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Abstracts Oral Presentations

Abstract 1

Epidemiology of HIV Transmission

Population attributable fraction of genital inflammation and ulceration in HIV transmission in discordant couples, Lusaka, Zambia, 1994-2012

*K. Wall*¹; *W. Kilembe*²; *B. Vwalika*²; *S. Lakhri*²; *R. Chavuma*²; *L. Haddad*³; *H. T. Naw*⁴; *I. Brill*⁵; *C. Vwalika*²; *L. Mwananyanda*²; *E. Chomba*⁶; *J. Mulenga*²; *A. Tichacek*³; *S. Allen*³

¹Emory University, Epidemiology, Atlanta, USA; ²Emory University, School of Medicine, Lusaka, Zambia; ³Emory University, School of Medicine, Atlanta, USA; ⁴Emory University, School of Medicine, Bangkok, Thailand;

⁵University of Alabama, Epidemiology, Birmingham, USA;

⁶Zambian Ministry of Health, Community Development Mother and Child Health, Lusaka, Zambia

Background: To quantify the risk associated with genital abnormalities in HIV transmission within Zambian serodiscordant couples.

Methods: From 1994-2012, HIV discordant heterosexual couples enrolled through couples' voluntary HIV counseling and testing were followed 3-monthly and provided with free outpatient care including routine genital exams. Composite variables were created for genital inflammation and ulceration including self-report, routine physical exam with laboratory screening, and interim treatment. Linked transmission was confirmed with sequencing. HIV+ partners were referred to antiretroviral treatment (ART) services and censored when ART was initiated.

Results: 207 HIV infections occurred in women over 2,848 couple-years (7.3/100CY;95%CI:6.3-8.3), while 171 infections occurred in men over 3,367CY (5.1/100CY;95%CI:4.3-5.9). Infection among women was associated with woman's genital inflammation (adjusted hazard ratio, aHR=2.3; 95%CI:1.6-3.4), ulceration (aHR=2.5; 95%CI:1.6-3.7), and man's inflammation (aHR=2.9; 95%CI:1.9-4.5) controlling for time since enrollment, age, and viral load; the population attributable fraction (PAF) was 23% for

female inflammation, 34% for male inflammation, and 11% for female ulceration. Infection among men was associated with man's genital inflammation (aHR=3.4; 95%CI:2.0-5.6), ulceration (aHR=2.7; 95%CI:1.7-4.3), and woman's inflammation (aHR=2.9; 95%CI:1.8-4.5) controlling for the above, circumcision, and unprotected sex; the PAF was 26% for male inflammation, 14% for male ulceration, and 29% for female inflammation. Candida, bacterial vaginosis (BV), and trichomonas were common causes of inflammation (3-12% of intervals) among women, three-quarters of which was asymptomatic for discharge. Inguinal adenopathy (IA) was common in men (29-59% of intervals), especially uncircumcised versus circumcised HIV-men ($p<0.05$) with foreskin smegma ($p<0.001$). Syphilis and HSV were the most common cause of ulcer (2%-17% of intervals).

Conclusions: This analysis is novel in showing the independent contribution of causes of genital inflammation and ulceration to both transmission from the donor and acquisition by the recipient. Educational (including increased awareness of genital abnormalities and home-based hygiene), screening (including microscopic exam of self-administered vaginal swabs for discharges; routine screening for syphilis and examination of IA), and/or low-cost easily-accessible treatment interventions for genital tract infections are warranted as these interventions may profoundly reduce HIV transmission risk.

No conflict of interest

Abstract: 2*Virology of HIV Transmission***Heterosexual Transmission of Subtype C HIV-1 Does Not Require Increase Replicative Capacity or Interferon-alpha Resistance**

Z. Ende¹, M. Deymier¹, A.E. Fenton-May², D.A. Dilemia³, W. Kilembe⁴, S.A. Allen⁵, P. Borrow², E. Hunter³

¹Emory University, Emory Vaccine Center, Atlanta Georgia, USA; ²Oxford University, Nuffield Department of Medicine, Oxford, United Kingdom; ³Emory University, Emory Vaccine Center, Atlanta, USA; ⁴Zambia Emory HIV Research Project, Lusaka, Zambia; ⁵Emory University, Pathology, Atlanta, USA

Introduction: HIV-1 transmission is associated with a genetic bottleneck that selects a single viral variant, the transmitted/founder (TF), during 80-90% of heterosexual transmission events. These breakthrough TF viruses, unique in each transmission event, may have properties that confer a higher capacity to transmit. Determining TF properties could inform interdiction strategies by increasing our understanding of the initial selection pressures operating during viral transmission. To investigate potential viral correlates of transmission we analyzed viruses from six epidemiologically linked pairs from the Zambia-Emory HIV Research Project (ZEHRP). For each transmission pair, we constructed infectious molecular clones (IMC) of the transmitted variant from the newly infected recipient and non-transmitted variants from the donor partner near the estimated date of infection.

Material & Methods: We sequenced 167 near full-length viral genomes and generated a representative panel of infectious molecular clones (IMC) including TF variants and 3-8 non-transmitted (NT) HIV-1 subtype C variants from six linked heterosexual transmission pairs near the time of transmission. We built maximum likelihood phylogenetic trees to assess single variant transmission and measured the distance to consensus for each sequenced variant. We then measured *in vitro* phenotypic traits that may be relevant to transmission, including particle infectivity, replicative capacity and interferon

resistance. Statistics were performed with Wilcoxon matched-pairs signed rank tests comparing the TF to the median of the NT variants.

Results: Consensus-like genomes ($p=0.047$) sensitive to donor antibodies ($p = 0.031$) were generally selected for during transmission in these six transmission pairs, supporting previous observations in this cohort. However, TF variants did not demonstrate increased viral fitness in terms of particle infectivity or viral replicative capacity in activated peripheral blood mononuclear cells (PBMC) and monocyte-derived dendritic cells (MDDC). In addition, TF variants were not more resistant to the antiviral effects of interferon- α (IFN- α) compared to the matched NT variants.

Conclusions: Neither replicative capacity nor IFN- α resistance discriminated the transmission potential of viruses in the quasispecies of these HIV-1 subtype C chronically infected individuals. Nevertheless, our findings generally support the hypothesis that within-host evolution of HIV-1 in response to adaptive immune responses reduces viral transmission potential.

Abbreviations: HIV-1=Human Immunodeficiency Virus type 1, TF=Transmitted/Founder Virus, NT=Non-transmitted Variant, IMC=Infectious Molecular Clone, PBMC=Peripheral Blood Mononuclear Cells, MDDC=Monocyte Derived Dendritic Cells, IFN- α =Interferon-alpha

No conflict of interest

Abstract: 3*Biology of HIV Transmission***Conserved molecular signatures in gp120 are associated with the genetic bottleneck in SIV, SHIV, and HIV-1 transmission***M.W. Gonzalez¹, A.L. DeVico², G.K. Lewis², J.L. Spouge¹*

¹NCBI/NLM/NIH, Computational Biology Branch, Bethesda, USA; ²Institute of Human Virology University of Maryland School of Medicine, Division of Vaccine Research, Baltimore, USA

Background: HIV transmission typically results from infection by a single transmitted/founder (T/F) variant. Our work address the following question: Are T/F variants chosen uniformly at random from the donor pool or are they selected for advantageous traits facilitating transmission? Evidence for selection during transmission would single out the corresponding phenotypic and/or genetic properties of the viruses as potential targets for vaccines or immunotherapies.

Material & Methods: Here, we systematically survey several extant sequence datasets for statistically significant differences between the Env proteins of SIV/SHIV stock and T/F variants, to search for the 'signature sites' of SIV/SHIV transmission. We also survey HIV amino acids corresponding to the signature sites.

Results: The present study finds statistically significant evidence for selection on the gp120 molecules of SIV/SHIV T/F viruses. Four sites of gp120 showed significant selection, and two additional sites showed similar trends. The six sites therefore differentiate T/F viruses statistically from the viral variants in the stocks. Two of the signature residues display complete conservation across the SIV, SHIV, and HIV variants we examined. Five of the signature residues map to the C1 region of gp120 and one to the signal peptide.

Conclusions: With our methods, we could reasonably infer the selection of SIV/SHIV viruses across both vaccinated and unvaccinated

subjects, across infections resulting from vaginal, rectal, and intravenous routes of transmission, and across viral dosages. The statistical evidence for selection in SIV and SHIV T/F variants is strong and plentiful. Commensurate with the availability of suitable data for analysis, our datasets also provide ancillary evidence suggesting the same sites are under selection in HIV. In addition, in governing the association between gp120 and gp41, the C1 region on gp120 might modulate transmission efficiency, replicative fitness, and/or host cell tropism at the level of virus-cell attachment and entry.

Our findings therefore suggest that the signature sites are involved in increasing the transmissibility of infecting viruses, providing potential targets for developing a vaccine or other measures protective against HIV. A recent study identified some of the same signature sites in T/F variants, but interpreted them as an effect of neutralization resistance. In fact, the T/F motif is present in non-immune subjects, so it has functional significance beyond neutralization sensitivity. In addition, a vaccine regimen popular in animal trials might have possibly increased the transmission of variants with otherwise low transmission fitness. Our observations might therefore explain why many animal vaccine trials have not faithfully predicted outcomes in human vaccine trials, and they suggest a need to re-examine current practices in vaccine design accordingly.

No conflict of interest

Abstract: 4*Transmission by Intravenous Drug Users***Genetic complexity of HIV-1 among drug users in Kenya***K. Gounder¹, M. Onger², N. Padayachi¹, K. Sojane¹, J. Wylie³, T. Ndung'u¹*¹*University of KwaZulu Natal-Nelson Mandela School of Medicine and KwaZulu-Natal Research Institute of HIV and TB, HIV Pathogenesis Programme, Durban, South Africa;*²*University of Nairobi, Department of Human Pathology, Nairobi, Kenya;* ³*University of Manitoba, Department of Medical Microbiology, Manitoba, Canada*

Background: Key populations such as drug users are playing an increasingly important role in fueling the HIV-1 epidemic in sub-Saharan Africa but the molecular epidemiology of HIV-1 in these groups has not been fully described. We analyzed the prevalence, distribution and diversity of HIV-1 subtypes and intersubtype recombinants among drug users in Kenya.

Methods: 55 drug users, including 17 intravenous drug users were identified with HIV-1 infection in Kisumu, Nairobi and Mombasa in Kenya. Full-length HIV-1 *gag* and *env* clonal sequences were obtained from plasma. HIV-1 subtypes were determined by phylogenetic relatedness patterns using Maximum-likelihood trees together with the REGA subtyping tool (www.bioafrica.net). Intersubtype recombination and breakpoint prediction were investigated by bootscanning using SimPlot software. Coreceptor prediction was determined using the web-based bioinformatic Geno2pheno tool.

Results: 237 (median=4/participant) *gag* clonal sequences from 54/55 and 160 (median=3/participant) *env* sequences from 47/55 study subjects were analyzed. Gag analysis revealed 61.1% of individuals were infected with pure subtypes [A (42.6%), C (14.8%) and D (3.7%)], 31.5% with intersubtype recombinants and 7.4% with mixed subtypes while *env* analysis showed that 68.1% of individuals had pure subtypes [A (46.8%), C (14.9%) and D (6.4%)], 27.7% with intersubtype recombinants and 4.3% with mixed subtypes.

Analysis of the 237 *gag* clonal sequences revealed that 96 (40.5%) were A, 35 (14.8%) were C, 9 (3.8%) were D and 97 (40.9%) were intersubtype recombinants. Analysis of the 160 *env* sequences indicated that 78 (48.8%) were A, 28 (17.5%) were C, 8 (5%) were D and 46 (28.8%) were intersubtype recombinants. Comparative analysis of *gag* and *env* regions showed that 28 participants (61%) had concordant subtypes while the remaining 18 (39%) were discordant subtypes. Subtype A predominated across the three study sites (Kisumu, Nairobi and Mombasa). The highest percentage of subtype C was detected in Mombasa (24.2% in *gag*, 29.3% in *env*). Kisumu had minimal subtype C infections (4.9% *gag* and 4.3% *env*) while in Nairobi, subtype C was only detected in our *env* sequences (12.1%).

Two cases were found to be epidemiologically linked and two cases of dual infection were confirmed. Coreceptor usage prediction showed that 80% of *env* sequences were of the R5 phenotype [A, 89 (81.7%); C, 19 (90.5%); and D, 9 (69.2%)].

Conclusions: HIV-1 genetic diversity among these drug users is indicative of ongoing generation of newly emerging recombinants of HIV-1 in Kenya and high genetic complexity where subtyping in one genetic region may not be fully informative. Furthermore, compared to historical data from Kenya, our data suggests a decline in HIV-1 subtype D prevalence compared to subtype C. HIV-1 R5 tropic strains were most prevalent in the study population with indications of higher X4 prevalence among subtype D compared to A or C. Further studies to better understand HIV-1 epidemiology, transmission patterns and implications for prevention strategies for key population groups in sub-Saharan Africa are warranted.

No conflict of interest

Abstract: 5

Virology of HIV Transmission

High levels of intra-subtype recombination and multiple variant transmission in a Kenyan MSM acute infection cohort

G. Macharia¹, L. Yue², D. Dilernia², E. El-Badry², J. Gilmour³, E. Sanders⁴, E. Hunter⁵

¹Imperial College London, IAVI Human Immunology laboratory, London, United Kingdom; ²Emory University, Emory Vaccine Center, Atlanta, USA; ³Imperial College London, Department of Medicine, London, United Kingdom; ⁴KEMRI Wellcome Trust, Clinical Research, Kilifi, Kenya; ⁵Emory University, Department of Pathology, Atlanta, USA

Background: HIV-1 heterosexual transmission involves a severe genetic bottleneck, with ~90% of transmission events in Zambian and Rwandan heterosexual transmission cohorts being initiated by single virus variant, the transmitted-founder (TF) virus, derived from the chronically infected donor. More than 90% of acute infections in Zambia were identified as subtype C, and 84% of TF viruses in Rwanda were defined as subtype A. In this study we have now analyzed the transmission pattern and genetics of the infecting viruses in an acute infection MSM cohort in Kilifi Kenya using near full-length genomic amplification and sequencing.

Material & Methods: Amplicons were produced by near full-length (>9000bp) single genome amplification of HIV-1 from patient plasma viral RNA. Full-length viral sequences obtained using PacBio Sequencing. All plasma were collected from patients at acute phase (<30 days post estimated date of infection), to allow the definition of the Transmitted/Founder virus sequence and determine the number of transmitted variants.

Result: In this study, we have PCR amplified 90 NFLG HIV-1 amplicons from 19 acute infection cases. Based on phylogenetic analysis, we determined that 32% of transmissions involved more than one T/F virus. In addition 37% of the acutely infecting viruses represented unique

recombinant forms. These included the following inter-subtype recombinants - A1/D, A1/C, A1/B, A1/A2/D.

Conclusion: The generation of full-length genome sequences from this MSM acute infection cohort has allowed characterization of the genotypic and phenotypic characteristics of the viruses involved. In contrast to transmissions in heterosexual couples in Rwanda and Zambia, we observed a much higher rate of dual infection and recombinant forms, consistent with a high prevalence of sexually transmitted infections and multiple subtypes in this MSM population. These data also provide important information for vaccine design and HIV prevention.

No conflict of interest

Abstract: 6

Transmission of HIV Drug Resistance

Inference and characterization of a transmission network in an opioid-driven HIV-1 outbreak in rural Indiana

E. Campbell¹, S. Blosser³, R. Galang^{1,2}, J. Gentry³, W. Heneine¹, H. Jia¹, S. Masciotra¹, P. Peters¹, M. Spiller¹, W. Switzer¹

¹Division of HIV/AIDS Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, CDC, US; ²Epidemic Intelligence Service, CDC, US; ³Indiana State Department of Health, US

Background: In January 2015, investigation of a sudden upsurge in new HIV-1 infections in a rural county in Indiana linked to injection drug use (IDU) identified a large outbreak (n=181). Here we describe the integration of epidemiologic and laboratory data to infer and characterize the transmission network to inform future prevention efforts.

Methods: Serum specimens were used to determine recency of infection and to obtain HIV-1 polymerase (pol) sequences for genetic analysis. Putative undirected transmission links were drawn between sequences with genetic distances <1.5%. Standardized interviews were conducted with HIV-positive patients to collect high-risk behavior and contact data. High-risk contacts (sexual, IDU, or both) were considered as continuous risk factors, regardless of reporting direction. The reported contact network and inferred transmission network were compared. A decision tree was generated and logistic regression performed concerning contact type and occurrence with respect to infection status.

Results: HIV-1 pol sequences were obtained from 157 persons epidemiologically linked to the outbreak. Phylogenetic analysis inferred a monophyletic pol clade with limited diversity. Of 123 specimens available for avidity testing, 113 (91.9%) were recent (<8 months prior to collection). Network analysis showed that each type of high-risk contact was correlated with HIV

infection ($p < 0.01$). However, 82.3% of potential transmission events corresponded to a reported IDU contact, as opposed to 11.0% for reported sexual contacts. Decision tree analysis revealed that the likelihood of infection for persons with >3 recent injection partners was 93.2% (109/117). In contrast, 18.3% (68/371) of persons with • 3 recent injection partners were infected. 87.1% (27/31) of persons with multiple sexual partners with whom they also share injection equipment, and who also had >1 additional recent injection partners, were infected.

Conclusion: A single HIV-1 strain was detected in this outbreak of HIV among persons who inject drugs, suggesting recent and rapid local transmission. Comparison of reported contact and transmission networks reveal that IDU drove transmission and led to explosive growth of the outbreak. Integration of sequence and epidemiologic data facilitated a better understanding of the HIV transmission dynamics in a rural community of persons who inject prescription opioids.

No conflict of interest

Abstract: 7

Virology of HIV Transmission

Multiplexed sequencing of HIV-1 env as a measure of viral diversity in HIV-1 infected transmission pairs

E.O. El-Badry¹, D.A. Dilernia¹, L. Yue¹, E. Hunter²

¹Emory University, Emory Vaccine Center, Atlanta GA, USA;

²Emory University, Pathology and Laboratory Medicine, Atlanta GA, USA

Introduction: Understanding the viral diversity of HIV following transmission is essential for the ongoing development of therapeutic and preventative strategies. HIV envelope exhibits the highest diversity of all HIV-1 genes and can therefore be a useful predictor of overall viral diversity. Here we present a novel approach to multiplexed sequencing of HIV *env* genes in both chronically and acutely infected individuals in order to more accurately characterize the diverse HIV genomes within an individual.

Material & Methods: Plasma samples from three HIV+ Clade C Zambian transmission pairs, identified with the recipient in Fiebig stage I/II, were diluted to equal viral loads and viral RNA was extracted. HIV *env* genes were amplified from cDNA using Q5 High Fidelity DNA Polymerase (NEB) and sequenced using Pacific Biosciences Single Molecule, Real-Time (SMRT) Sequencing technology. Amplicons from all donors were pooled in a single multiplexed analysis. The same process was repeated for amplicons from all recipients. Novel computer algorithms (described by Dilernia, et al. 2015) were used to reconstruct *env* variant sequences present in the mixture of amplicons.

Results: In these initial experiments multiple distinct *env* sequences were defined for each of the chronically infected partners. For acutely infected recipients, up to 8,000 reads were generated per individual and confirmed our previous finding from single genome amplification that all three recipients were infected by a single genetic variant. Two these acutely infected

recipients had a single *env* sequence and the third had two closely related sequences that differed by a single nucleotide consistent with the Fiebig I/II stage of infection.

Conclusions: Our findings indicate that this approach of multiplexed sequencing of HIV *env* genes can be used to efficiently characterize viral diversity within chronically and acutely infected individuals by providing a large number of reads of individual sequences. This novel method can be used to further examine the relative abundance of specific HIV variants within an individual, mutations in specific regions of *env*, and instances of multiple variant transmission.

No conflict of interest

Abstract: 8*Virology of HIV Transmission***Applications of Next-Generation Sequencing for Identification of Transmitted HIV-1**

K. Brooks¹, D. Dilernia¹, D. Claiborne², M. Deymier¹, W. Kilembe³, S. Allen³, E. Hunter¹

¹Emory University, Emory Vaccine Center, Atlanta, USA;

²Ragon Institute, MGH MIT & Harvard University, Boston,

USA; ³Rwanda Zambia HIV Research Group, Zambia-Emory HIV Research Project, Lusaka, Zambia

Background: Characterizing the genetic bottleneck associated with HIV-1 transmission is critical for identifying potential virologic correlates of transmission. To study the transmission bottleneck, identification of the transmitted/founder virus or viruses [TFV(s)] is essential. TFV(s) can be inferred through high-fidelity amplification and sequencing of HIV-1 genomes from recently infected individuals and subsequent phylogenetic analysis. In single variant transmission, genome sequences form a star-like phylogenetic tree, and the TFV is defined as the consensus of the sequences. Here we employ next-generation, multiplexed sequencing with Pacific Biosciences (PacBio) to sequence near full-length genomes (NFLGs) of recently infected individuals.

Materials & Methods: We amplified HIV-1 NFLGs from the plasma of eight Zambian seroconverters with limiting dilution PCR to amplify single genomes. Nucleotide barcodes were then designed for both the 5' and 3' ends of the NFLGs, and amplicons were reamplified with a distinct pairing of barcodes; barcoding permitted amplicon identification in the multiplexed analysis. Thirty-nine barcoded amplicons were combined in a library preparation according to PacBio protocol, and library sequencing was performed on the PacBio RS II machine. Nucleotide reads generated by RS II single molecule real-time (SMRT) sequencing were analyzed with a MatLab code (Dilernia et al. 2015), which builds sequences from the reads through an iterative read alignment and phasing process. NFLGs previously sequenced with Sanger sequencing

were included in the library for comparison of the PacBio sequences to those obtained by Sanger sequencing.

Results: A total of 35 sequences built from more than 60 reads and with greater than 80% agreement per nucleotide position among the contributing reads were generated from the library. One or more amplicons were sequenced for all the seroconverters, and for 25 of the NFLGs. Eight sequences were generated for NFLGs previously sequenced with Sanger sequencing; four of the eight were identical to the appropriate Sanger reference sequence. In the remaining four sequences, there was a median of two and range of one to six insertions/deletions/mutations in the PacBio sequences across the >9000 nucleotide Sanger reference sequences. Maximum-likelihood phylogenetic trees built from five or more sequences of NFLGs from the same individual all exhibited star-like diversity.

Conclusions: Next-generation sequencing of barcoded amplicons with Pacific Biosciences permits high-throughput sequencing of near full-length HIV-1 genomes with high accuracy and at a lower cost (approximately \$25/genome) than Sanger sequencing. We have applied this technology toward inferring transmitted/founder viruses, which can be further studied for genotypic and phenotypic properties.

No conflict of interest

Abstract: 9

Epidemiology of HIV Transmission

Genome-wide population genomics of inpatient HIV-1 evolution

J. Albert^{1,2}, F. Zanini³, J. Brodin¹, L. Thebo¹, C. Lanz³, G. Bratt⁴, R.A. Neher³

¹Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, Stockholm, Sweden; ²Department of Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden; ³Evolutionary Dynamics and Biophysics, Max Planck Institute for Developmental Biology, Tübingen, Germany; ⁴Department of Clinical Science and Education, Vårdhögskolan, Stockholm South General Hospital, Stockholm, Sweden

Background: Within an infected host HIV-1 rapidly accumulates mutations, in part to evade immune recognition.

Methods: To characterize this evolutionary process, we performed whole genome deep sequencing of HIV populations in 9 untreated patients with 6-12 longitudinal samples spanning 5-8 years of infection. The first samples were obtained within 18-167 days from estimated date of infection. RNA templates were quantified by limiting dilution and correlated with plasma RNA levels. Illumina sequencing provided more than 100 Million sequence reads and an average coverage exceeding 1000x. Quality control experiments showed that mutations as rare as 0.3% could be tracked (in samples with sufficient RNA templates) and linkage information was retained over the length of the reads (approximately 500bp).

Results: Minor genetic variation within patients mirrored global HIV-1 diversity, suggesting that universal fitness costs control the level of diversity at individual nucleotide positions. Almost one third of mutations that evolved following transmission were reversions towards the ancestral HIV-1 sequence represented by a HIV-1 group M consensus. Reversions were observed throughout infection and their rate increase with conservation of the nucleotide position. Non-

synonymous mutations away from the global consensus were highly overrepresented at predicted CTL epitopes. Frequent recombination limited linkage disequilibrium to about 100 base pairs. However, hitch-hiking due to remaining short range linkage causes levels of synonymous diversity to be inversely related to the speed of evolution.

Conclusions: We report one of the most complete portraits of inpatient evolution HIV-1 available to date. By analysing divergence, diversity, linkage and recombination, we show that within the infected individual, HIV-1 is an extensively recombining population in a constant struggle between immune evasion and maintenance of virus function.

No conflict of interest

Abstract: 10*Biology of HIV Transmission***Mechanisms by which anti-Alpha4Beta7 integrin reduces mucosal transmission in SIV macaque model**S. Byrareddy¹¹Department of Pharmacology and Experimental Neuroscience, Durham Research Center, Nebraska Medical Center, Omaha

Introduction: We have recently shown that in vivo administration of an $\alpha 4\beta 7$ mAb but not normal rhesus IgG to groups of 12 rhesus macaques (RM) during SIV exposures using a repeated low dose intra-vaginal SIV challenge model led to inhibition of transmission in 6/12 RMs and markedly lower gastro-intestinal tissue viral loads (GIT-VL) in the remaining 6/12 SIV infected RM.

In efforts to understand the mechanisms underlying reduced transmission we measured correlates of protection by using immunological, biochemical and imaging techniques and found the following underlying mechanisms that we submit led to reduced transmission

1) Plasma viral loads were significantly correlated with levels of a series of pro-inflammatory markers (Pearson $\rho > 0.8$) indicating that the changes were coincident with the median time to infection

2) The plasma levels of sMAdCAM-1 were significantly lower in anti- $\alpha 4\beta 7$ -mAb treated animals vs. control animals ($P < 0.05$).

3) Since, select lineages with in GALT synthesize retinoic acid (RA) from vitamin A and it has been documented that RA promotes cells to home to the gut tissues via the up regulation of $\alpha 4\beta 7$, we found that anti $\alpha 4\beta 7$ -mAb treated animals prevented the decrease of plasma RA as compared the control IgG treated animals ($P < 0.05$) 4) the anti $\alpha 4\beta 7$ -mAb treated monkeys showed a significantly lower signal in the large as

compared with the small intestine as compared with the control IgG treated animals with similar viral loads using Immuno-PET/CT with SIV gp120 as probe., In addition, only the control treated monkeys showed a clear PET/CT signal in lymph nodes surrounding the genital tract suggesting that treatment with anti $\alpha 4\beta 7$ -mAb prevents viral replication in this tissue, leading to different patterns of tissue localization of the virus between the two groups and 5) using Immuno-PET/CT with CD4 imaging, it appears that anti $\alpha 4\beta 7$ -mAb treated animals show preserved and higher CD4+ levels in the colon, small bowel, and lymph nodes compared with IgG-treated animals.

In conclusions, anti $\alpha 4\beta 7$ -mAb treatment appears to protect the gut during both acute and chronic infection, by preserving CD4+ cells, by targeting different tissues and organs, by lowering pro-inflammatory cytokines and by reducing RA synthesis and underscores the potential utility of $\alpha 4\beta 7$ -mAb -directed intervention in SIV and HIV disease.

No conflict of interest

Abstract: 11*Virology of HIV Transmission***Co-infection of HTLV-1 permits HIV-1 direct infection of female genital epithelium in vitro despite antiretroviral therapy: Implications for HIV-1 vaginal transmission**

Y.T. Tang¹, A.G. Alvin George¹, O.P. Oksana Petrechko¹, F.N. Franklin Nouvet¹, S.S. Stephanie Sweet¹, Y.T. Yuetsu Tanaka², B.I. Brian Imbiakha³, J.E.K.H. James E.K. Hildreth^{1,4}

¹University of California Davis, Molecular and Cellular Biology, Davis, USA; ²University of the Ryukyus, Immunology, Okinawa, Japan; ³University of California Davis, Molecular and Cellular Biology, Davis, USA; ⁴Meharry Medical College, Microbiology and Immunology, Nashville, USA

Introduction: Young females are at high risk of HIV infection and constitute 75% of infected individuals in some sub-Saharan Africa countries. The regions with high prevalence of HIV-1 are often highly endemic for HTLV-1. HIV/HTLV-1 co-infection in Africa, Latin America and the Caribbean basin is increasing and has emerged as a global health problem. We propose that HIV-1 is able to acquire envelope glycoproteins of HTLV-1 during co-infection in a process we call 'natural pseudotyping', which expands HIV-1's cellular tropism and enable it to infect primary female genital epithelial cells. Natural pseudotyping may dramatically increase risk of HIV-1 transmission in females during sexual intercourse and therefore could be a biological factor contributing to the devastating spread of HIV in young females in Africa and other regions of the world.

Material & Methods: Primary CD4+ T cells were co-infected with HIV-1 and HTLV-1. Primary female genital epithelial cells isolated from endocervical and vaginal tissues were exposed to the progeny virions. Infection by HIV-1 and HTLV-1 was confirmed by immunofluorescence staining with anti-HIV-1 Gag and anti-HTLV-1 Gag

antibodies respectively. Viral release from epithelial cells was measured by ELISA and qRT-PCR.

Results: Progeny HIV-1 from HTLV-1/HIV-1 co-infected CD4+ T cells was capable of infecting primary female lower genital (vaginal and cervical) epithelial cells (FLGECs) via both cell-associated and cell-free routes. No HIV-1 infection was observed in epithelial cells exposed to progeny virus from CD4+ T cells infected with HIV-1 alone. Infection of primary genital cells was significantly reduced by neutralizing antibodies against the HTLV-1 glycoprotein, indicating that HIV-1 infection was mediated by the HTLV envelope protein. Active HIV-1 replication in primary genital epithelial cells was confirmed by inhibition with protease inhibitors. However, HIV-1 reverse transcriptase inhibitors AZT and Rilpivirine only partially blocked HIV-1 infection in FLGECs. Further analysis indicated that AZT or Rilpivirine treatment blocked HIV-1 infection in cells infected with HIV-1 alone but not in cells infected by both HIV-1 and HTLV-1. The infected FLGECs were able to spread HIV-1 to natural target T cells or to uninfected FLGECs via another round of natural pseudotyping. These results support the hypothesis of direct infection of primary female lower genital epithelium by naturally pseudotyped HIV-1 and transfer to intraepithelial hematopoietic cells as a mechanism of enhanced transmission of HIV-1 in women in areas highly endemic for both HIV-1 and HTLV-1. The resistance of HIV-1 to RT inhibitors in the setting of HTLV-1 co-infection could complicate current strategies to prevent HIV-1 sexual-transmission.

Conclusions Co-infection with HIV-1 and HTLV-1 produces naturally pseudotyped HIV-1 capable of directly infecting female lower genital tract epithelial cells *in vitro* and HIV-1 infection in this setting may be resistant to RT inhibitors.

No conflict of interest

Abstract: 12

Biomedical Approaches of HIV prevention of transmission

Safety and pharmacokinetics of quick dissolving polymeric vaginal films delivering antiretroviral combinations for pre-exposure prophylaxis

P. Srinivasan¹, J. Zhang², A. Martin¹, J.M. McNicholl¹, R.W. Buckheit Jr³, J.M. Smith¹, A.S. Ham³

¹Centers for Disease Control and Prevention, Division of HIV/AIDS Prevention, Atlanta, USA; ²Total Solutions Inc, Biology, Atlanta, USA; ³ImQuest BioSciences Inc, Formulation Science, Fredrick, USA

Introduction: Topical pre-exposure prophylaxis (PrEP) with antiretrovirals can be highly effective in reducing HIV infection, as demonstrated by the CAPRISA vaginal Tenofovir (TFV) gel trial which reduced HIV acquisition by 54% among highly adherent women. However, subsequent trials with TFV gel established no protection in women due to low adherence. These data demonstrate the direct relationship between adherence and efficacy and provide the rationale for expansion of prevention options. PrEP delivery through polymeric films may be more acceptable to women due to their small size making them more discreet, and no need for an applicator. The ease of insertion and fast dissolving time may provide a viable alternative to gels as a self-initiated prevention option for women. We report in pigtailed macaques the safety and bioavailability of IQP-0528, a potent non-nucleoside reverse transcriptase inhibitor, and IQP-0528 in combination with TFV, in a quick-dissolving film. We also investigated whether poly(lactic-co-glycolic acid) nanoparticle encapsulation would increase mucosal tissue penetration of IQP-0528.

Methods: To test the bioavailability of IQP-0528 when delivered alone or in combination with TFV, polyvinyl alcohol based vaginal films (1.5% w/w, 22x44x0.1mm, surface area 75% of a human dose) with IQP-0528 (with and without nanoparticle encapsulation) or IQP-0528+TFV were inserted into pigtailed macaques (n=15 and

6, respectively). IQP-0528 and TFV were quantified in vaginal fluid (1, 4, and 24 hours) and tissue (24 hours) by LC-MS/MS. We monitored the effect of the IQP-0528 film on vaginal microflora, pH, and bio-markers of inflammation (cytokines and chemokines).

Results: Median vaginal fluid concentrations of IQP-0528 obtained with the IQP-0528 only films at 1, 4, and 24 hours post film application were 160.97, 181.79, and 484.50 µg/mL, respectively. The IQP-0528+TFV combination film yielded similar concentrations of IQP-0528 with 1,155, 250.06 and 181.9 µg/mL at 1, 4, and 24 hours. TFV was not detected in the vaginal fluid. Median vaginal tissue IQP-0528 concentrations at 24 hours with the IQP-0528 and IQP-0528 +TFV films were 3.10 and 7.95 µg/g respectively, and values were similar proximal, medial and distal to the cervix. Seventy percent of the vaginal tissue samples had detectable TFV (median 0.3 µg/gm, range 0.008 to 19.37 µg/gm). Overall, in vaginal tissue and secretions, the nanoparticle formulation was not found to be superior to the base formulation. A single application of the IQP-0528 formulation did not disturb the vaginal microflora or the pH (7.24±0.84). Mucosal cytokine and chemokine concentrations, in particular pro-inflammatory cytokines, remained stable throughout the study.

Conclusions: We demonstrate that the quick-dissolving IQP-0528 vaginal film formulations exhibit promising safety and pharmacokinetics. While the film resulted in high mucosal levels of IQP-0528, between 1 and 5 logs higher than the *in vitro* IC₉₀ of 0.146 µg/mL, the low TFV concentration indicates that further development is required to optimize the release of TFV from the combination films. The nanoparticle encapsulation did not provide any greater advantage for IQP-0528 distribution. The rapidly obtained levels and excellent coverage support further investigation of the efficacy of IQP-0528 quick-dissolving film in protecting macaques against repeated low-dose SHIV transmission studies.

No conflict of interest

Abstract: 13

Biomedical Approaches of HIV prevention of transmission

HIV epidemics could be eliminated by using treatment as prevention: a proof-of-concept study in Copenhagen

D. Robbins¹, J. Okano¹, L. Palk¹, J. Gerstoff², N. Obel², S. Blower¹

¹David Geffen School of Medicine at UCLA, Center for Biomedical Modeling, Los Angeles, USA; ²Copenhagen University Hospital, Department of Infectious Diseases, Copenhagen, Denmark

Background: The WHO has proposed using 'treatment as prevention' (TasP) to eliminate HIV, and UNAIDS has proposed treatment targets to be met by 2021. However, the effectiveness of TasP in the 'real-world' remains unknown. Here we conduct a proof-of-concept study to determine whether TasP could be an effective elimination tool. We determine the impact that TasP has had on the HIV epidemic in the MSM community in Copenhagen over the past two decades. UNAIDS has identified Copenhagen as a priority city, and the MSM community as a priority risk group, for HIV elimination. The WHO elimination threshold is one new infection per 1,000 individuals per year.

Material & Methods: We use ~20 years of treatment and diagnosis data from the Danish HIV Cohort Study (DHCS). We use a Bayesian CD4-staged back-calculation approach to analyze a historical dataset spanning almost two decades: we begin in 1996 when effective therapies were introduced. We then use a predictive model that simulates transmission dynamics from 2013 to 2025. The model is parameterized to reflect the epidemiological conditions in the MSM community in Copenhagen. The back-calculation model and DHCS treatment data provide initial conditions for the model.

Results: Our results show, between 1996 and 2013, the number of MSM capable of transmitting HIV decreased by ~63%: from 2,218 (median, 95% Bayesian credible interval, BCI: 1,955-2,381)

to only 819 (median, 95% BCI: 463-1,065). In addition, the annual number of new infections decreased by ~36%: from 117 (median, 95% BCI: 94-140) in 1996 to 75 (median, 95% BCI: 20-117) in 2013. We estimate by 2013 treatment coverage had reached 73% (median, 95% BCI: 67-83%). We found a strong negative correlation between coverage and incidence: coverage increased as incidence decreased. Using our transmission model we predict the WHO elimination threshold will be reached in the MSM community in Copenhagen by 2021. We predict the annual incidence in 2021 will be 0.9 (median, BCI: 0.7-1.2) new HIV infections per 1,000 MSM. Although this infection rate is below the WHO elimination threshold, it translates to a fairly high number, 51 (median, BCI: 39-64), of new infections in 2021.

Conclusions: Our study is the first to demonstrate that TasP can substantially reduce an HIV epidemic in the 'real-world'. It provides a proof-of-concept that TasP could be effective in eliminating HIV in resource-rich settings. Our results have significant implications for the use of TasP as a public health intervention and tool for HIV elimination. Notably, the conditions in Copenhagen have been optimal for TasP to have a significant effect: high treatment coverage, high viral suppression rates, and high retention. Even under these optimal conditions, it has taken several decades for TasP to have a population-level effect. Our results imply that it will be essential to use other interventions, such as pre-exposure prophylaxis, in combination with TasP. Most importantly, our results show that Copenhagen is very close to achieving the WHO elimination threshold in their MSM community. This provides hope that it will be possible to achieve the global elimination of HIV.

No conflict of interest

Abstract: 14

Biomedical Approaches of HIV prevention of transmission

Optimizing the rollout of “treatment as prevention” in Sub-Saharan Africa: efficiency in prevention versus equity in access to treatment

S. Blower¹, B.J. Coburn¹, J.T. Okano¹

¹UCLA David Geffen School of Medicine, The Semel Institute, Los Angeles, USA

Background: WHO, and UNAIDS, have proposed using 'treatment as prevention' (TasP) to eliminate HIV in sub-Saharan Africa (SSA). This would be extremely challenging. HIV prevalence is high, epidemics 'hidden', and TasP expensive. We address all three issues. We design rollout strategies for TasP based on different optimization criteria: maximizing efficiency in prevention versus ensuring equity in access to treatment. Strategies are calculated in terms of the division of a supply of treatment among - and within - healthcare districts (HCDs). We focus on Lesotho, where HIV prevalence in the general population is 40% and treatment coverage is ~30%.

Material & Methods: We use kriging and adaptive bandwidth kernel density estimation to construct a concentration of infection (Col) map that reveals Lesotho's 'hidden' HIV epidemic. We use georeferenced HIV-testing data from ~7,000 individuals (aged 15 to 49 years old), and high-resolution demographic data. We then use the Col map and optimization techniques to calculate treatment allocation strategies that maximize either the effectiveness of TasP, or equity in access to treatment.

Results: Our Col map shows the number and geographic location of all HIV-infected individuals (aged 15 to 49 years old) in Lesotho, at a resolution of 0.01 km². We estimate there are ~188,000 HIV-infected individuals; ~70% live in rural areas where the average Col is 4-10 HIV-infected individuals/km². The remaining 30% lives in urban areas where there are ~400 HIV-infected individuals/km². We identified a quantitative

relationship between the Col and treatment coverage goals. We used this relationship to compare rollout strategies. We found significant differences in the geospatial allocation of treatment depending upon whether it was being allocated in order to maximize efficiency in prevention or to ensure equity in access to treatment. Differences were in terms of the allocation amongst the HCDs, and in the division of resources between urban and rural communities. We found that in some HCDs more treatment is needed if the objective is to maximize the efficiency in preventing HIV infections than if the objective is to achieve equity in access to treatment. However in other HCDs, the opposite holds true. In these HCDs less treatment is needed if the objective is to maximize efficiency than if the objective is to achieve equity in access.

Conclusions: Our results have significant implications for global health policies, TasP implementation, and HIV elimination in SSA. Our results apply to other countries in SSA with generalized HIV epidemics and a large rural population. Our results clearly show it will not be possible to maximize the efficiency of TasP and to ensure equity in access to treatment. Choosing to maximize efficiency will be more beneficial for uninfected individuals in urban areas (their risk of infection would be reduced). Choosing to ensure equity in access will be more beneficial for HIV-infected individuals in rural areas (their mortality risk would be reduced). Choosing to optimize the rollout of TasP based on efficiency would increase the probability of eliminating HIV, but would exacerbate the already significant health disparities between urban and rural communities.

No conflict of interest

Abstract: 15

Biomedical Approaches of HIV prevention of transmission

Pre-Exposure Prophylaxis Modality Preferences among Men who Have Sex with Men

E. Hall¹, W. Heneine², T. Sanchez¹, C. Sineath¹, P. Sullivan¹

¹Emory University, Rollins School of Public Health, Atlanta, USA; ²Centers for Disease Control and Prevention, Division of HIV/AIDS Prevention, Atlanta, USA

Background: Pre-Exposure Prophylaxis (PrEP) is currently available as a daily pill for Human Immunodeficiency Virus (HIV) prevention. Innovative methods of administering PrEP systemically or topically are being developed and we sought to assess attitudes towards the different modalities.

Methods: From April to July 2015, we recruited 1109 HIV-negative men who have sex with men (MSM) through online social media advertisements and surveyed them about their likelihood of using different PrEP modalities. Participants responded to 5-point Likert items indicating how likely they were to use each of the following potential PrEP modalities: a daily oral pill, 'on-demand' pills, periodic injection, penile gel (either before or after intercourse), rectal gel (before/after) and rectal suppository (before/after). Wilcoxon signed rank tests were used to determine if the stated likelihood of using any modality differed from daily oral PrEP. Related items were combined to assess differences in likelihood of use based on tissue or time of administration. Participants also ranked their interest of using each modality and the modified Borda count (MBC) method was used to determine consensual rankings.

Results: The majority of participants indicated they would be somewhat likely or very likely to use PrEP as an on-demand pill (62%), daily oral pill (51%), injection (53%) or penile gel (58% before intercourse; 54% after). The stated likelihood of using on-demand pills (mean score=3.63) or a penile gel before intercourse (mean=3.44) were

both higher than a daily oral pill (mean=3.31, both p-values <0.01). Compared to a daily oral pill, participants reported a significantly lower likelihood of using any of the four rectal modalities (all p-values <0.001). When combined by application method, the reported likelihood of using a penile gel was higher than using a rectal gel (p-value<0.001), which was higher than using a rectal suppository (p-value<0.001). The MBC ranked on-demand pills as the most preferred modality of PrEP. There was not a difference in likelihood of use of PrEP (as a gel or suppository) before or after intercourse.

Conclusions: Participants typically prefer systemic PrEP and are less likely to use a modality that is administered rectally. While most of these modalities are seen as favorable or neutral, attitudes might change as information about efficacy and application become available. Further data on modality preference across risk groups will better inform PrEP development.

No conflict of interest

10th International Workshop on HIV Transmission

5 – 6 December 2015, Atlanta, USA

**Abstracts
Poster Presentations**

Abstract: 16

Behavioral risk factors affecting HIV Transmission

Understanding the Factors Associated with Alcohol Use among Female Sex Workers (FSWs) In a High HIV Prevalence Northeast State of India

S. Sharma¹

¹*International Institute for Population studies, Dept. of mathematical Demography and Statistics, Mumbai, India*

Introduction: The paper illustrates the factors associated with alcohol use among FSWs in Dimapur, an important commercial hub of Nagaland, which is a high HIV prevalence state of India.

Materials & Methods: Analysis is based on 417 FSWs aged above 18 years who participated in round 2 of *Integrated Behavioural and Biological Assessment (IBBA)*. There is significant association between ever consumption of alcohol use among female sex worker and education, ever drug use, needle / syringe changing behaviour, age at first sex, age at first started sex work, volume of clients per week and condom use with occasional, regular clients ($p < 0.05$).

Results: Binary logistic regression of Alcohol use among Female sex workers found that greater than 25 years of FSWs (2.2 times, $P \leq 0.10$), divorced/Separated (0.41 times, $p \leq 0.10$), >10 the standard of education (0.311 times, $p \leq 0.001$), drug use (5 times, $p \leq 0.001$), sharing of injecting drugs with the partner (3.7 times, $p \leq 0.001$) were independently associated with Alcohol use respectively. Those FSWs have first sex and first started sex work at age 15-20 years were 6.3 times ($p \leq 0.05$) and 2.4 ($p \leq 0.05$) times more likely to use alcohol respectively. One important finding is those female sex workers were less likely to use alcohol consumption that has 5-9 clients per week. Alcohol using older (25+ years) FSWs were more likely to have HIV seropositivity almost 9 times. It was also reported that married FSWs those using alcohol, were less

likely to have HIV, And those alcohol using female sex worker aged at first sex was 15-20 years, they are at 5 times more likely to have HIV seropositivity.

Conclusion: Alcohol using female sex worker were 30 times more likely to have HIV seropositivity those who served their client at lodge/ Hotel and they were less likely to use condom with all type of sexual partner.

No conflict of interest

Abstract: 17

Behavioral risk factors affecting HIV Transmission

The Use of Mobile Apps and Automated Messages by Health Facilities in low-resource areas to enhance Antiretroviral adherence and delivery of HIV treatment and care in Nigeria

S. Akwafuo¹, A. Eze², K. Ogungbemi³

¹*National Agency for the Control of AIDS, Monitoring and Evaluation, Abia State, Nigeria;* ²*National Agency for the Control of AIDS, PMTCT, Abia State, Nigeria;* ³*National Agency for the Control of AIDS, Monitoring and Evaluation, Abuja, Nigeria*

Background: Adherence to treatment schedule is one of the major priority areas of care and support to People Living with HIV/AIDS (PLWHA) in Nigeria. This is necessitated by the recent revelations about low adherence rate to treatment. There are real concerns about low adherence rate in Nigeria. Concerted efforts are being made at devising an effective means of increasing adherence rate. There is increasing research interest into the use of mobile phone and apps to enhance services by public health professionals. This article presents a study undertaken across the six geographical zones of Nigeria on the

effectiveness of the recently introduced text messaging services to people enrolled in treatment and care by health facilities.

Methodology: The study was facilitated by the National HIV/AIDS Resource Centre (NHRC). Two states were selected in each of the zones for the pilot study. Two health facilities were further selected in each of the states. The 24 facilities were visited and names of people who initially indicated no objection and interest to be part of researches and survey were contacted. From each of the health facilities, six respondents were picked and placed on observation list for 14 days. They were given forms to fill times of receiving the text and when they took their medications. They were various questions as indicated in the appendix.

Results: 89% of participants took their medication within five minutes of receiving the test message. 66% would have missed their medication or considerably delay their medication if the text message had not arrive. All of the participants reported having positive feelings about receiving text messages from the clinic staff, and many reported that the calls were helpful reminders in relation to medication adherence. It was also observed many of the participants previously set up reminders on their mobile phones. However, 33% of them will always snooze the reminder and forget to take their medication. 69% indicated interest in receiving an interactive voice response call.

Conclusion and Recommendations:

As shown in this study, the use of mobile messaging and internet app services has gone a long way in improving adherence to antiretroviral treatment by PLWH across Nigeria. This has further strengthened the Nigerian National Response to HIV/AIDS epidemic. The use of Information technology in the delivery of public health services has proved to be effective. The study also concluded that the use of phones could reduce loss to follow-up, which is a considerable problem confronting treatment adherence. It is therefore recommended that this service be sustained and continually proved upon. Efforts should be made at ensuring confidentiality of messages sent out, such that only the receiver will decipher the meaning and implications of the content. Efforts should also be made at

incorporating picture messages and interactive voice response calls into the scheme.

No conflict of interest

Abstract: 18

Behavioral risk factors affecting HIV Transmission

Occupational Exposure of HIV among outreach workers: a qualitative study in Sindh, Pakistan

J. Hasnain¹, S. Hussain¹, M. Badshah², S.A. Shah³, S. Vermund⁴

¹Bridge Consultants Foundation, Community Home Based Care, Karachi, Pakistan; ²Pak International Medical College, Anatomy, Peshawar, Pakistan; ³Bridge Consultants Foundation, Executive Director, Karachi, Pakistan; ⁴Vanderbilt University, VIGH, Nashville, USA

Background: With the increase in the Pakistani HIV/AIDS epidemic, healthcare providers such as outreach workers (ORWs) working with injection drug users (IDUs) are being exposed to HIV infection by needle-sticks. This risk can be substantially reduced by taking appropriate measures to prevent needle stick injuries and following the guidelines and proper implementation of post-exposure prophylaxis (PEP). We studied risk factors related to needle stick injuries, assessing knowledge, attitudes, and practices of HIV PEP among the ORW working with IDUs in different hot spots of Karachi district of Sindh Province.

Material and Methods: Insight was gained from a focus group discussion (FGD), in-depth interviews (IDIs) and through a 'Thinking Group of Safe Injection Practices'. This group consisted of experts in injection safety, IDUs and Public Health and provided their professional views on the subject. Feedback was also sought through a FGD that was conducted with seven ORWs. Five

IDIs were also conducted with ORWs including one IDI that was carried out with an ORW who received a needle stick injury. These FGDs and IDIs were conducted using semi-structured questionnaires. Information about occupational exposures of HIV, risk factors related to needle stick injuries, knowledge regarding HIV PEP, its uses, timeline restrictions and access were collected and analyzed thematically.

Results: Six main themes emerged from the data: (1) most occupational exposure accidents happened either during service delivery at the hot spots when the ORWs were approached by more than five IDUs and the ORW did not have time to consider self-protection or because of hostile behavior of IDUs towards the ORWs; (2) though ORWs were given some information related to needle stick injuries and HIV PEP, they did not receive proper training such that knowledge and compliance were poor; (3) negligence on the part of ORWs regarding self-protection was admitted; (4) barriers to better compliance included the lack of any medical background among the ORWs and unavailability of appropriate protection gear (gloves, safety glasses, gowns, and protective footwear); (5) although a proper system regarding HIV PEP exists in Karachi, most ORWs were not aware of it, resulting in delayed initiation of PEP; (6) exposure of HIV caused ORWs severe adverse psychological pressure, such as stress, anxiety and post-traumatic stress disorder (PTSD).

Conclusion: This study highlights the need to strengthen the existing HIV prevention strategies and safety of ORWs and institutional support in promoting compliance with PEP guidelines among the ORWs. Further it emphasizes the need of training on precautionary measures that may reduce the risk of occupational HIV infections among these workers.

No conflict of interest

Abstract: 19

Behavioral risk factors affecting HIV Transmission

Acceptability of couples' voluntary HIV testing among HIV-infected patients in care and their HIV-negative partners

K. Wall¹, L. Canary², K. Workowski³, A. Lockard³, J. Jones¹, P. Sullivan¹, K. Hills³, K. Fofana⁴, R. Stephenson⁵, S. Allen³

¹Emory University, Epidemiology, Atlanta, USA; ²CDC, Division of Viral Hepatitis, Atlanta, USA; ³Emory University, School of Medicine, Atlanta, USA; ⁴Georgia State University, Public Health, Atlanta, USA; ⁵University of Michigan, Health Behavior and Biological Sciences, Ann Arbor, USA

Background: Couples' voluntary HIV counseling and testing (CHTC) is an HIV risk reduction strategy adapted for use in the US and supported by the CDC. However, no current data exists on the acceptability of CHTC among HIV-infected clinic patients, heterosexual or MSM, who are either newly diagnosed or already established in care. Additionally, the willingness of partners of HIV-negative persons to participate in CHTC with their positive partners has not been evaluated.

Methods: We assessed willingness to participate in CHTC among US HIV-infected clinic patients via tablet-based survey and among HIV-negative persons with HIV-infected partners in care via mixed-method in-depth phone interviews.

Results: Most of the N=64 HIV-infected partners surveyed were men (89%), on ART (92%), and many self-identified homosexual (62%). We observed high levels of willingness to participate in CHTC (64%) among HIV-infected partners. Reasons for not wanting to participate included perceived lack of need (26%), desire to self-disclose their status (26%), and fear of being asked sensitive questions with their partner present (17%). HIV-infected partners were interested in discussing ART (48%), other STIs (44%), and relationship agreements like monogamy (31%) during CHTC sessions. All N=15 HIV-negative partners interviewed were men, most identified as homosexual (73%), and

about half (54%) reported consistent condom use with HIV-infected partners. We observed high levels of willingness to participate in CHTC (87%) among HIV-negative partners, who were also interested in discussing ART (47%), other STIs (47%), mental health services (40%), and relationship agreements (33%). Most negative partners (93%) indicated that they believed their HIV-infected partner was virally suppressed, but in the event that they were not, many (73%) were willing to take PrEP.

Conclusions: Our results indicate that CHTC for serodiscordant couples is acceptable and should emphasize aspects most pertinent to these couples, such as discussion about ART/PrEP, STIs, relationship agreements, and how to best support the partner in care. It may be necessary to highlight the benefits of CHTC beyond serostatus disclosure to this population, and dispel myths about what sensitive information may be elicited during CHTC. Expansions of CHT service should consider the needs of known serodiscordant couples in both marketing and service provision.

No conflict of interest

Abstract: 20

Behavioral risk factors affecting HIV Transmission

Does Participation in Harm Reduction Program Exclude Risky Behaviours of PWIDs?

M. Gogia¹, K.H. Kutateladze¹, G. Jikia¹

¹*Georgian Harm Prevention Network, HIV prevention, Tbilisi, Georgia*

Background: Needle sharing practice remains the main factor for spreading HIV (47.3%) among people who inject drugs (PWIDs) in Georgia. Estimated number of injecting drug users have been increasing during recent years in Georgia and is considered to be 49700. Needle-Syringe Programs (NSP) has been functioning in the country since 2005 but in limited volume. NSP

program coverage of PWIDs had increased 3 times during last 2 years. Significant amount of PWID have never tested on HIV (40-75%). The objective of this study is to analyse risky injection and sexual behaviour of PWIDs who are clients of NSP, to assess how participation in NSP program results in their risky behaviour.

Methods: Snowball sampling was used to recruit PWIDs during 5 months in 2015. The selection criteria was: a) Drug injection practice during last month; b) be a beneficiary of NSP program for more than 6 month; c) age should be 18 or more and d) willing to participate voluntarily into study. Sample size was 1032, totally beneficiaries of 13 NSP sites participated in the study. Structured standard questionnaire of Risk Assessment Battery (RAB) was used to assess Drug Risk and Sex Risk Items separately and calculate RAB Score (Drug Risk Total+Sex Risk Total). SPSS 15.0 was used for data analyses.

Results: PWIDs mostly inject drugs with more than 2 people (57.66%). 38% of study participants had Needle sharing practice during last month, among them 43.8% shares with only 1 and 56.1% with more than 2 persons ($p<0.05$). Among those who used non-sterile syringe, 15.8% cleaned it by soap and water, 17.9% by hot water, 3% by Alcohol. Sharing rate of other injecting equipment is 58.6%, sharing of cotton 25.8%; sharing of syringe cleaning water 32.7%. As referring to sexual practice, 32.2% PWIDs had more than 2 sex partners during 6 months and with whom they use condoms mostly 32.2%, sometimes 14.1%, always 28.7. Meaningful was to reveal, that 20.6% of study participants had never tested on HIV and 32.3% was tested more than a year ago. Total RAB Scale Score is equal to 0.6 (Range=0-1).

Conclusion: The study results demonstrate that PWIDs practice risky behaviour despite involvement in harm reduction program. Although condom use practice is relatively improved since 2012 among NSP beneficiaries. HIV testing rate among PWIDs is attempted and needs further investigation of reasons for it. The findings of this study will be used to address needs, modify program direction or implement new approaches to increase safe behaviours of PWIDs.

No conflict of interest

Abstract: 21*Modeling of HIV Transmission***The use of a mathematical model and risk ratio to estimate the impact of HIV/AIDS intervention programs on FSW and their communities: The Nigerian Experience**

S. Akwafuo¹, D. Ogbang², K. Ogungbemi³, M. Morka⁴

¹National Agency for the Control of AIDS, Monitoring and Evaluation, Abia State, Nigeria; ²United Nations Joint Programs on AIDS (UNAIDS), Monitoring and Evaluation, Abuja, Nigeria; ³National Agency for the Control of AIDS, Strategic Knowledge Management, Abuja, Nigeria; ⁴Federal Ministry of Health, National AIDS/STIs Control Program, Abuja, Nigeria

Introduction: Having determined that Female Sex Workers (FSWs) are one of the most important drivers of the HIV epidemic in Nigeria, a combination prevention program for FSW was recently launched. As part of the evaluation of the impacts of these programs, a mathematical modelling Technical Working Group (MMTWG) was set up. Various sub-groups of the TWG worked on different interventions of the program. The FSW sub group developed a mathematical model to estimate the impact of the intervention programs on the rate of new infections for HIV and Sexually Transmitted Diseases (STIs).

Objectives: The objective of the MMTWG was to develop a mathematical model to estimate how many indirect HIV infections would be inverted among clients of FSWs and the general population, attributable to prevention programs targeting female sex works in Nigeria. Risk ratio was used to estimate the impact of the programme on new HIV infections among the FSWs while a mathematical model was used to estimate the number of new HIV infections in clients and members of the general population that were averted as a result of the effect of the intervention among the sex workers.

Methodology: A mathematical model (as shown in the appendix) was developed, using python programming language. Variables were selected and the current values of the variables served as baseline inputs to the model. The variables include initial prevalence of HIV among FSW, their clients and general female (GF); proportion of sex acts that are protected; Initial population of the target group; duration of the intervention; number of sexual contacts per FSW and average number of sexual acts. Specific risk equations were developed for the FSWs and other groups. Three Scenarios of the model was estimated over a period of five years. Putting more persons on treatment by increasing CD4 baseline (from present 350 cells/microls to 500 cells/microls) and keeping other variables constant; Putting 80 of eligible FSWs; and universal access to treatment (TaP). An uncertainty analysis was also carried out as part of the running of the model.

Results: It was observed that if the status quo (37% of eligible positive FSW on treatment) is maintained, the new infection rate will gradually increase to 3.6 in five years' time. Putting 80% of eligible positive FSWs on treatment will avert 2789 new infections in the same duration and reduce the current rate of new infections to 0.7. A slight decrease of 0.3% would be experienced in the general female population. Putting all FSWs on treatment returns a 89.7% reduction on the number of new infections among clients of FSW.

Conclusion and Recommendations: The mathematical model reveals the efficiency of treatment in reducing the rate of new infections among FSWs, their clients and general female. The models reveals the importance of the investing in the FSW intervention programs. The model outputs can be used to calculate the Quality Adjusted Life Years (QALY) to be gained during the intervention. Further modelling scenarios are required to effectively infer on the efficiency of the intervention programs.

No conflict of interest

Abstract: 22*Mother to Child Transmission***Prevention of Mother to Child Transmission of HIV; Socio-demographic determinants of knowledge, attitude and practice among pregnant women in Abia State, Nigeria**A. EZE¹, E.C. Aniwada²¹National Agency for Control of AIDS (NACA/SURE-P HIV Programme), PMTCT/HCT, Umuahia, Nigeria; ²University of Nigeria, Department of Community Medicine College of Medicine, Enugu, Nigeria

Introduction: Transmission of HIV from pregnant mothers to their children is a key route of HIV transmission. Nigeria contributes about 30% of the global PMTCT gap and coverage of PMTCT services remained low at <19% - falling short of both the universal access and National Strategic Plan targets. The study aims to identify factors underlying the low uptake of PMTCT interventions in rural and urban public primary health facilities of Abia state, Nigeria.

Material & Methods: A cross-sectional analytical study design was used with three stage sampling method to select 350 clients in 10 of 74 health centers that offer PMTCT services in the state. Clients were women who attended antenatal care in the facilities. Outcome measures are knowledge, attitude and practice of PMTCT services.

Results: Their mean and standard deviation for knowledge score were 17.85(3.61) and 13.85(4.33), attitude 4.83(0.51) and 4.22(1.07), practice 6.89(1.44), 6.74(2.29), for urban and rural areas respectively. There were significant differences for knowledge and attitude $p < 0.001$ but not significant for practice $p = 0.451$. There were significant associations between socio-demographics: (education in urban $p < 0.001$, employment $p < 0.001$ and income for rural $p = 0.039$) and mean knowledge, (age in urban $p = 0.001$ and income for rural $p = 0.017$) with mean

attitude and income for urban $p = 0.043$ with mean practice. Predictors of PMTCT uptake were income (AOR=4.7, 95% CI: 1.9-11.5) for knowledge and employment (AOR=3.7, 95% CI: 1.2-10.7) for attitude.

Conclusion: Education, employment and income influences PMTCT uptake. Empowering women with quality education will go a long in PMTCT as it determines their employment status as well as earnings.

*No conflict of interest***Abstract: 23***Mother to Child Transmission***Mother-to-child HIV transmission and its predictors among HIV-exposed infants under prevention of mother to child transmission program in Ethiopia***M. Jano¹, B. Birlie², T. Akaba², K. Sisay², A. Gurmessa²*¹Jimma University, Department of Medical Laboratory Sciences and Pathology, Jimma, Ethiopia; ²Jimma University, Department of Statistics, Jimma, Ethiopia

Background: Despite the marked progress in coverage of prevention of mother to child transmission (PMTCT) of HIV programs, high rate MTCT was documented among exposed infants. This raised questions about the effectiveness of PMTCT program and the need for more research on identifying predictors of MTCT of HIV. This study aimed to quantify MTCT rate of HIV and to identify predictors among HIV-exposed infants at PMTCT clinic in Southwest Ethiopia.

Methods: Institution based retrospective follow up study was carried at Jimma University Specialized Hospital PMTCT clinic. Data were

extracted from medical records of HIV-infected women and exposed infants between September 2010 and December 2011. Univariate and multivariate logistic regression analyses were carried out to identify potential infant and maternal factors predicting infant infection status.

Result: A total of 146 infants born to HIV-infected mothers were included in the analysis. MTCT of HIV occurred in 17.1%, (95% CI: 11.01- 23. 23) of exposed infants. In the adjusted multivariate logistic regression model, mothers being on HAART or use of single dose NVP during labour (OR = 0.21; 95% CI: 0.051-0.435), antenatal care (ANC) (OR = 0.19; 95% CI: 0.035 - 0.571), mixed feeding (OR=0.1; 95% CI: 0.011 - 0.515), and exclusive complementary (replacement) feeding (OR=0.08; 95% CI: 0.010 - 0.428) reduced the risk MTCT of HIV, while mothers being at HIV stage 3 or 4 (OR = 9.83; 95% CI: 1.686 - 16.505) amplify risk of MTCT.

Conclusion: There is a high risk of MTCT among exposed infants on follow up at the PMTCT clinic in Southwest Ethiopia. Increasing coverage of ANC, putting HIV infected pregnant mothers on HAART and counseling mothers about the risk of breast feeding could reduce HIV MTCT rates.

No conflict of interest

Abstract: 24

Mother to Child Transmission

Evaluation of in-house nested HIV-1 DNA PCR test using dried blood spots for the early diagnosis of HIV-1 infection in infants in South India.

A. Durairaj¹, S. Mini Jacob¹, K. Siva Sangeetha¹, K. Mary Sush¹, A. Ganesan¹, G. Thatchinamoorthy¹, G. Patchiappan¹, R. Vanitha¹, D. Shantharam²

¹Tamilnadu Dr MGR Medical University, Department of Experimental Medicine, Chennai, India; ²Tamilnadu Dr MGR Medical University, Vice-Chancellor, Chennai, India

Introduction: HIV-1 DNA PCR is the gold standard for diagnostic testing of infants and children younger than 18 months born to HIV seropositive mothers. The objective of this study was to compare the results between in-house nested HIV-1 DNA PCR test and Roche Amplicor HIV-1 DNA test using dried blood spots (DBS) in the early diagnosis of HIV-1 infection in infants.

Methods: This was a cross sectional study. HIV exposed infants born to HIV seropositive mothers were enrolled from PMTCT centres of Namakkal district, South India. After obtaining written informed consent from the parent/guardian, the blood samples of HIV exposed infants were directly spotted onto Protein saver Whatman 903 cards by heel/ toe and finger prick. After drying, the DBS was kept in an individual ziplock bag along with the desiccants and was transported into the Department of Experimental Medicine for testing. DNA was extracted from the DBS with 10% Chelex-100 resin and in-house nested PCR amplification was performed using gag gene. The commercially available Roche Amplicor® HIV-1 DNA test kit was used for the same DBS samples. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) was calculated.

Results: A total of 125 infants samples were collected and tested. Their age ranged from 15 days after birth to 12 months. Thirteen (10.4%) infants were found to be HIV-1 positive and 112 (89.6%) were HIV-1 negative using the two methods of PCR. Among the HIV positive infants, 7 were males and 6 were females. When compared with Roche Amplicor DNA Test, the sensitivity, specificity, PPV and NPV of in-house PCR test was 100 %.

Conclusion: The study suggests that in-house nested HIV-1 DNA PCR using DBS can be used as an alternative reliable test for the diagnosis of HIV infection in infants in the resource limited settings.

No conflict of interest

Abstract: 25*Virology of HIV Transmission***Reduced incidence of intra-subtype superinfection is associated with more frequent counseling***E. Woodson¹, D. Basu¹, P. Hrabec², H. Khu³, W. Kilembe⁴, J. Mulenga⁴, S. Allen⁵, E. Hunter⁶*

¹Emory University, Emory Vaccine Center, Atlanta GA, USA; ²Los Alamos National Laboratory, Theoretical Biology and Biophysics, Los Alamos, USA; ³Emory University, Department of Global Health Rollins School of Public Health, Atlanta, USA; ⁴Emory University, Zambia Emory HIV Research Project, Lusaka, Zambia; ⁵Emory University, Pathology, Atlanta, USA; ⁶Emory University, Emory Vaccine Center, Atlanta, USA

Introduction: Understanding the factors that predispose individuals to HIV-1 intra-subtype superinfection may help identify key determinants of transmission/acquisition. In a previous comparative analysis, we showed that 3/22 individuals became superinfected (SI) within the first year of infection. These individuals mounted a statistically significantly lower antibody (Ab) response to their respective founder viruses compared to non-superinfected controls, which likely contributed to increased susceptibility to re-infection.

Materials and Methods: In the current study, an additional 55 newly infected individuals from the Zambia-Emory HIV Research Project (ZEHRP) transmission cohort were screened for evidence of SI. Briefly, viral RNA was extracted from longitudinal plasma samples and reversed transcribed. Nested PCR amplifications were performed for both HIV-1 gp41 and p17 regions. Population sequences from amplicons were analyzed by phylogenetic analysis, and degenerate bases were counted for evidence of mixed viral populations. Suspected cases of SI were confirmed by single genome amplification of full-length *Env* genes from plasma at longitudinal time points.

For neutralization assays, full-length *Env* sequences were directionally cloned, sequence

verified, and co-transfected with an env-deficient subtype B provirus to generate env-pseudoviruses. Titered env-pseudoviruses were tested for neutralization against autologous plasma. Luciferase expression was quantified as in a standard JC53-BL neutralization assay. Percent viral infectivity and correlating neutralization IC50 values (representing plasma dilution resulting in 50% viral infectivity) were determined using a linear-regression-least squares fit method.

As in our previous analysis, behavioral risk factors were compared between SI and non-SI individuals using the Wilcoxon rank sum test or the Fisher's exact test as appropriate. All analyses were performed using SAS (Cary, NC) and p-values <0.05 were considered statistically significant.

Results: Compared to the incidence of intra-subtype SI in Zambian seroconverters in the initial study, the incidence of SI measured here was significantly lower (13.6% to 1.8%). This marked difference in frequency of SI between the two studies is likely due to an increased number of early study visits (7, [range of 5-17] vs 5.6, [range of 4-9]) and decreased risk behaviors (as assessed by genital inflammation) within the first year of primary infection in the current study [$p < 0.0001$ and $p = 0.04$, respectively]. The single case of intrasubtype SI identified in the current study showed an earlier, more robust Ab response to founder virus than previously identified SI individuals.

Conclusions: These data support the extended benefit of couples voluntary counseling and testing (CVCT) to reduce the behavioral risk of SI in a heterosexual cohort. In some instances, SI can occur in the presence of a moderately robust Ab response to the primary virus, suggesting there may be alternate factors contributing to susceptibility in HIV-1 SI. Additional experiments are currently underway to evaluate the role of the CTL response in protection against HIV-1 intra-subtype SI.

No conflict of interest

Abstract: 26*Virology of HIV Transmission***Quantitative Real-Time PCR Assay Reveals Differences in HIV RNA Quality Among Clade C Virus Donors and Recipients***S. Connolly¹, M. Deymier¹, E. El-Badry¹, E. Hunter¹*¹*Emory University, Emory Vaccine Center, Atlanta GA, USA*

Background: Acute HIV infection is characterized by a genetically uniform population of viruses, established by a single transmitted founder virus (TFV) from the donor quasispecies. By investigating samples from heterosexual transmission pairs, it is possible to define both TFV and non-transmitted variants in both the donor and recipient using single genome amplification (SGA) and sequencing. Traditionally, SGA has been achieved via a time-consuming series of cDNA dilutions, followed by gel electrophoresis.

Methods: In order to increase the efficiency of near full-length genome SGA, we developed a clade C specific quantitative real-time PCR (qPCR) assay to quantify the amount of cDNA generated based on three specific regions of the HIV genome: *gp41*, integrase, and *gag*. We infer the quality of HIV RNA by simultaneously measuring amplicons from the 3' end, center, and 5' end of cDNA using Sybr Green fluorescent chemistry (Life Technologies) and real-time detection. All three primer sets were optimized for concentration and annealing temperature so that all three targets could be tested simultaneously on the same reaction plate, reducing time and minimizing freeze/thaw cycles, which damage cDNA. Quantity is determined based on comparison to a standard curve using the linearized plasmid of a clade C TFV clone.

Results: In this assay, we have confirmed that plasma sample viral load is correlated to both the amount of *gag* ($R^2=0.45$, $p=0.0062$) and *gp41* ($R^2=0.5$, $p=0.0031$) detected in the cDNA, however there is a dichotomy in cDNA quality as shown by the *gag:gp41* ratio. We tested eleven

individuals, eight recipients and three donors, with varying viral loads (range: 2060 - 16.6×10^6 copies/mL). The ratio of *gag* to *gp41* varied for these individuals, with 5/8 recipients having the highest ratio and all 3 donors having a significantly lower ratio (two tailed t test, $p=0.05$).

Conclusion: Although a significant positive correlation of *gag* and *gp41* copies to plasma viral load exist in this real-time quantitative assay, the *gag:gp41* ratio of cDNA may be impacted by donor/recipient status due to differences in population diversity and alternative HIV transcripts. This real-time quantitative PCR technique will be used as a tool for optimizing RNA extraction and cDNA synthesis, with the goal of increasing throughput and efficiency in amplifying near full-length genomic HIV.

*No conflict of interest***Abstract: 27****Prevalence and clinical and biological profile of patients co-infected with HIV-HBV monitored at Ambulatory Treatment Center (ATC) in CHNU Fann, of Dakar***M. Ali Bolti¹, A. Sow Sall², N.F. Ngom², F. Diop³, M. Diop², L.F. Deguenonvo¹, N.A. Lakh², D. KA², K. Mbaye¹, M.P. Diallo¹, A. Diouf¹, C.T. Ndour¹, N.C. Touré-Kane³*¹*Service infectious disease Fann Dakar;* ²*Centre outpatient treatment Fann Dakar;* ³*Laboratory Bacteriology-Virology of the Aristide Le Dantec hospital in Dakar.*

Introduction: HVB chronic infection touched around 5-15% of 34 million of Persons Living With HIV AIDS in the World. The HIV infection alters the natural history of HBV infection and generally worsens the prognosis of chronic hepatitis B. The aim of this study was to assess prevalence, clinical and biological profile of HVB-HIV co-

infected patients of Ambulatory Treatment Center (ATC) of HIV in CHNU Fann, of Dakar.

Methods: It was retrospective study focused on HVB-HIV co-infected patients registered and followed between 1st January 2010 and 31th December 2014. Epidata 6 software and Khi2 test was used for comparisons of frequencies and Student test for means comparisons.

Results: 58 were co-infected among 457 detected. HVB-HIV co-infection prevalence was 12.7%. The sex-ratio was 1.23. The mean age was 39.62 ± 10.12 . The range age of [30-49] was most represented with 60.34%, [IC95%: 37.49-87.16]. The median CD4 was 235 cells/mm³ [3-936]. The median HIV viral load was 4.11log copies/mm³. The ALAT mean was 27.22 ± 25.11 . At baseline, 3 / 37 (8.11%) had HBV DNA greater than 10,000 copies / ml. One patient among 3 was HVB viral load undetectable at 6 months. Of the 58 patients, 46 (79.31% [66.65 to 88.83%]) were under ARV therapy containing TDF / FTC or TDF / 3TC.

Conclusion: The seroprevalence of viral hepatitis B remains high (12.70%) among Persons Living With HIV AIDS in Dakar. It confirms the geographic distribution of HBV endemicity, that the prevalence of HBV would be 8% in Sub-Saharan region. Co-infected patients are predominantly young males, severely immunosuppressed, admitted to stage III and II of the WHO, about little replicative HBV.

No conflict of interest

10th International Workshop on HIV Transmission

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