SESSION 6: SEXUALLY TRANSMITTED DISEASES

Organizer: *Dr. Samoel. Khamadi*

ORAL PRESENTATIONS

1. Prevalence and correlates of nevirapine resistant virus in HIV-1 infected infants

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Background: Nevirapine (NVP) provided as either single-dose NVP (sd-NVP) or with zidovudine (AZT) for prevention of mother-to-child transmission (PMTCT) of HIV-1 commonly leads to development of resistance to non-nucleoside reverse transcriptase inhibitors (NNRTI), which may compromise future NNRTI based antiretroviral therapy (ARV). We determined the prevalence and correlates of NVP-R virus in infants (< 12 months of age), prior to initiation of ART therapy.

Materials and Methods: HIV-1 infected infants were screened from PMTCT clinics and hospital wards in an ongoing clinical trial. Previous exposure to NVP for infants was based on self-report from mothers. An 'in-house' genotypic population based sequencing method was used to detect the presence of drug resistance mutations in the reverse transcriptase viral genome from plasma samples. Infants <5 months old were enrolled into an ongoing trial examining empiric early ART, and demographic information was obtained; CD4 cell counts and viral loads were monitored for mother-infant pairs. A Fisher's exact test was used to compare the prevalence of resistance in the younger versus older infants. Within the enrolled younger cohort, correlates of resistance were evaluated using Fisher's exact and Wilcoxon rank sum statistical tests.

Results: At screening, 97 mother-infant pairs had reported exposure to NVP. 13 of 42 (31%) infants <5 months of age, and 11 of 55 (20%) infants >=5 months of age, had detectable NNRTI resistance mutations (p=0.242). The common NNRTI resistant mutations detected in the younger (<5 months olds) NVP exposed infants were Y181C (N=6), K103N (N=4), G190A (N=2), and dual K103S and Y181C mutations (N=1), while in the older infants were mainly K103N ((N=9) and Y181C (N=2), that causes high level resistance to NVP and other NNRTI. Detection of NVP resistance in the younger infants was not associated with HIV-1 subtype, maternal CD4 cell count, infant CD4 cell percent, or infant sex or age. There was a trend for higher prevalence of NNRTI resistance in the younger infants exposed to sd-NVP (13/36, 36%) compared to infants with NVP plus AZT prophylaxis (0/6, 0%) (P=0.153).

Conclusions: Among NVP-exposed infants, prevalence of NNRTI resistance was higher in young infants and less likely among those with NVP-AZT rather than SD-NVP exposure.

2. HIV Resistant commercial sex workers have a gene expression signature pattern reminiscent of a lowered immune activation state

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Abstract

To identify biomarkers for HIV-1 resistance, including pathways that may be critical in in design of novel anti-HIV-1 preventive strategies, we carried out a gene expression analysis on blood samples obtained from HIV-1 highly exposed seronegative (HESN) volunteers from a commercial sex worker cohort in Nairobi and compared their profiles to HIV-1 susceptible negative controls. Whole blood samples were collected from HIV-1 resistant sex workers and a similar number of controls.

Total RNA was extracted and hybridized to the Affymetrix HUG 133 Plus 2.0 micro arrays

(Affymetrix, Santa Clara CA) and output data was analyzed through ArrayAssist software (Agilent, San Jose CA). More than 2,274 probe sets were differentially expressed in the HIV-1 resistant sex workers as compared to the control group (fold change ≥1.3; p value ≤ 0.0001, FDR <0.05) . Unsupervised hierarchical clustering of the differentially expressed genes readily distinguished HESNs from susceptible controls.

Pathway analysis genes through the KEGG signaling database revealed a majority of the impacted pathways (13 of 15, 87%) were significantly down regulated. The most down expressed pathways were glycolysis/gluconeogenesis, pentose phosphate, Phosphatidyl inositol, Natural Killer cell cytotoxicity and T-cell receptor signaling.

We infer that the hallmark of HIV-1 resistance is down regulation of genes in key signaling pathways that HIV-1 depends on for infection and suggest that promising anti-HIV-1 preventive strategies may need to incorporate components that induce an immune quiescent environment.

3. Diagnostic Utility Of The Exavir Drug; An Enzyme Phenotypic Assay For Monitoring Of Phenotypic Resistance Of HIV To Nevirapine

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Introduction: Phenotypic drug resistance testing is valuable in the management of HIV/AIDS disease. It measures either the inhibition of viral replication in culture in the presence of drugs or inhibition of the activity of viral derived enzymes in the presence of drugs. This ability to detect Reverse Transcriptase (RT) activity associated with any retroviruses, including HIV-1 and HIV-2, provides a unique tool that is strain or subtype independent unlike genotypic assays. This study proposed to determine the diagnostic utility of the Exavir Drug; an enzyme phenotypic assay for monitoring of phenotypic resistance of HIV to Nevirapine.

Methods: RT enzyme was extracted from a cohort of 210 HIV infected patients at Mbagathi District Hospital. Based on an IFU threshold of 10fg/ml enzyme quantity preceding testing, 48 were successfully phenotyped. A correlation was done between the two reporting formats i.e. Fold Change (FC) and the concentration of drug that inhibited the enzyme's activity by 50% (IC50). Of the 48 successfully phenotyped, 30 were successfully genotyped.

Results: The prevalence of Nevirapine resistance was 18.75%. The correlation between FC and IC50 was linear with the FC values predicting the IC50 values by 97.3%. The correlation equation was y=2.57x+0.237 and R^2 of 0.973 when IC50 is plotted on the x axis and y=0.3786x-0.039 and R^2 of 0.973 when FC is plotted on the x axis. The IC50 range was 0.14 -28.67 while the range for FC was 0.1 – 12.5.A concordance of 80% was observed between the phenotypic and the gold standard genotypic assay for detection of Nevirapine resistance. This improved to 92.5% when partial agreement was considered.

Conclusion: This data strongly suggests that the phenotypic assay is suitable for use in the determination of drug resistance to RT inhibitors like Nevirapine.

4. **Virologic and Clinical Responses to First Line Antiretroviral Therapy and Immune Reconstitution Syndrome in Patients with CD4 Counts < 100 cells/mm3: Interim Summary of the Kericho IRIS Study**

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ABSTRACT

Background: Limited systematically collected, prospective data are available regarding virologic and clinical responses (including Immune Reconstitution Syndrome, IRIS) in patients with CD4 counts less than 100 cells/mm^3 starting first line antiretroviral therapy (ART) in rural settings in sub-Saharan Africa.

Objective: To describe early (up to 6 months) virologic and clinical responses to ART including development of IRIS in patients with advanced HIV disease starting ART in rural Kericho, Kenya.

Methods: 200 adults in the ongoing IRIS observational cohort study were followed for 24 weeks. HIV-1 positive men and women above 18 years, ART naïve with CD4 count less than 100cells/mm3 were included in the study. Baseline and interval follow-up data were examined to determine rates of virologic and clinical responses including IRIS events. Routine descriptive statistics and analyses were used.

Results: Participants entering the IRIS study included 53% females with a mean age of 36.5±8.2 years, CD4=43.2±35.8 cells/mm^3, HIV-1 RNA=347,327±266,135 copies/ml, BMI=18.6 ±3.6 kg/m^2, Hb 11.2±2.1 gm/dL, Creatinine 66.6±21.0 µmol/L, and ALT 34.3±37.5 U/L. Virologic response to ART was rapid and cumulative with proportions of participants with HIV-1 RNA <400 copies/ml 13% at 2 weeks, 31.5% at 4 weeks, 53.5% at 8 weeks and 69.0% at 12 weeks. Fifteen (7.5%) participants had virologic failures identified at 24 weeks of follow up. Of those with virologic failure, 2 (13.3%) had subsequent viral suppression following adherence counseling and remained on first line ART, 10 (66.7%) have started second line treatment and only three have not had viral suppression but continue to be monitored. Among the 200 participants followed, 18.5% experienced an IRIS event before week 24 with tuberculosis (Tb) IRIS accounting for 45.9% of the events. IRIS was also observed in relationship to Herpes Zoster (16.2%), CNS toxoplasmosis and Pneumocystis jiroveci pneumonia (10.8% each), Kaposi's Sarcoma (8.1%), hepatitis B (5.4%), and Cryptoccoccal meningitis (2.7%). The majority (70.5%) of the IRIS cases were unmasking in origin. Twenty-one deaths occurred before 24 weeks of follow-up resulting in a 6-month mortality rate of 10.5%.

Conclusions: Rapid and sustained virologic responses with low virologic failure rates can be achieved in patients with advanced HIV who are initiating first line ART in rural Kenya. IRIS, most often related to Tb, is commonly seen in this patient population

5. Title: RV 217b: Early Capture HIV Cohort (ECHO): Acute HIV infection in most at risk populations (MARPs) in Kericho

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Background: Events occurring in acute HIV infection critically influence prognosis. Understanding the nature of host control of viral replication will provide crucial insights to guide vaccine development. ECHO is a prospective cohort study among high risk adults designed to define incidence, retention in these populations and acquire samples from acutely infected persons prior to the advent of detectable HIV antibody.

Objectives: The purpose of the study is to characterize recruitment and retention, and determine HIV prevalence and incidence in MARPs, and to describe host-virus interactions during early HIV infection.

Methods: HIV uninfected, female sex workers and females considered at high risk for HIV infection based upon a screening questionnaire are enrolling and will be followed for two years. Small blood volumes (SBV) are collected twice a week by finger stick with testing for HIV-1 RNA by the APTIMA Qualitative Assay to detect acute infection. Volunteers are seen every 6 months for larger blood draws to include HIV diagnostics, immunoassays, and host/viral genetics. Volunteers with reactive APTIMA findings are entered into an intensive one-month diagnostic verification phase to establish HIV status definitively. Volunteers

with acute HIV infection are followed for an additional five years to correlate clinical outcome with acute events.

Results: About 700 volunteers have been screened so far and 88% were found to be at high risk by our pre-defined criteria. Baseline HIV prevalence is 30% (179 of 594). SBV collection adherence has been 97% through 3- 12 months of monitoring. Thirteen volunteers have been identified with acute HIV infection, giving a crude incidence of approximately 3.38/100 PYs (95% CI: 1.54-5.22%). Twelve were identified and samples collected prior to advent of detectable HIV antibody (Fiebig I-II).

Conclusion: ECHO has demonstrated that efficient detection of individuals with very early acute HIV infection is feasible in Kenya. This will provide an extremely powerful tool to study host-HIV interactions with direct relevance to HIV vaccine development.

SESSION 6: POSTER PRESENTATIONS

1. **Primary HIV-1 Drug Resistance Patterns in Persons With Advanced HIV Starting ART in the Southern Rift Valley Province After 6 Years of PEPFAR ART Roll-out: Interim Analyses from the Kericho IRIS Study**

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Background: Six years into the roll-out of the President's Emergency Plan For AIDS Relief (PEPFAR) program, data regarding primary HIV drug resistance (HIVDR) are unavailable in the southern Rift Valley Province of Kenya where HIV prevalence rates range from 6-14%. To date, over 25,000 persons have started first-line antiretroviral therapy (ART) largely consisting of dual nucleoside reverse transcriptase inhibitor (NRTI) and non- nucleoside reverse transcriptase inhibitor (nNRTI) antiretrovirals. Protease inhibitors (PIs) are in general reserved for second line ART. With such ART pressure and HIV prevalence, an understanding of primary HIVDR rates is important in assuring optimal first-line ART.

Objective: To describe initial results of baseline HIVDR analyses for 50/200 participants in the IRIS study.

Methods: HIVDR testing was conducted for research purposes on 50 HIV-1 infected patients, self reported as drug naïve, with CD4 count <100 cells/µL who initiated first-line ART in Kericho as part of the ongoing IRIS study. HIV-1 RNA was extracted from plasma using the Qiagen® RNA extraction kit, amplified, and sequenced using the Trugene® HIV-1 genotyping system. Portions of the protease and reverse transcriptase genes about 1000bp in size were successfully sequenced and analyzed. Routine descriptive statistics were used in analyses.

Results: At time of ART initiation for 50 patients analyzed, the mean age was 36.98 (+/-8.20) years; 53.4% were female; the mean CD4 count was 39.52 (+/- 31.74) cells/µL; and, the mean HIV-1 RNA was 303,974 (+/- 209,525) copies/ml. Overall, 3 (6%) of the 50 were found to have HIVDR mutations. All the three patients had reverse transcriptase mutations conferring resistance to antiretrovirals used in first-line ART: 3 patients (6%) with K103N (conferring resistance to efavirenz and nevirapine), 1 (2%) with M184I (conferring resistance to lamivudine), and 1 (2%) with both K103N and M184 mutations. For the 3 participants with HIVDR mutations currently on the study, only one had viral suppression. Further analysis of the sequences generated from all the 50 participants indicated that a majority of them were infected with HIV-1 subtype A1:74%, HIV-1 subtype D: 18% and HIV-1 subtype C: 8%.

Conclusions: Preliminary, interim analyses of patients with advanced HIV starting ART in Kenya's southern Rift Valley Province found a small but notable proportion with HIVDR mutations relevant to 1st line ART but without apparent clinical consequence. Additional, final analyses are needed on the entire

cohort in order to best inform clinicians and policy makers.

2. **Comparative Evaluation of the NucliSENS MiniMAG and EasyMAG Nucleic Acid Extraction Platforms for Plasma HIV-1 Viral Load Determination on the NucliSENS EasyQ System**

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Introduction: Extraction of nucleic acids is a key factor in the overall performance of nucleic acid amplification systems for clinical diagnosis and molecular research assays. Newly developed extraction methods address issues of cost effectiveness coupled with sample volume, shorter test turnaround times, minimal operator involvement and HIV RNA quality and yield. Efficient nucleic acid extraction coupled with robust amplification and detection methods facilitate detection of low numbers of target nucleic acids, reproducibility of clinical sample results and a decrease in rates of inhibited clinical samples that could lead to inconclusive or false negative results.
In this regard, we assessed the comparability in plasma viral loads quantified on the NucliSENS EasyQ Analyzer using nucleic acids obtained from the NucliSENS MiniMAG (NMM) manual extraction system and the automated NucliSENS EasyMAG (NEM) system.

Methods: A total of 19 residual repository plasma samples from chronically ill HIV-1 infected patients attending Coptic Mission hospital for routine HIV care in 2009 were extracted on the NEM platform according to the manufacturer's instructions. Quantitative PCR for HIV RNA was determined on the NucliSENS EasyQ Analyzer. Results previously obtained from the corresponding plasma samples isolated on the NMM platform according to the manufacturer's instructions served as a reference.

Results: Of the 19 NEM nucleic acid eluates, 10(0.53%) had detectable viral loads whereas 9(0.47%) yielded results below the detection limit of the NucliSENS platform of 25 copies per ml comparable to the corresponding NMM extracted samples. The percentage of samples with inhibited amplification after initial nucleic acid extraction by NMM and NEM were 0.053% and 0% respectively.

Conclusion: Our analysis showed comparable extraction efficiencies of the NEM and NMM extraction methods. Both systems can thus be used interchangeably for study protocols although the NMM manual extraction requires more laborious handling and enhanced proficiency.