SATuRN: Report 2011

Southern Africa Treatment and Resistance Network

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PRELIMINARY REPORT 2011

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Foreword

While policy makers, experts and advocates debate whether widespread antiretroviral therapy (ART) can reverse the direction of the epidemic, treatment is unequivocally saving lives across Africa (1). The massive scale up of ART as treatment, pre and post exposure prophylaxis are changing the course and outcomes of infection and transmission. Systematic research is necessary to track the impact of treatment and antiretroviral drugs on health, drug resistance, development, sustainability and governance in Africa.

The Southern Africa treatment and Resistance Network (SATuRN) seeks to build innovative collaborations between researchers, clinicians and policy makers focused on the monitoring, evaluation and delivery of ART. We have developed an approach to virologic failure, delivering genotyping, interpretation and clinical management to remote clinics, without elaborate computer systems or infectious diseases specialists at each clinic. Applying the concepts of telemedicine, laboratorians and specialists, in medical centers throughout the world can review cases, including clinical and resistance data contend provide feedback and advice to the physician/nurse managing the patient at the primary clinic (more info on pages 16 to 18). This system also allows the collection of complete treatment and clinical information to be used in surveys that can be harnessed to systematically track the transmission and acquisition of drug resistance. Results from three settings are seen on pages 4 to 9 in this report.

Another objective of the SATuRN project is to increase access to efficient, lower cost genotyping and drug resistance testing in Africa. To collect, curate, interpret and disseminate sequence and drug resistance data, we have installed in South Africa two of the best HIV drug resistance databases in the world (The Stanford HIV Drug Resistance Database and RegaDB Clinical Management and Drug resistance Database). These databases are public accessible at our bioinformatics servers (via www.bioafrica.net/saturn/ website). To advance access to drug resistance testing to patients, providers and programs, we have been working with a network of academics laboratories to develop and implement a cheaper resistance genotype test. This activity has received support from Life Technologies, with the provision of a discount of 25% for reagents needed for resistance genotyping (please see page 22 of this report).

SATuRN currently includes 24 research partners in southern Africa that support our initiatives and we have collated over 3,200 resistance genotypes linked to treatment and clinical information. These datasets are open for researchers and post-graduate students through the submission of a data request and proposal to a research steering committee. We have also delivered training in drug resistance testing and management to nearly 1,000 physician and nurses in southern Africa. Our long-term goals are to expand clinical and laboratory training and capacity building to build research capacity and enhance treatment throughout Africa.

Tulio de Oliveira, David Katzenstein and Christopher Seebregts on behalf of SATuRN.

A survey of 20 years of primary drug resistance in South Africa

Introduction:
The development of HIV-1 drug resistance poses a major threat to sustaining the achievements of antiretroviral therapy (ART) programmes in Africa, particularly in settings where limited resources prevent regular laboratory monitoring of responses to ART and complex ARV regimens.

Objective:
To describe the trend in the prevalence of transmitted drug resistance (TDR) in South Africa over the past 20 years and to compare the results obtained from the Africa Centre’s 2010 HIV surveillance in rural KwaZulu-Natal.

Results:
Eight published data sets (Pillay et al., 2002; Gordon et al., 2003; Bessong et al., 2005, 2006; Seioghe et al., 2007; Jacobs et al., 2008; Pillay et al., 2008; Huang et al., 2009) were selected for analysis from a total of 32 HIV drug resistance studies. Additional sequences published through non-drug resistance articles (Matthews et al., 2008) were also retrieved from Genbank and included in the analysis. The total number of sequences analyzed was 1650.

The prevalence in transmitted drug resistance over the past 20 years has remained low. There was no evidence of transmitted resistance prior to the year 2000 (n = 32). The year with the highest level was 2002 (6.67%, 95% confidence interval (CI):3.09-13.79%; n: 6/90). After 2002, the prevalence remained below 5% (WHO low-level threshold) and did not significantly vary statistically overtime. The Africa Centre’s transmitted drug resistance surveillance among sero-converters identified in the 2010 surveillance round showed a prevalence of 1.39%, 95% CI: 0.25-7.46% (n: 1/72).

Data source and Methods:
A comprehensive literature search was conducted on PUBMED to identify papers published on the past 20 years in South Africa on drug resistance in treatment naïve patients. The key search terms used were “HIV-1 AND Drug resistance AND South Africa”. Seventy two (72) sero-positive samples from recent sero-converters from northern rural KwaZulu-Natal were genotyped. They were selected from participants of Africa Centre’s 2010 annual adult population based HIV surveillance.
**Figure:** Trend in the prevalence of TDR between 2000 and 2010. The 2010 results were extracted from the Africa Centre’s transmitted drug resistance surveillance among seroconverters and estimated a prevalence of 1.39% (n=1/73). The unique individual had the NNRTI (Y181C) and NRTI (M184V) resistance mutation.

**Poster with complete results at SA AIDS Conference:**

**Background:** Surveillance of HIV-1 transmitted drug resistance (TDR) was conducted among pregnant women in South Africa over a 5 year period after the initiation of a large national antiretroviral treatment program.

**Methods:** All participants were from Gauteng (GP) and KwaZulu-Natal (KZN) Provinces and were part of the 2005 to 2009 annual antenatal HIV seroprevalence survey conducted by the National Department of Health. HIV-1 positive serum specimens were tested using the Aware™ BED™ EIA HIV-1 Incidence Test. TDR was assessed in primigravid women age <25 years. Genotyping was performed on viral RNA by sequencing the protease and reverse transcriptase genes.

**Results:** A total of 354 sequences were analyzed from 9 surveys. In GP, the levels of TDR were <5% for all drug classes while in KZN levels were <5% in 2007 but appeared to be increasing for NNRTI as these reached 5-15% in 2009. A total of 12 (3.4%) sequences had TDR including K103N and M184I/V. Use of the BED ELISA suggested that samples selected for inclusion into the study were enriched for those with incident infection.

**Conclusions:** Analysis of the levels of TDR in 2 provinces of South Africa over 5 years suggested that in GP, TDR remains low while in KZN TDR levels may be increasing with the most recent survey showing moderate levels of resistance to the NNRTI drug class. Serological tests for incident infections may be a useful additional parameter to include in surveys of TDR.
Table: Transmitted HIV Drug Resistance Threshold Surveys Performed in Gauteng and KwaZulu-Natal Provinces between 2005 and 2009

<table>
<thead>
<tr>
<th>Year</th>
<th>Province</th>
<th>Number tested</th>
<th>Number sequences analyzed</th>
<th>Amplification rate</th>
<th>Median Age (Range)</th>
<th>HIV subtype</th>
<th>Number with mutations</th>
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<td>GP</td>
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References:

Mutational patterns:
- **GP**: K103N, Y181C, ND*
- **KZN**: K103N, V106M

Threshold levels:
- **GP**: C (1A), L10I, M184V
- **KZN**: C (1D), K101P, K103N
HIV drug resistance in first and second line patients in South Africa: EC Free State and Pretoria cohorts.

**Introduction:**
There has been a lack of effective interaction between South Africa’s research and prevention/treatment policies. To facilitate exchange of information between researchers and policy makers, SATuRN, in collaboration with researchers from the United States and Europe, has developed two HIV-1 drug-resistance databases (Stanford HIVdb and RegaDB) in southern Africa. These rapidly expanding databases (currently > 2,500 genotypes), which serve a resource for regional and global HIV-1 research, have the capacity to enhance the large scale systematic monitoring of antiretroviral rollout programs throughout southern Africa.

**Results:**

**Free State 1st line:** 116 of 131 (88.5%) patients experiencing virological failure an average of 874 days after the initiation of ART had resistance mutations. NRTI and NNRTI, the most prevalent mutations, were detected in 76.3% and 83.9% of patients, respectively. M184V/I, the most common NRTI mutation, was present in 71.7% of patients, followed by a range of NNRTI mutations (K013N, V106M, G190A, Y181C) present at levels ranging from 43.5% to 17.6%. Although 17.6% of sequences contained TAMs, only 7.6% had more than two TAMs, which limit the effectiveness of second line regimens.

**Pretoria 1st line:** Analysis of 111 patients (113 genotypes) failing first line (NRTI/NNRTI-based) ART indicated that 75.2% of sequences contained at least one NRTI or NNRTI mutation. M184V/I, the most prevalent resistance mutation, was detected in 71/113 genotypes (62.8%). This was followed, in order of decreasing prevalence, by mutations at K103N (40.7%), V106M (16.8%), G190A (16.8%) and Y181C (13.3%). At least one Thymidine Analog Mutation (TAM) was detected in 23/111 (20.7%) of patients.

**Data source and Methods:**
The databases are being populated with a large number of HIV-1 sequences from South Africa and neighbouring countries. Researchers in the University of the Free State (UFS) School of Medicine and the Departments of Family Medicine and Immunology at the University of Pretoria (UP) are instrumental in supplying data on patients failing therapy. The UFS and UP component of the database currently consists of two datasets that provide detailed information on the patients’ treatment and clinical history, together with the patients’ genotypic data.
Free State 2nd line: Resistance mutations were detected in 16/45 (35.5%) patients experiencing virological failure on average 714 (interquartile 245-955) days after initiation to second line ART containing at least one PI. Major PI mutations were identified in only 11.1% of patients. NRTI, NNRTI and TAMs mutations were more common and present at a frequency of 24.4%, 22.2% and 8.9%, respectively.

Pretoria 2nd line: 8/17 (52.9%) adults and 3/33 (9.1%) children had no detectable resistance, suggesting non-compliance. Major PI mutations (V32I, M46L, I47A, L90M) were detected in only of 1/17 (5.9%) adults compared to 7/33 (21.2%) pediatric patients. 5/33 (15.1%) pediatric sequences contained >3 PI mutations. The most prevalent, M461 and V82A, were detected in 18.2% of sequences, followed by I54V, L24I, I50V and L76V at a frequency of 3.0% each. Children also had more NNRTI and NRTI mutations (39.4% vs 29.4%), especially those related to NVP (K103) and 3TC (M184V/I) (27.3% vs 17.7% and 75.8% vs. 29.4%, respectively).

Results to be presented at SA AIDS Conference and IAS 2011:


HIV-1 drug resistance at antiretroviral treatment initiation in children previously exposed to single-dose nevirapine

Objective: To describe the prevalence of HIV-1 drug resistance mutations at the time of treatment initiation in a large cohort of HIV-infected children previously exposed to single dose nevirapine (sdNVP) for prevention of transmission.

Design: Drug resistance mutations were measured pre-treatment in 255 infants and young children under 2 years of age in South Africa exposed to sdNVP and initiating ritonavir-boosted lopinavir-based therapy. Those who achieved viral suppression were randomized to either continue the primary regimen or to switch to a nevirapine-based regimen. Pre-treatment samples were tested using population sequencing and real time allele-specific PCR (AS-PCR) to detect Y181C and K103N minority variants. Those with confirmed viremia >1000 copies/ml by 52 weeks post-randomization in the switch group were defined as having viral failure.

Results: Non-nucleoside reverse transcriptase inhibitor (NNRTI) mutations, predominantly Y181C, were detected by either method in 62% of infants less than 6 months of age, in 39% of children 6-12 months of age, 22% 12-18 months, and 16% 18-24 months (p=<0.0001). NNRTI mutations detected by genotyping, but not K103N or Y181C mutations detected only by AS-PCR, were associated with viral failure in the switch group.

Conclusions: The prevalence of mutations known to compromise primary NNRTI-based therapy is high in sdNVP-exposed children, supporting current guidelines recommending use of PI-based regimens for young children. Standard genotyping is adequate to identify children who could benefit from switching to NNRTI-based therapy.
Figure: Overall prevalence of NNRTI mutations among 255 sdNVP-exposed children initiating antiretroviral therapy. Data show the prevalence of NNRTI mutations by genotyping (black bars) plus the additional prevalence when including samples that were only identified using the Y181C or the K103N AS-PCR (grey bars). Samples classified as indeterminate by Y181C AS-PCR were excluded.

References:


Phenotypic Resistance to Etravirine in an HIV-1 Subtype-C Background

**Background:** South Africa has an estimated 5.7 million people infected with HIV-1 of whom 919,923 were receiving antiretroviral treatment by the end of 2009. The first-line regimen includes a non-nucleoside reverse transcriptase inhibitor (NNRTI), either efavirenz or nevirapine. Both drugs share similar mutation profiles and exhibit cross-resistance. Here we examine the phenotypic sensitivity of single and double NNRTI mutations found in patients failing either nevirapine or efavirenz, to a second generation NNRTI, etravirine which has an unrelated resistance profile.

**Methods:** Single and double NNRTI resistance mutations were introduced into an HIV-1 expression plasmid containing a ~3.7 kilo-base gag-pol insert from a subtype-C reference strain. The NNRTI mutation list from the International AIDS Society was used for selection of single mutants. Double mutants with significant covariation were identified by performing a Jaccard analysis on sequences from NNRTI experienced patients. Mutant plasmids were transfected into 293T-cells for the production of HIV-1 resistance vectors, and used to infect 293T-cells in serial dilutions of efavirenz, nevirapine and etravirine. Fold-change (FC) values were deduced for each virus-drug combination. Phenotypic resistance was classified by use of the Monogram PhenoSense™ and Virco Antivirogram® cut-off values.

**Results:** Of the 30 single NNRTI mutations tested, only Y181I (FC>40) and Y181V (FC>40) caused high level resistance to etravirine. Mutations K101E/P, E138A/K, Y181C, Y188L and M230L gave a low to intermediate level of resistance. Mutations K101P, K103N, V106M, Y188L, G190S and M230L caused high-level resistance to efavirenz and nevirapine, while Y181C/I/V, Y188C/L and G190S conferred high-level resistance to nevirapine only. Mutation V179F conferred hyper-susceptibility to all three NNRTIs (FC=0.004-0.151). Mutation Y188C, although conferring high level resistance to nevirapine (FC>40), conferred hypersusceptibility to etravirine (FC=0.144). All eight double mutations caused high-level resistance to nevirapine (FC>40), and some to efavirenz. Interestingly, the combination of V179F with Y181C caused a high level of resistance to both etravirine (FC=40) and nevirapine (FC>40). The Y181C and G190S double mutant was the only other combination that caused high-level resistance to etravirine (FC>40).

**Conclusion:** NNRTI resistance mutations, either singly or in combination, that arise in response to nevirapine or efavirenz rarely conferred high levels of resistance to etravirine. However, the combinations of V179F/Y181C and G190S/Y188C conferred high level resistance to ETV, as has previously been predicted. As combinations are not prevalent in currently failing individuals, etravirine is a suitable option for first-line NNRTI-based regimen salvage.
Reference:
In adult patients failing a second-line protease inhibitor regimen lopinavir concentration measurement in plasma or hair are helpful to exclude patients with poor adherence from unnecessary resistance testing

**Background:** South African patients receiving a regimen of LPV/r, didanosine and zidovudine as a second regimen have a high prevalence of virological failure (30-40%). This could be due to the poor tolerability of the regimen. However, problems with adherence are often not disclosed.

**Objective:** Therefore objective measures of drug exposure such as plasma concentration (recent drug use) or hair concentration (longer term drug use) could be helpful in identifying those patients with failure despite good adherence who would need resistance testing. In a study of patients failing this second-line regimen in 2009 conducted in two public healthcare sites, only 2 of 93 patients on this regimen had protease inhibitor resistance.

**Results:** A high lopinavir plasma or hair concentration had a negative predictive value of 86% and 89%, for virological failure and the use of both plasma and hair concentrations could detect all patients with inadequate drug exposure and who did not have resistance while having virological failure of the regimen.

**Conclusion:** We therefore propose that lopinavir hair and or plasma measurement should form part of the work-up of patients failing a second-line boosted protease inhibitor regimen to exclude patients with inadequate adherence from genotypic antiretroviral resistance testing.
**Figure:** Scatterplot of Lopinavir (ng/mg) and plasma concentrations [(μg/mL)] in patients with virologic failure (triangles) and nonfailure patients (open circles). The dashed lines indicate the respective concentration cut-offs: LPV plasma concentration of 1 μg/mL and LPV hair concentration of 3.64 ng/mg.

**Reference:**
HIV clinical management: Integrating virtually failure clinics in southern Africa.

Introduction:
Several years into the ART rollout program in South Africa, some patients are beginning to fail their first or second-line drug regimens. Data on HIV drug resistance and its impact on the management of patients with subtype C viruses is limited. Information on resistance associated mutations at clinical outcome of patients is needed to guide treatment options and ensure that South Africa’s ART program is highly effective.

HIV Drug Resistance Interpretation:
HIV Drug Resistance Testing Process: RT and protease were sequenced using a discounted in-house genotyping method, which is freely distributed by SATuRN (more info on this method on page 14 of this report). Data was submitted to SATuRN RegaDB Clinical Database for confirmation of sequence quality and identification of PI, NNRTI and NRTI resistance mutations. A resistance report is produced together with clinical tests and treatment information (an example is seen on page 10 of this report).

Virtual Failure Clinic meetings:
A virtual failure clinic meeting is scheduled twice a month on Wednesdays, 8am-9am. During these meetings, two clinical cases are presented together with literature on the subject. These meetings are chaired by Dr. Theresa Rossouw (UP), Dr. Cloete Van Vuuren (UFS) and Dr. Kevi Naidu (Africa Centre).

HIV Treatment Failure Clinic Interpretation Model:
A resistance report is generated using RegaDB/Stanford HIVDB algorithms. The clinical chart and resistance results are interpreted by an Infectious Disease (ID) specialist in Pretoria (Dr. Theresa Rossouw) or Bloemfontein (Dr. Cloete van Vuuren), who suggests the best possible treatment option based on the drugs that are available through the Department of Health in South Africa. The report is sent to the physician managing the patient, who can contact the I.D. specialist for further discussion.
How to participate in the virtual failure clinics:
These meetings are targeted at clinicians, clinical virologists, researchers and post-
graduate students who are currently involved in the treatment of patients with ARVs in
Southern Africa. Participants need to register with SATuRN in advance and will join via conference call.

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knaidu@africacentre.ac.za

**Figure 2:** Trend in the prevalence of TDR between 2000 and 2010

**Subtype Result:** HIV-1 Subtype C (Rega Subtyping tool v2.0)

**Algorithm interpretation:** HIV Stanford HIV Drug Resistance DB 6.0.5

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<th>Mutations</th>
<th>Description</th>
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<th>GSS</th>
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<td>Intermediate resistance</td>
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**Dotted line, Resistance Genotype (05 June 2008)**

Southern Africa Treatment and Resistance Network

SATuRN: Report 2011
**Representative clinical chart and interpretation of resistance.** This paediatric patient had resistance mutations directed against three of the four antiretroviral drugs he/she was receiving. Fortunately, the pattern did not include any PI (LPV/r) mutations that would exclude the use of this class of drugs. The patient exhibited high-level resistance to Lamivudine (3TC), intermediate resistance to Zidovudine (AZT) and low-level resistance to Abacavir (ABC). His/her HIV population had the characteristic 3TC resistance mutation, M184V, in addition to three TAMs, 67N, 70R and 219Q.

The patient’s last six VLs prior to resistance genotyping ranged between 1,000 and 50,000 copies/mL of plasma. The presence of multiple TAMs and relatively low level of viral replication (ie. for a paediatric patient) suggest that, although the child appeared to be taking his/her antiretroviral drugs, adherence may have been suboptimal, or that the drug dosage was not appropriate.

As would be expected, the CD4 T cell count decreased during virological failure.

**Infectious Disease (I.D.) Specialist Comments:** The presence of multiple TAMs limits this patient's options for a subsequent regimen. In the absence of a novel drug class, such as an entry inhibitor or integrase inhibitor, an augmented standard second-line regimen is suggested. This is achieved by adding 3TC to a NRTI-combination of AZT and ddi in order to 'cripple' the virus and make it hyper-susceptible to AZT. This augmented backbone is combined with the protease inhibitor, lopinavir/ritonavir. Special attention should be paid to adherence issues that might have compromised the first regimen, with special attention to family support structures, the use of any concomitant medication or alternative remedies, and drug dosage. Ongoing adherence support should be provided because of the high pill burden and side-effect profile of this combination of drugs.

**Clinical Outcome:** Complete viral suppression was achieved by switching to an augmented second-line regimen. If this viral suppression is sustained, simplification of the regimen can be considered with removal of 3TC or ddi, depending on the clinical picture and side-effect profile of the child.
Southern African HIV Drug Resistance and Clinical Management Workshop

Introduction:
The Southern African Drug Resistance and Clinical Management Workshop is presented every year. The workshop includes theoretical lectures and practical sessions on the usage and interpretation of HIV-1 drug resistance genotyping in the management of HIV patients on anti-retroviral (ARV) treatment. This workshop is targeted at clinicians, clinical virologists, nurses, medical students and researchers working in the public and private sector who are currently involved in the treatment of patients with ARVs in Southern Africa.

Successful past and promising future:
The 4th and 5th workshops were presented at the University of the Free State (UFS) Medical School in 2009 and 2010. This workshop, to the best of our knowledge, is now considered the top regional meeting on HIV drug resistance and clinical management. In total we have trained nearly 1,000 physicians and nurses as part of this workshop.

For example, in 2010 we got 436 applications and 215 participants attended the workshop, representing in total 17 countries. We also had 22 presenters. These included the CDC/PEPFAR Chief of the AIDS Treatment and Care Branch in South Africa, Prof. Jeffrey Klausner, the director of the HIV resistance program from the World Health Organization, Dr. Michael Jordan and some of the top international and national HIV clinicians and researchers.

1st to 5th workshop was organized by:

Funded by:

bioafrica.net/saturn
SATuRN and Life Technologies (ABI) partnership provide a discounted HIV resistance genotyping system.

Introduction:
Antiretroviral (ARV) drugs are becoming increasingly available to treat HIV-1 infected individuals in the developing world. The goal of many governments and non-governmental organizations is to sustain the effective ARV treatment of > 5 million people in Africa. Pharmaceutical companies are reducing both prices and international trade restrictions on patented drugs to allow more equitable access to essential medicines for AIDS, TB and Malaria. However, the widespread increase of treatment is threatened by the appearance of drug resistance. Public health and patient benefit may be limited by the increase in selection and transmission of broadly ARV resistant viruses. Drug resistance viruses can currently be identified with genetic sequencing of two HIV-1 genes. However, the price of an individual test using commercial methods (ZAR 2,500 – US$ 300) makes it too expensive for public health implementation in southern Africa.

SATuRN HIV Resistance Genotyping
We have developed an in-house HIV resistance genotyping system in collaboration with the Stanford HIV Drug Resistance Database team and it has been internationally validated by the French AIDS Research (French acronym: ANRS).

Our in-house sequences are generated with the Sanger ABI sequencing technology. This process involves the production of cDNA, which is reverse transcribed from the viral RNA. Our in-house genotyping test reagents currently costs around ZAR 750 (US$ 100) per sample, a great part of the cost of which ZAR560 (US$80) is due to the cDNA synthesis and the ABI sequencing process. SATuRN members would like to ask ABI to reduce the price of the reagents needed for HIV drug resistance genotyping for the members of our network.

Life Technologies (ABI) and SATuRN partnership:
Life Technologies has agreed to provide a 25% discount for reagents for HIV genotyping to SATuRN members. This partnership aims to produce a ‘discounted genotypic test kit’ to southern African partners that will be available soon. For the moment, partners can request discounted reagents from ABI (Seshnee Pillay, Seshnee.Pillay@lifetech.com) and the protocol and validation samples from SATuRN partners at the UFS (Dr. Dominique Goedhals, gnvrdg@ufs.ac.za) and Africa Centre (Justen Manasa, jmanasa@africacentre.ac.za).
HIV Drug Resistance Satellite Meeting of the 5th South African AIDS Conference

The HIV Drug Resistance Satellite session:
We would like to bring to your attention the HIV Drug Resistance Satellite Meeting of the 5th South African AIDS Conference, on 7 June 2011 (9am – 1pm), at the International Conference Centre (ICC), Durban, South Africa. The meeting includes theoretical lectures and clinical cases on the usage and interpretation of HIV-1 drug resistance genotyping in the management of HIV patients on anti-retroviral (ARV) treatment. This meeting is targeted at clinicians, clinical virologists, nurses, medical students and researchers working in the public and private sector who are currently involved in the treatment of patients with ARVs in Southern Africa.

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**Publications:**
**SATuRN: Report 2011**

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**Publications:**
Publications:

Abstracts:
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