potential mechanism might be. One hypothesis posited that the presence of anti-Ad5 antibodies at baseline was a marker for the presence of Ad5-specific CD4 T cell responses, and immunization with the Ad5 vector activated Ad5-specific CD4 T cell responses, thereby increasing the number of potential target cells for HIV infection in vaccine recipients. This hypothesis was explored to limited extent in one of the papers presenting the STEP results that was published in *The Lancet* last year by Juliana McElrath and colleagues; in that paper, analyses of Ad5-specific CD4 T cells showed that responses tended to be

lower in vaccine recipients who became HIV infected, at least in peripheral blood.

Two new studies just published online in *Nature Medicine* now offer a more detailed absolution of Ad5-specific CD4 T cells. [1]

The results show that baseline antibody titres did not correlate with the magnitude of the Ad5-specific CD4 T cell response (as measured by production of IL-2, interferon gamma, TNF-alpha, MIP-1beta and perforin - Th2-type cytokine production by Ad5-specific CD4 T cells has not yet been evaluated). Furthermore, most individuals who lacked anti-Ad5 antibodies nevertheless displayed Ad5-specific CD4 T cell responses. The studies also demonstrate that receipt of the vaccine rapidly induced both Ad5-specific CD4 T cells and antibodies in people who lacked them at baseline, suggesting that if these responses enhanced the risk of HIV infection then the enhancement should have been seen in all vaccine recipients after the initial immunisations, not just the subset with detectable anti-Ad5 antibodies at baseline.

While these papers make an important contribution to the analyses of what occurred in STEP, there are some caveats. Most critically – and contrary to what has been written in one media story on *The Scientist* website [2] – the new results do not absolve the Merck HIV vaccine of significantly enhancing the risk of HIV acquisition among uncircumcised men with pre-existing antibodies against Ad5. It is disheartening to read quotes from Alan Bernstein, Executive Director of the Global HIV/AIDS Vaccine Enterprise, erroneously stating otherwise. Also in *The Scientist* article, Nelson Michael is quoted as saying that including circumcision status as a variable in the multivariate analyses of STEP "washes out" the enhancing effect of vaccination. Based on Susan Buchbinder's talk at Keystone earlier this year, this is simply not true; what the circumcision data show is that the enhancement effect was most significant in the uncircumcised subgroup with anti-Ad5 antibodies. Additionally, Buchbinder noted that continued follow-up of STEP participants indicates the enhancement effect has waned over time, which adds to the evidence that receipt of the Ad5 vector was responsible.

Another more speculative caveat is that the new data may not entirely rule out a role for Ad5-specific CD4 T cell responses in the trial outcome. One possibility that remains to be studied is whether the presence of persistent Ad5 infection alters the behaviour of Ad5-specific CD4 T cell responses after immunization (i.e. if natural Ad5 antigens are being expressed somewhere in the body, Ad5-specific CD4 T cells would be expected to traffic to those sites). Recent research from Linda Gooding's group at Emory (abstracts and links appended at the end of the post) has employed PCR to confirm that Ad5 infection can persist in humans. The main cell type infected by Ad5 in these studies was T cells, and activation of infected T cells stimulated Ad5 replication. In the context of the STEP results, these data suggest several questions:

- Can persistent Ad5 infection be detected in the foreskin?
- Is there any correlation between Ad5 serostatus and detection of persistent Ad5 infection?
- Does immunisation with an Ad5 vector lead to any detectable changes in the interactions between Ad5-specific CD4 T cells and Ad5-infected cells?

Juliana McElrath's *Lancet* paper cites the need to consider events in the mucosa, stating that Ad5-specific CD4 T cells "could have

trafficked to mucosal sites—a process known to occur in natural infection—and thus increased the number of susceptible CD4+ T-cell targets for HIV... To address this possibility, studies are planned to examine lower gastrointestinal tissue and foreskin after immunization for enhanced T-cell activation.” This idea is not unprecedented, as studies from Larry Corey’s laboratory have strongly implicated the persistent presence of activated HSV-2-specific CD4 T cells interacting with HSV-2-infected cells in the mucosa as the explanation for the association between HSV-2 infection and increased susceptibility to HIV acquisition. At the Keystone conference earlier this year, Corey showed that these interactions continue to be detectable even when HSV-2-infected individuals are on chronic suppressive therapy with acyclovir, offering a reason for the failure of the drug to reduce the risk of HIV infection in several large trials.

There is one other slightly uncomfortable caveat to the Nature Medicine papers: both groups of researchers include people working on vaccines using alternative adenovirus serotypes. If evidence did suggest that Ad5-specific CD4 T cells played a role in enhancing risk of HIV acquisition in STEP, this could potentially impact their work because Ad5-specific T cell responses have been shown to cross-react with multiple adenovirus serotypes. Because the alternative adenovirus-based vaccines are being developed for neglected diseases that do not represent profitable vaccine markets, it’s not a case of suspecting significant financial conflicts-of-interest, but the issue should perhaps have been acknowledged in the papers.

Source: TAG basic science project (22 Jul 2009).

<http://tagbasicscienceproject.typepad.com>

References:

1. Hutnick NA et al. Baseline Ad5 serostatus does not predict Ad5 HIV vaccine-induced expansion of adenovirus-specific CD4+ T cells. *Nature Medicine*. Brief Communication abstract. Published online: 20 July 2009 | doi:10.1038/nm.1989. <http://www.nature.com/nm/journal/vaop/ncurrent/abs/nm.1989.html>
2. <http://www.the-scientist.com/blog/display/55828>

## Tracing HIV reservoirs

**Richard Jefferys, TAG**

Two new papers offer differing perspectives on the reservoirs of HIV that persist despite effective antiretroviral therapy.

Nicolas Chomont and colleagues demonstrate that when memory CD4 T cells containing integrated HIV proliferate (as most memory CD4 T cells do occasionally in a process known as homeostatic self-renewal), they copy the HIV provirus along with their own genomes. [1]

When CD4 T cell numbers decline, homeostatic proliferation occurs more frequently and Chomont’s paper shows that this is associated with an increase in the number of latently infected memory CD4 T cells. The researchers describe the cells that undergo more frequent proliferation in this setting as “transitional memory” T cells. At earlier stages of infection when the CD4 T cell pool is relatively intact, the reservoir of infected memory CD4 T cells is found to be far smaller and integrated virus is primarily located in “central memory” cells that divide less frequently.

Based on these findings, the study authors suggest that anticancer drugs that interfere with memory T cell proliferation

should be studied for their potential to deplete the HIV reservoir. However, given the potential toxicities associated with inhibiting T cell proliferation, the risk/benefit of such trials would need to be carefully evaluated. A more ideal therapy would be one that only targeted dividing CD4 T cells containing HIV DNA, but it is currently unclear whether such an approach is within the realm of possibility.

The second paper - by Timothy Brennan and colleagues from Bob Siliciano’s laboratory - uses genetic analyses of HIV sequences to show that there is a reservoir of virus that seems to be coming from a cell type other than memory CD4 T cells. [2]

The study finds that in most cases, the residual virus detectable in individuals on suppressive ART is genetically distinct from the virus found in memory CD4 T cells. The authors note in their conclusion: “Numerous laboratories are actively pursuing various eradication strategies, most of which involve some aspect of targeting and purging the latent reservoir in resting memory CD4+ T cells. If much of the residual viremia of patients undergoing HAART comes from another reservoir or compartment as suggested here, then eradication strategies will have to include ways to target and purge this additional reservoir to be successful.”

Source: TAG basic science project (24 Jun 2009).

References

1. Chomont N et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nature Medicine*. Published online: 21 June 2009 | doi:10.1038/nm.1972 <http://www.nature.com/nm/journal/vaop/ncurrent/abs/nm.1972.html>
2. Brennan TP et al. Analysis of HIV-1 Viremia and Provirus in Resting CD4+ T Cells Reveals a Novel Source of Residual Viremia in Patients on Antiretroviral Therapy. *JVI Accepts*, published online ahead of print on 17 June 2009. *J. Virol.* doi:10.1128/JVI.02568-08 <http://jvi.asm.org/cgi/content/abstract/JVI.02568-08v1>

## Illuminating early events in HIV infection using single genome amplification

**Richard Jefferys, TAG**

Two papers in the current *Journal of Experimental Medicine* offer unprecedented insight into the initial interactions between virus and host after HIV infection. An accompanying commentary by Zabrina Brumme and Bruce Walker eloquently articulates what these studies have achieved: “By identifying persons before seroconversion, pinpointing the transmitted virus, and assessing immune responses to that particular variant as it evolves, they provide a novel view of host and viral dynamics during the earliest stages of infection.” [1]

In both studies the researchers use an optimised version of a technique called Single Genome Amplification (SGA), originally developed by Sarah Palmer and colleagues at the National Cancer Institute. While costly and labour-intensive, this technique allows sequencing of the HIV genome without many of the potential confounding errors that can occur with standard PCR. The researchers also used the sequences obtained by SGA to synthesise peptides for CD8 T cell response assays; this allowed detailed tracking of the impact of CD8 T cell responses on the virus genome.

The study results echo prior work from these groups suggesting that most HIV transmission events involve a single isolate; in 11

out of 12 cases SGA showed that all detected sequences were related to a single infecting virus. The remaining individual was infected with two viruses that could be unambiguously identified based on their sequences. In terms of viral evolution after infection, the researchers found that between transmission and peak viraemia, diversification of HIV sequences was essentially random and showed no evidence of selection pressure from host immune responses. Subsequently, between 9-16 days later, the effects of selection became obvious, particularly effects attributable to HIV-specific CD8 T cell responses. By 32-45 days postinfection, almost the entire replicating virus population in each subject studied was replaced by viruses with mutations at two to five distinct loci in the genome, evincing selection pressure from both CD8 T cell and neutralizing antibody responses (and other unidentified sources also, perhaps innate and/or CD4 T cell immune responses).

The level of detail involved in the study also allowed the researchers to document virus escape from CD8 T cell responses earlier than has previously been reported. Mathematical modelling of the data indicated that HIV-specific CD8 T cells are more efficient at killing virus-infected cells during acute infection than prior estimates have suggested. Discussing the implications of their findings for T-cell-based vaccines, the authors state: "Modelling implied that a single T cell response was contributing as much as 15-35% of viral decline with multiple T cell responses. The implication of these observations is that vaccine-induced HIV-1-specific T cells will contribute to control of acute viraemia if they are activated early in subsequent HIV-1 infection. However, because of the very rapid escape that occurs within the first few weeks of infection, T cell vaccines will need to stimulate a considerable breadth of T cell responses, clearly greater than the median of three epitopes induced by the Merck vaccine."

Source: TAG basic science project (22 Jun 2009).

#### References

1. Brumme ZL and Walker BD. Tracking the culprit: HIV-1 evolution and immune selection revealed by single-genome amplification. Commentary. *Journal of Experimental Medicine*, Vol. 206, No. 6, 1215-1218. Published online 1 June 2009. doi:10.1084/jem.20091094.  
<http://jem.rupress.org/cgi/content/abstract/206/6/1215>
2. Goonetilleke N et al. The first T cell response to transmitted/founder virus contributes to the control of acute viremia in HIV-1 infection. *Journal of Experimental Medicine*, Vol. 206, No. 6, 1253-1272.  
<http://jem.rupress.org/cgi/content/abstract/206/6/1253>

## FUTURE MEETINGS

The following listing covers some of the most important upcoming HIV-related meetings and workshops.

Registration details, including for community and community press are included on the relevant websites, where available.

5 -8 October 2009: IVth Meeting Latin American Society for Mycobacteria and Tuberculosis, Rosario Argentina

29 October-1 November 2009: 47th IDSA, Philadelphia.

<http://www.idsociety.org>

3-7 December 2009: 40th Union World Conference on Lung Health, Cancun Mexico

<http://www.worldlunghealth.org/Conf2009/website/>

16-19 February 2010, 17th CROI San Francisco

<http://www.retroconference.org/2010/>

17-19 March 2010, 8th European Drug Resistance Workshop, Sorrento Italy

7-9 April 2010, 11th International Workshop on Clinical Pharmacology of HIV Therapy, Sorrento Italy

1-4 June 2010, 2nd South African TB Conference, Durban

<http://www.tbconference.co.za/>

18-23 July 2010, World AIDS Conference, Vienna

<http://www.aids2010.org/>

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## HIV i-BASE

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HIV i-Base is an HIV-positive led treatment information service. We produce information both for clinicians and other health workers and for people with HIV.

Our publications are used and have been adapted in many countries and settings.

Our fully searchable website is designed to be fast to access, easy to use, and simple to navigate.

All i-Base publications are available online.

<http://www.i-base.info>

i-Base produce five non-technical treatment guides, which are available online as web pages and PDF files.

<http://www.i-base.info/guides>

- Introduction to combination therapy
- A guide to changing treatment
- Avoiding & managing side effects
- HIV, pregnancy & women's health
- Hepatitis C for People living with HIV

The site also includes a web-based Q&A section for people to ask questions about treatment:

<http://www.i-base.info/questions>

Recent questions include:

- Is it OK to take probiotic cultures with HIV meds?
- Is d4T+3TC+EFV good enough?
- Can I take these supplements with my HIV treatment?
- Can I use Zyban to stop smoking if I'm on HIV treatment?
- What would happen if somebody starts with a CD4 count of zero and on entry inhibitor?
- Do I have a natural resistance to HIV?
- How to remove genital warts?
- Switching back to Kaletra after efavirenz...
- Is it OK to use muscle gain powder?
- Do we have to start using condoms?

We have also posted online a set of generic clinic forms, developed with the Royal Free Centre for HIV Medicine, which may be a useful resource for other hospitals.

<http://www.i-base.info/clinicforms>



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**[www.i-Base.info](http://www.i-Base.info)**



**Southern African HIV Clinician's Society**  
**[www.sahivsoc.org](http://www.sahivsoc.org)**