P15.02 HUMAN HAEMOGLOBIN DERIVED PEPTIDE PREVENTS HIV-1 INFECTION AND PROTECTS CELLS FROM HIV-1 INDUCED INFLAMMATION

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Introduction HIV/AIDS pandemic is one of the leading cause of death worldwide and a matter of serious concern to the scientific world. Most of new HIV infections spread through heterosexual mode and leads to HIV-1 induced immune system activation. This renders infected individuals more susceptible to HIV-1 pathogenesis and opportunistic infections. Hence, preventing HIV infections at early stages and neutralising its effect on host cells is essential in combating AIDS epidemic.
Methods Human haemoglobin derived peptide, named HbAHP-25, was designed *in silico* against CD4 binding domain of gp120 by molecular docking methods. HbAHP-25 was characterised for its inhibitory activity on various strains of HIV-1 in PBMCs in the presence and absence of seminal plasma and vaginal fluid. Specificity of action of HbAHP-25 was determined by HIV-1 pseudotyped assays. Immunofluorescence, Multiplex Cytokine assay and Dual Chamber assays were performed to evaluate safety of HbAHP-25 and its role in modulating immune response to HIV.
Results HbAHP-25 has significant anti-HIV activity against various strains of HIV-1 in a dose dependent fashion. HbAHP-25 binds to a site proximal to CD4 binding site on gp120, has partial epitope similarity with VRC01 on gp120 and inhibits gp120-CD4 interaction. Flow cytometery analysis showed that HbAHP-25 specifically binds to gp120 expressing HL2/3 cells. HbAHP-25 inhibits HIV-1 and doesn't inhibit HIV-1 pseudotyped virus from entering cells. Further, HbAHP-25 didn't affect cell viability even at higher concentrations; nor did it have any effect on epithelial monolayer integrity. HbAHP-25 doesn't elicit any pro-inflammatory response and protects cells from HIV induced inflammation. Results indicate that HbAHP-25 prevents HIV-1 from activating NF-kB pathway, thus limiting its ability to induce cytokines.
Conclusion HbAHP-25 protects cells from HIV-1 entry and HIV-1 induced inflammation by binding proximal to CD4 binding site of gp120. HbAHP-25 maintained good safety profile and can be a potential molecule for pre-clinical development of prophylactic/anti-HIV drug.
Disclosure of interest statement Nothing to declare.

P15.03 DEVELOPMENT OF A NEW CEM REPORTER T-CELLS (GXR-CELLS) VIRAL INHIBITION ASSAY (VIA) FOR ELUCIDATING THE ROLE OF CLASS-I-HLA ALLELES ON THE INHIBITORY CAPACITY OF HIV-1-SPECIFIC CD8$^+$T-CELLS

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Introduction Standard immunogenicity assays, such as ELISpot and intracellular cytokine staining, fail to correlate HIV-1-specific CD8$^+$T-cells responses with HIV-1 replication *in-vivo*. Therefore, it is essential to develop assays that can determine antiviral potential of vaccine elicited CD8$^+$T-cells. The current ELISA-VIA measures HIV-1-p24 production overtime in autologous CD4$^+$T-cells. However, it is not designed to identify the class-I-HLA-allele involved in mediating the response. We developed a new FACS based VIA that can investigate CD8$^+$T-cells antiviral potential in the context of restricting class-I-HLA alleles. The assay measures the ability of CD8$^+$T-cells to kill HIV-1 infected GXR-cells over-expressing class-I-HLA allele of interest. The assay utilises a GXR-cell engineered to express GFP upon HIV-1 infection.
Methods CD8$^+$T-cells were co-cultured with HIV-1 infected GXR-cells for 3 days. Reduction in the infected GXR-cells expressing GFP measured by FACS was used to evaluate the CD8$^+$T-cells killing activity. The assay was validated using a panel of 9 HIV-infected samples and were concurrently assayed with the ELISA-VIA. The tested results on each assay were categorised into four groups namely: true-inhibition (TI ≥50%), doubtful-inhibition (DI ≥20% to ≤49.99%), false-inhibition (FI ≥10% to ≤19.99%) and non-inhibition (NI≤ 9.99%). These results were used in a 2 by 2 table to compute sensitivity (TI/TI+DI) and specificity (FI/FI+NI).
Results True inhibition was observed in 44% of samples analysed using GXR-VIA compared to 33% with ELISA-VIA. 11% with GXR-VIA had doubtful result compared to 33% with ELISA-VIA. 22% with GXR-VIA were categorised as false inhibition compared to 33% with ELISA-VIA. Interestingly, no sample showed non-inhibition with GXR-VIA whereas 22% showed no inhibition by ELISA-VIA. Collectively, GXR-VIA is very specific (100%) but less sensitive (57%) at detecting virus inhibition activity.
Conclusion The specificity of GXR-VIA and its marginal sensitivity indicates that the assay is capable of identifying CD8$^+$T-cells-mediated inhibition of HIV-1 replication. Overall, the GXR-VIA provides a platform to assess the influence of different restricting class-I-HLA alleles on HIV-1-specific CD8$^+$T-cells antiviral function.

P15.04 GENITAL TRACT CELLULAR ACTIVATION AND INFLAMMATION ASSOCIATED WITH HIGHLY PREVALENT SEXUALLY TRANSMITTED INFECTIONS AND BACTERIAL VAGINOSIS IN ADOLESCENT WOMEN AT RISK FOR HIV INFECTION

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Introduction The biological mechanisms underlying HIV risk in younger women is unclear. HIV is primarily transmitted across the genital mucosa and preferentially infects CD4$^+$ T-cells. We investigated the influence of asymptomatic sexually transmitted infections (STIs) and bacterial vaginosis (BV) on CD4$^+$ T-cell

activation and inflammation in the genital tracts of adolescents from South Africa.

Methods Cervical cytobrush mononuclear cells were isolated from 148 adolescents (16–22 years) from Cape Town, and expression of T-cell activation and proliferation markers (CD38, HLA-DR, Ki67, CCR5) was measured by FACs. Adolescents were screened for BV (Nugent) and STIs (*C. trachomatis*, N. gonnorhoea, *T. vaginalis*, *M. genitalium*, HSV-2) by PCR. For comparison, 11 HIV-negative adult women were included. Concentrations of 48 cytokines, chemokines and growth factors were measured in matching menstrual cups by Luminex.

Results Adolescents (median 18 years; IQR 17–20) had significantly higher frequencies of activated $CD4^+$ T-cells ($CD38^+$, $HLADR^+$, $CD38^+HLADR^+$: each $p < 0.0001$) from cervical cytobrushes than adults although CCR5 expression was higher in adults. STIs and BV prevalence was very high, with 71% of adolescents having ≥1 STI and/or BV, and 42% being *C. trachomatis* positive. Adolescents with an STI, despite these being asymptomatic, had higher frequencies of activated and proliferating T-cells compared to those with no STI/BV ($CD4^+CD38^+HLADR^+$: $p = 0.047$; $CD4^+Ki67^+$: $p = 0.020$). Women positive for chlamydia had significantly higher frequencies of $CD4^+CD38^+$ T-cells ($p = 0.006$). Women with both STIs and BV had the most pronounced increase in $CD4^+$ T-cell activation ($CD38^+$: $p = 0.002$; $CD38^+HLADR^+$: $p = 0.001$; Ki67+: $p = 0.002$). Higher cervical T-cell activation marker expression was directly associated with increased genital cytokine profiles.

Conclusion Heightened levels of genital immune activation and inflammation found in South African adolescent females, partly due to the presence of asymptomatic STIs and BV could increase their risk for HIV infection.

P15.05 PERFORMANCE EVALUATION OF THE APTIMA HIV-1 QUANT DX ASSAY FOR DETECTION OF HIV-1 IN PLASMA AND DRIED BLOOD SPOTS (DBS)

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Introduction The Aptima HIV-1 Quant Dx presented on the Hologic PANTHER^{TM} system provides continuous and random access processing of molecular samples for groups M HIV-1 RNA viral load testing. Significant efficiencies are realised through 3.5 h to first result with over 275 samples processed within 8 h. This study assessed the performance of the system in routine plasma samples and whole blood presented as dried blood spot (DBS).

Methods A total of 181 plasma samples were tested over the analytical range and compared to a benchmark real time PCR system. The study focused on the lower analytical range <5,000 copies/mL HIV-1 RNA (55%). HIV-1 viral load equivalence in non-B subtypes of regional geographical significance was assessed where subtype was available (72%). A further 20 DBS (single 10 mm punch, whole blood) with HIV-1 RNA 500–5,000 cpy/mL and were eluted using a variety of methods, tested and compared with plasma RNA.

Results Overall, Aptima HIV-1 Quant Dx correlated with the routine analytical platform (r2 = 0.9605). Samples ranged undetectable (16, 8.8%), below the benchmark test lower limit of detection (<20 cpy/ml) (16, 8.8%), low range (20–5,000) (84, 46.4%), medium (5,000–50,000) (36, 19.9%) and high range (>50,000 cpy/ml) (29, 16%). Samples in the lower analytical range <1,000 cpy/ml showed little variance when compaired with the Roche (CAP/CTM) assay using Bland-Altman correlation analysis. Reproducibility was assessed in the high, medium and low range within 1–2SD of mean. DBS samples with HIV-1 RNA results >1,000 were well correlated with plasma.

Conclusion The Aptima HIV-1 Quant Dx automated random access platform correlated with a commonly used HIV RNA test in plasma and offered significant workflow advantages. Promising results obtained using DBS samples could potentially overcome logistics encountered with conventional plasma. Further correlations and limit of detection studies are needed to validated DBS.

Disclosure of interest statement No conflict of interest to declare.

P15.06 MOLECULAR VALIDATION OF PUTATIVE ANTIMICROBIAL PEPTIDES FOR IMPROVED HUMAN IMMUNODEFICIENCY VIRUS DIAGNOSTICS VIA HIV PROTEIN P24

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Introduction The Human Immunodeficiency Virus-1 (HIV-1) is responsible for causing Acquired Immunodeficiency Syndrome (AIDS), and to date remains a pandemic. More than 40 million people are infected globally, with 60% of the infected people residing in Sub-Saharan Africa. Earlier detection translates into earlier treatment, which ensures improved quality of life. However, difficulties remain in the field of HIV diagnostics. The p24 antigen detection tests are preferred due to its ability to decrease the window period. The current p24 diagnostic assay displays great insensitivity, due to the p24 antibody produced by the body, binding to the C-terminal of the p24 antigen. This interaction obstructs detection, the basis of the current p24 test. Using *in silico* approaches, novel antimicrobial peptides (AMP) were identified which bind to the N-terminal, instead of the C-terminal domain (antibody binding pocket) of the p24 antigen (provisional patent). This is important because if the p24 antibody binds to the C-terminal, the unoccupied N-terminal domain would provide a binding pocket for the AMP. Successful conjugation of nanoparticles to the positively validated AMP, can lead to the development of a diagnostic lateral flow device.

Methods In silico site-directed mutagenesis and docking studies to identify additional AMPs that bind the N-terminal domain of protein p24 with increased binding affinity.

Preliminary study: Lateral flow design with identified AMPs to test HIV positive sera.

P24 recombinant protein expression.

P24 protein-AMP binding studies.

Results In silico studies identified 9 AMPs which could be used to bind p24 antigen for HIV diagnostics.

Preliminary study: Lateral flow successfully detected HIV in HIV positive sera.

Successful p24 recombinant protein expression.

Successful validation of binding AMPs against the p24 protein.

Conclusion Binding interaction between AMPs and p24 protein is validated. Subsequently a sensitive lateral flow device could be developed that successfully detects HIV in positive HIV sera.