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ORAL PRESENTATIONS

O1 The genetic landscape of immune-competent and HIV lymphoma
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Burkitt lymphoma (BL) and diffuse large B cell lymphoma (DLBCL) are aggressive forms of lymphoma in adults and demonstrate overlapping morphology, immunophenotype and clinical behavior. The risk of developing these tumors increases ten to hundred-fold in the setting of HIV infection. The genetic causes and the role of specific mutations, especially in the setting of HIV, are largely unknown.

The decoding of the human genome and the advent of high-throughput sequencing have provided rich opportunities for the comprehensive identification of the genetic causes of cancer. In order to comprehensively identify genes that are recurrently mutated in immune-competent DLBCL and BL, we obtained a total of 92 cases of DLBCLs and 40 cases of BL. These cases were compared to a set of 5 DLBCLs and BL tumors derived from patients with HIV. The DLBCL cases were divided into a discovery set (N=34) and a prevalence set (N=61). The Burkitt cases were also divided into discovery and prevalence sets (N=15, N=45 respectively). For each of the discovery set cases we also obtained paired normal tissue. We performed whole-exome sequencing for all of these using the Agilent solution-based system of exon capture, which uses RNA baits to target all protein coding genes (CCDS database), as well as ~700 human miRNAs from mirBase (v13). In all, we generated over 6 GB of sequencing data using high throughput sequencing on the Illumina platform. We identified a total of 432 genes that were recurrently mutated in DLBCL and BL. We found that each tumor had an average of 20 gene alterations, which is fewer than most other solid tumors sequenced to date. Commonly implicated biological processes comprising these genes included signal transduction (e.g. PIK3CD, PDGFRα), immune response (e.g. B2M, CD83, IRF8) and chromatin modification (e.g. MLL3, SETD2). We found that lymphomas that arose in the setting of HIV had fewer mutations overall and had a paucity of mutations related to immune response. These data implicate the depressed immune response by HIV as a contributing risk factor for the development of lymphomas and suggest that HIV lymphomas are genetically less complex than their immune competent counterparts. This study represents one of the largest applications of exome sequencing in cancer, and provides early clues to the genetic causes of HIV-lymphomas.

O2 Analysis of the miRNA targetome in EBV-infected B cells
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microRNAs (miRNAs) are ~22 nt, non-coding regulatory RNAs expressed by all metazoans and several viruses. During latent infection, Epstein-Barr virus (EBV) expresses 25 pre-miRNAs and influences the expression of cellular miRNAs, such as miR-155 and miR-21, all of which potentially have roles in viral oncogenesis. To date, only a limited number of EBV miRNA targets have been identified; thus, the role of viral miRNAs in viral pathogenesis and/or oncogenesis is not well defined. Using photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP) [1] combined with high-throughput sequencing and computational analysis [2], we
interrogated the miRNA targetome in EBV-infected B cells. We identified miRNA binding sites in over 5,700 cellular 3’ untranslated regions (UTRs), 25% of which contained sites for EBV miRNAs. miRNA binding sites were also identified at a lower frequency in coding regions. Our results reveal that EBV miRNAs predominantly target host cellular transcripts, thereby reshaping the host environment. Furthermore, viral miRNA targets are involved in multiple biological processes that are directly relevant to EBV infection, including modulation of immune responses, cell proliferation, and cell survival. Finally, we identified a number of viral transcripts that contained conserved binding sites for cellular miRNAs, including members of the myc-regulated mir-17/92 cluster. This comprehensive survey of the miRNA targetome in EBV-infected B cells is a positive step towards identifying novel therapeutic targets for EBV-associated malignancies.

References

O3
EBV-regulated global changes in mRNA isoform usage
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Approximately 90% of the global adult population is latently infected with Epstein-Barr Virus (EBV). Latent EBV infections are normally asymptomatic due to a robust cytotoxic T cell response. However, in the event of immunosuppression, as observed in HIV/AIDS patients, these latent infections can lead to B-cell lymphomas. In vitro EBV has the capacity to transform primary B-cells into immortalized Lymphoblastoid Cell Lines (LCLs). In order to assess changes in both overall mRNA abundance and mRNA isoform usage, we queried resting, primary human B cells and LCLs using Human Exon (HuEx) and conventional Affymetrix U133 arrays. Using a novel computational algorithm, SplicerEX, we identified 433 genes whose miRNAs undergo changes in alternative isoform usage during the transformation from primary B-cells to LCLs. Isoform changes were largely orthogonal from expression changes as only ~1/3 of mRNA isoform changes were also changed at the level of overall abundance. Isoform changes were classified into alternative 5’ initiation, internal exclusion/ inclusion of exons, 3’ terminal exon choice, and 3UTR alterations. The most striking mRNA isoform change was 3’UTR shortening, accounting for ~25% of all changes. Gene ontology analysis of mRNA isoform changes revealed a strong enrichment for nucleic acid binding proteins, including splicing and transcription factors. We have confirmed a subset of the predictions made by SplicerEX using isoform-specific RT-PCR. Importantly, many mRNA isoform changes observed were in fact regulated by EBV latent infection, not just proliferation per se, as they were also observed in the conversion of EBV-negative Burkitt’s Lymphoma cells (BL41) to latency III expressing BL41/B95-8 cells. Our preliminary results further indicate that two transcription factors, the E2 family member TCF4 and the plasma cell differentiation factor XBP1, are both regulated by EBV at the level of alternative isoform usage. These proteins both impact activation of the BZLF1 lytic promoter and our data thus suggest a novel mechanism by which EBV maintains latent infection in immortalized B cells. These data may point to new approaches in regulating the latent/lytic switch crucial to the pathogenesis of EBV-associated AIDS lymphomas.

O4
Comprensive analysis of the KSHV miRNA targetome by Ago-HITS-CLIP
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Infectious Agents and Cancer 2012, 7(Suppl 1):O4

The gamma-herpesvirus Kaposi’s Sarcoma-associated Herpesvirus (KSHV) is the etiological agent of Kaposi’s Sarcoma (KS), Primary Effusion Lymphoma (PEL) and a subset of Multicentric Castleman’s Disease (MCD) in immunocompromised individuals. As all herpessviruses KSHV has a latent and a lytic life cycle. Malignant cells in KS, PEL, and MCD are latently infected with KSHV. Interestingly, the virus encodes 12 miRNA genes giving rise to 25 mature miRNAs that are predominantly expressed during latency, i.e. in malignant cells. This, together with the increasing evidence for the involvement of miRNAs in cancer, suggests a potential role for the KSHV miRNAs in viral tumorigenesis. However, to date, very little is known about the function of these viral miRNAs. To address the question we performed Ago-HITS-CLIP [1] using the anti-Ago antibody ZAB 12 [2] to identify RISC complexes from KSHV-infected lymphoma cells (BCBL1, BC3). RNAs extracted from these complexes were analyzed by Illumina sequencing to identify viral and cellular miRNAs and their target genes. The search for canonical seed sequence matches (nt 2-7) of the KSHV miRNAs within the mRNA-derived sequencing tags revealed more than 1000 cellular targets. Gene ontology analysis revealed that KSHV miRNA targets are enriched in genes involved in apoptosis, lymphocyte activation, cell cycle regulation, and transcriptional control. Importantly, we reproducibly obtained clusters of reads on experimentally confirmed KSHV miRNA target sites in several known target genes (e.g. Bach1, BCLAF1, and THBS1). New target genes are on the process of experimental validation, with 4 confirmed targets so far: TP53INP1, TPDS2, ANXA2, C/EBPB. Target analysis for non-canonical seeds as well as for cellular miRNAs is currently ongoing.

References

O5
Genetic variation in KSHV encoded microRNAs affects microRNA expression and is associated with multicentric Castleman’s disease risk
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Kaposi sarcoma-associated herpesvirus (KSHV) encodes 12 pre-microRNAs which potentially yield 25 mature microRNAs and have been shown to play prominent roles in the viral lifecycle including maintaining viral latency, evading the host immune response, and controlling lytic replication. We previously reported phylogenetic analysis of the microRNA-coding region of KSHV from Kaposi sarcoma (KS), primary effusion lymphoma (PEL), and multicentric Castleman disease (MCD) patients. We showed a high level of conservation for most sequences, but also a divergent cluster of 5 KSHV sequences including 2 from MCD patients [1]. We additionally observed single nucleotide polymorphisms (SNP) in the sequence of KSHV encoded mature and pre-miRNAs from clinical samples including a SNP in mir-K12-5 reported to result in increased expression of the mature miRNA [2]. To determine whether SNPs in other KSHV encoded miRNAs resulted in differences in microRNA processing and expression we used four custom ABI real time qPCR assays. Luciferase reporter assays were performed following transfection of transformation defective B cells with mir-K12-5. Additionally, in vitro maturation assays were performed to assess differences in Drosha/DCGR8 and Dicer cleavage between wild type and variant pre-miRNAs. Our
results indicate that polymorphisms within the pre-miRNA sequence can cause subtle expression differences as in the case of KSHV mir-K12-6 or more profound changes as observed in mir-K12-5. Our data clearly shows that SNPs can affect pre-miRNA processing resulting in changes in mature miRNA expression levels. To extend our studies on miRNA variation in MCD patients, KSHV miRNA sequences from 23 MCD patients and 7 patients with a newly described KSHV-associated inflammatory cytokine syndrome (KICS) were examined by amplification, cloning, and sequencing of a 646-bp fragment of KI2/T0.7 encoding miRNA-K12-10 and miRNA-K12-12 and a 2.8-kbp fragment containing the remaining 10 pre-miRNAs. Phylogenetic analysis showed a distinct variant cluster consisting exclusively of MCD and KICS patients in all trees. Pearson’s chi-squared analysis revealed 40 single nucleotide polymorphisms (SNPs) at various loci were significantly associated with MCD and KICS risk. Additionally, cluster analysis of these SNPs generated several combinations of three SNPs as putative indicators of MCD and KICS risk. Taken together, these findings show that MCD and KICS patients frequently have unusual KSHV microRNA sequences and suggest association between the observed sequence variation and risk of MCD and KICS.

References

06
KSHV infection of endothelial cells manipulates CXCR7-mediated signaling: implications for Kaposi’s Sarcoma progression and intervention

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CXCR7 was recently characterized as an alternative receptor for the chemokine CXCL12/SDF-1, previously thought to bind and signal exclusively through CXCR4. We recently identified CXCR7 as a key cellular factor in the endothelial cell (EC) dysfunction associated with KSHV infection. CXCL12 signaling is critically associated with tumor growth, angiogenesis and metastasis in several diverse tumors and is one of the most studied chemokine/chemokine receptor interactions in cancer systems. The tumorigenic activity of the CXCL12 signaling axis offers an attractive target for therapeutic intervention in multiple cancers including Kaposi’s Sarcoma (KS). However, most of the research to date was based on the assumption that CXCR4 was the sole CXCL12 receptor, and thus focused on the development of CXCR4-targeted treatments. CXCR4 participates in important homeostatic functions including hematopoiesis and mucosal immunity, while CXCR7 is rarely expressed in normal adult cells. As a result, CXCR7 may be a more specific chemotherapeutic target for tumor cells and tumor-associated vasculature with fewer adverse effects than treatments targeting CXCR4. CXCR7 is poorly studied throughout the cancer literature and although CXCR7 expression has been found in tumor-associated vasculature, no studies comprehensively examine the biology of CXCR7 in EC and its implications for tumor biology. We seek to define the role of CXCR7-mediated CXCL12 signaling in EC biology, and in the context of KSHV infection, in order to determine potential contributions of CXCR7 signaling to KSHV-mediated EC transformation and KS tumorigenesis. We demonstrate that CXCR7 is strongly expressed on LANA+ spindle cells in KS biopsy tissue at all stages of tumor progression. We further demonstrate that CXCR7 induction by KSHV in vitro is specific to lymphatic EC lineages and occurs coincident with the acquisition of spindle morphology. Detailed examination of CXCR7 functions in EC biology reveals multiple roles for CXCR7 that could impact KS tumorigenesis, including effects on cellular proliferation, junctional integrity, cell survival and metastatic capacity. Specifically, we determine that CXCR7 expression results in a loss of PECAM/CD31 expression, perturbing the formation and maintenance of EC monolayers. Moreover, CXCR7+ EC display significant SDF-1 dependent hypermotility, as measured via Electrical Cell-Substrate Impedance Sensing (ECIS). We also demonstrate that SDF-1 signaling through CXCR7 expression is enhanced in EC undergoing anchorage-deprivation, affecting EC cell survival and invasion into SDF-1 rich niches. Taken together, these results demonstrate that CXCR7 is a novel KSHV-induced oncogene with the capacity to influence multiple aspects of KS pathogenesis including tumor growth, seeding and metastasis.

07
Efficacy of a latency- and productive infection-deficient Gammaherpesvirus as a vaccine strategy

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Background: Human gamma-herpesviruses, Epstein-Barr virus (EBV or HHV-4) and Kaposi’s sarcoma-associated herpesvirus (KSHV or HHV-8), are associated with several malignancies, especially in AIDS patients. Although highly active antiretroviral therapy (HARRT) has significantly reduced the incidence of EBV- and KSHV-associated tumors, it does not eliminate EBV or KSHV infection, and the tumor risk remains high for HIV-1-infected individuals who are also carriers for EBV and KSHV. Tumorigenesis of gamma-herpesviruses is associated with the persistence of infection. Thus, vaccination to elicit protective immunity that inhibits the establishment of viral persistence will prevent the occurrence of virus-associated cancers. However, currently there are no effective vaccines available for KSHV or EBV.

Materials and methods: For proof of concept vaccination experiments, we utilize a mouse gamma-herpesvirus infection model. Previously, we have shown that vaccination with a non-persistent highly lytic live attenuated virus (AC-RTA) provides effective protection against a challenge infection by the wild type virus. To increase the safety of vaccination, the in vivo lytic replication capacity of a live gamma-herpesvirus needs to be significantly weakened without losing immunogenicity. We hypothesize that removal of the viral genes that inhibit the host immune responses will reduce the fitness of the virus but potentially increase its immunogenicity. For this purpose, we have removed immune evasion genes from AC-RTA along with other modifications.

Results: The resultant virus named DIP (Deficient in Immune evasion and Persistence), replicates in cell culture but is severely attenuated in mice, more deficient in acute productive infection and latency. However, immunization of the DIP virus prevents latency establishment by the challenge wild type virus. Next, we aim to test strategies to improve the immunogenicity in immunocompetent and CD4-deficient hosts by incorporating expression of co-stimulatory molecules into the DIP virus.

Conclusions: The non-persistent DIP virus that undergoes limited in vivo viral replication provides us a novel vaccine strategy for preventing infection of human gammaherpesviruses. The "proof of concept" study in the mouse infection model is necessary to provide fundamental insights into the development of vaccines for the tumor-associated human herpesviruses.

08
Herpesviruses control the DNA damage response through Tip60
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Infectious Agents and Cancer 2012, 7(Suppl 1):O8

Herpesviruses control the DNA damage response through Tip60

RC1
Background and results: Herpesviruses establish life-long persistent infections that result in clinical manifestations ranging from mild cold sores, to pneumonitis and cancers. Immunocompromised populations, including AIDS patients, are at risk for more serious disease outcomes. Although the α-, β-, and γ-herpesviruses infect different tissues and cause distinct diseases, they confront many of the same challenges in producing new virions and spreading infection. The herpesvirus families each encode a conserved serine/threonine kinase that plays an important role in virus replication and spread. Despite the potential of these kinases as pharmacological targets, the extent of substrate conservation and the key common cell signalling pathways targeted by these enzymes are unknown. We applied a human protein microarray, high-throughput
During 1980-2007, a total of 25,011 anal cancers occurred in 2.1 million person-years of follow-up. Of these, 1087 were incident and 456 were prevalent cases in people with AIDS: cases that occurred after AIDS diagnosis (incident) and cases that were prevalent at the time of AIDS diagnosis (prevalent). All rates were standardized to the 2000 U.S. population by age, sex and race. Poisson regression was used to estimate changes in rates over time.

**Results:** During 1980-2007, a total of 25,011 anal cancers occurred in 2.1 billion person-years of follow-up. Of these, 1087 were incident and 456 were prevalent cases in people with AIDS. Among men, the anal cancer rate increased 2.0% per year from 0.69 to 1.06/100,000 during 1980-2007 (Figure 1A). Excluding cases in people with AIDS, the rate only increased 0.77% per year to 0.77/100,000 in 2007. Among women, the anal cancer rate increased 2.1% per year from 1.09 to 1.71/100,000 during 1980-2007 (Figure 1B). Removal of cases with AIDS changed the trends very little (increase of 2.1% per year). Among 20-49 year olds, AIDS cases strongly influenced trends in men. Overall rates increased 4.0% per year, but rates
During 1980-2007, the U.S. anal cancer epidemic in young men was strongly influenced by the HIV epidemic; however, among women, the anal cancer epidemic was independent of HIV. Effective anal cancer prevention in HIV-infected men would have a substantial impact on U.S. anal cancer rates.

Conclusions: During 1980-2007, the U.S. anal cancer epidemic in young men was strongly influenced by the HIV epidemic; however, among women, the anal cancer epidemic was independent of HIV. Effective anal cancer prevention in HIV-infected men would have a substantial impact on U.S. anal cancer rates.

O11
Differential modulation of human beta-defensin-3 expression in human oral epithelial cells by HPV oncoproteins E6 and E7: potential implication in oral cancer
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Background: Human papillomaviruses (HPVs) are small, non-enveloped DNA viruses that infect stratified squamous mucosal and cutaneous epithelia, causing diseases ranging from benign warts to invasive tumors. Failure of the immune system to detect and clear persistent HPV infections frequently leads to the development of oral warts and cancer. HPV infection has been etiologically linked with oral warts and a subset of oral squamous cell carcinoma, particularly in HIV infected patients. The incidence of HPV-related oral lesions is increased in HIV+ subjects on highly active antiretroviral therapy (HAART). We previously showed that tumor cells in oral carcinoma in situ (CIS) lesions overexpress human beta-defensin-3 (hBD-3), an antimicrobial peptide with immunomodulatory capabilities. Expression of hBD-3 in CIS contributes to the local pro-tumor immune response by selectively chemoattracting tumor-associated macrophages and by enhancing tumor development and progression.

Results: To elucidate mechanisms by which high-risk HPV could evade immune detection and clearing via infected epithelial cells, we investigated if oncoproteins E6 and E7 derived from high-risk HPV-16 modulate the innate immune response of infected epithelial cells and the role of HPV-induced gene expression in orchestrating local immunity. We have found that cancer cells of HPV-related oral and oropharyngeal squamous cell carcinoma biopsies overproduce hBD-3. Introduction of an expression vector producing HPV-16 E6 or E7 oncogene into oral squamous cancer cell lines or primary oral epithelial cells increases the levels of hBD-3 mRNA and peptide. However, E6 derived from the low-risk HPV-11 is significantly less potent in promoting hBD-3 expression. Combination of oncopgenic E6 and E7 in oral epithelial cells also shows reduced induction of hBD-3. Furthermore, the transactivicity of an hBD-3 luciferase promoter construct is differentially stimulated by oncopgenic E6 and E7 compared with MEKK1, a known inducer of hBD-3 expression. Although the pharmacological inhibitors for MAPK and PI3K reduce the transactivicity of a 2.5 kb hBD-3 promoter reporter, they do not exhibit the inhibitory effect on the promoter reporter containing a 450 bp 3′-regulatory region. These data suggest that high-risk and low-risk early genes of HPV differentially modulate hBD-3 expression in oral epithelial cells.

Conclusion: Our results suggest that oncoproteins of high-risk HPV strains induce higher levels of hBD-3 expression compared with early genes of low-risk HPV. The oncogenic E6 and E7 genes may contribute to overexpression of hBD-3 in the early oral lesion, which then leads to recruitment of tumor-associated macrophages to further develop and promote the progression of cancer.

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O12
Human papillomavirus prevalence in invasive cervical carcinoma by HIV Status
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Background: Data on the prevalence of human papillomavirus (HPV) types in invasive cervical carcinoma (ICC) in women with HIV are scarce but are essential to elucidate the influence of immunity on the carcinogenicity of different HPV types, and the potential impact of prophylactic HPV vaccines in populations with high HIV prevalence.

Objectives: To compare the prevalence of HPV types in ICC by HIV status.

Methods: From 2007 to 2009, a multicentre case-case study was conducted at two referral hospitals in Nairobi, Kenya, and in Durban, South Africa. Women over 18 years of age presenting with ICC were recruited, and frozen biopsies were obtained and tested for HPV DNA.

Figure 1(abstract O12) Prevalence of human papillomavirus (HPV) in 225 women with cervical squamous cell carcinoma by HIV status and multiplicity of HPV infection 10 HPV-negative women were excluded; ∗Either 16 or 18 as single infection or in combination with any type as multiple type infection; HPV: human papillomavirus; HRX: uncharacterized high-risk type; LR: low-risk.
using GPS-6/4+PCR methodology. The present analysis was limited to the 235 squamous cell cancers (SCC) detected.

Results: We included 106 HIV-positive (mean age 40.8 years) and 129 HIV-negative women (mean age 45.7) with SCC. Among HIV-positive women, the mean CD4 count was 334 cells/μL and 48.1% were on combined antiretroviral therapy. HIV-positive women had many more multiple HPV infections (21.6% of HPV-positive carcinomas) compared to HIV-negative women (3.3%) (p < 0.001) and the proportion of multiple infections was inversely related to CD4 level. An excess of HPV18 of borderline statistical significance was found in HIV-positive compared to HIV-negative women (Prevalence ratio (PR) = 1.9, 95% confidence interval (CI): 1.0-3.7, adjusted for centre, age, and multiplicity of infection). HPV16/ and/or 18 prevalence combined, however, was similar in HIV-positive (66.7%) and HIV-negative women (69.1%) (PR = 1.0, 95% CI: 0.9-1.2). No significant difference was found for other HPV types (Figure 1).

Conclusions: Overall, our data suggest that current prophylactic HPV vaccines against HPV16 and 18 may prevent similar proportions of cervical SCC in HIV-positive as in HIV-negative women provided that vaccine-related protection is sustained after HIV infection.

Table 1(abstract O14)

<table>
<thead>
<tr>
<th>Status</th>
<th>N (%)</th>
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<tbody>
<tr>
<td>Treatment Completed per protocol</td>
<td>21 (62%)</td>
</tr>
<tr>
<td>Disease Progression</td>
<td>3 (9%)</td>
</tr>
<tr>
<td>Early termination due to adverse event*</td>
<td>5 (15%)</td>
</tr>
<tr>
<td>Early termination due to patient withdrawal**</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>Early termination – counts did not recover within time frame to begin cycle 4</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Treatment ongoing</td>
<td>2 (6%)</td>
</tr>
</tbody>
</table>

*1 pt with grade (gr) 4 thrombocytopenia and gr 3 infection: 1 pt with gr 3 left hemiparesis; 1 pt with gr 3 confusion unrelated to treatment; 1 pt with prior hepatitis B and cirrhosis had gr 3 encephalopathy and pulmonary infiltrates; 1 pt with gr 4 neutropenia and gr4 thrombocytopenia. **1 CR 2 yrs post treatment.

Regimens have a high relapse rate. We modified CODOX-M/IVAC hoping to preserve efficacy while improving tolerability, particularly treatment related mortality (TRM). Primary object: improving 1 year overall survival (OS) from the historical 65 to 85%.

Methods: Modifications of the US NCI regimen include rituximab (R), cyclophosphamide reduction [800 mg/m2 x 2 days], vincristine 2 mg cap, metrotrexate (mtx) 300 mg/m2, dual chemotherapy lumbar punctures and IVAC infusion (high risk pts). Antibiotic prophylaxis & growth factor support included 100% grade IV hematopoietic toxicities in the original regimen. HAART therapy at the discretion of the local MD. Pathology review included CD20, CD10, BCL2, BCL6, p53, Ki67, BLUMP1, IRF4/MUM1 and EBV EBER. (Table 1)

Results: Accrual of 33 planned pts by April 2010. Baseline: Classical Burkitt, 97%; Low/High Risk, 9/91%; Median (range) Age 42 (19 – 55); CD4 count 195 (0 - 721), CD4 <100, 7 (27%); HIV viral load 1819 (Undetectable – 1,187,968). Median follow up (fu) is 9 mos for surviving pt. Number of pts with gr3/4 toxicity: any 20 (61%), 13 (39%) hematologic, 16 (48%) infection including 7 febrile neutropenia, 6 metabolic with 1 tumor lysis syndrome, 4 neurologic, 2 thrombotic and 1 each coagulation, GI or pain. Only 2 gr 1/2 stomatitis/mucositis; 0 had gr 3/4. Six deaths: encephalopathy with hepatic failure, hepatitis A and pneumonia (1), disease progression (3) including 1 in the CNS; fungal infection (1). Median 1 year OS (n=34) was 81.7% (61.0%, 92.1%) with a 35 mo median survival. OS by non-BL defining proteins: EBER +/- (8/16) and p53 +/- (10/10) were not predictive. IRF4/MUM1 +/- (8/15) highly predictive in overall pts, but not in the confirmed Burkitt +/- (6/14) with only 1 IRF4/MUM1 neg pt dying of BL.

Conclusions: AMC 048 with a median fu of 9 mos has a 1 yr OS of 82% in BL. Relapses after 1 year are rare. TRM was zero. R did not appear to increase toxicity. Only 5 pts withdrew due to AEs. Grade 3/4 toxicities were markedly reduced. Results compare favorably with 2 studies of HIV neg pts. Magrath (1995) reported 100% grade 4 hematologic and 20% grade 4 mucositis in 39 adults, 33 children (92% 2 yr EFS). MRC/NCRI LY10 trial (Mead 2008) reduced mtx (3gr/m2), but reported 9% TRM (64% 2 yr OS). IRF4/MUM1 deserves further study in BL.

Acknowledgements: This study is presented on behalf of the AIDS Malignancy Consortium.
We show for the first time that Treg suppress CD8 T cell
activation-associated molecules (sCD23, AICDA-6, IL-10, CXCL13, sCD27, sCD30) were
expressed at a higher level in PBMC samples from patients with AIDS-NHL compared to non-AIDS defining
cancer patients. We present the results of this study to determine the proper dosing of new targeted chemotherapies in
patients with AIDS-NHL.

**Methods:** Patients with HIV and cancers refractory to standard therapy were stratified into two arms: (1) non-ritonavir based HAART or (2) ritonavir-based HAART. Six patients were to be enrolled on arm 1 and 1 receive the standard dose of sunitinib (50mg po qd). Arm 2 used a phase I pharmacokinetic (PK) study to determine the proper dose of these agents in HIV+ cancer patients. We present the results of this study to determine the proper dosing of new targeted chemotherapies in patients with AIDS-NHL.

**Results:** Between 8/09 and 4/11, 19 patients were enrolled and completed the AMC is performing a study to determine the proper dosing of new targeted chemotherapies in patients with AIDS-NHL. The AMC is performing a study to determine the proper dosing of new targeted chemotherapies in patients with AIDS-NHL.

**Conclusion:** The recommended dose of sunitinib for patients on ritonavir is 25mg per day.

**O16 Serum levels of several molecules that are associated with B cell activation and inflammation are elevated in AIDS-associated non-Hodgkin’s lymphoma (AIDS-NHL) and predict response to treatment**

Material and methods: Plasma and PBMC were obtained from AIDS-NHL patients (n=70) in the AMC 034 study, which evaluated treatment of AIDS-NHL with EPOCH chemotherapy and rituximab. Plasma was collected prior to the initiation of therapy, and post-treatment, at the first cycle of chemotherapy, and at 6 and 12 months following completion of treatment. Biomarkers were quantified by ELISA, AICDA expression by qPCR.

Results: Higher pre-treatment plasma levels of most of these B cell activation-associated molecules (IL-6, IL-10, CXCL13, sCD27, sCD30) were seen in AIDS-NHL patients, when compared to HIV+ and HIV-negative reference groups. However, sCD23 levels were lower post-AIDS-NHL than typically seen in the years preceding NHL diagnosis. Additionally, AICDA expression in PBMC was not detected in specimens collected after AIDS-NHL diagnosis. Treatment of NHL was seen to result in decreased plasma levels of these molecules, with decreased levels persisting for one year following the completion of treatment. CXCL13 and sCD27 decreased the most after treatment (mean levels were reduced from 847 pg/ml to 310 pg/ml, and 1,080 units/ml to 330 units/ml, respectively) and remained low at one year following the initiation of treatment (86.6 pg/ml and 362 units/ml, respectively). Pre-treatment levels of some of these molecules (IL-6, IL10, CXCL13) were associated with subsequent response to lymphoma therapy, and were lower in patients with high IPI scores. Using a normalizing transformation on CXCL13, sCD27 and LDH, logistic regression analysis showed that the only significant predictor was the pre-treatment level of CXCL13.

Conclusions: Biomarkers for AIDS-NHL identified in this study were further evaluated post-AIDS-NHL diagnosis, and decreased with treatment for NHL. Importantly, elevated pre-treatment CXCL13 was associated with a poorer subsequent response to treatment, and was a better predictor of response than LDH levels and IPI scores.

Reference

**O17 CD4 regulatory T cells Control CD8 T cell responses to human Herpesvirus 8 lytic and latency proteins**

Material and methods: Plasma and PBMC were obtained from AIDS-NHL patients (n=70) in the AMC 034 study, which evaluated treatment of AIDS-NHL with EPOCH chemotherapy and rituximab. Plasma was collected prior to the initiation of therapy, and post-treatment, at the first cycle of chemotherapy, and at 6 and 12 months following completion of treatment. Biomarkers were quantified by ELISA, AICDA expression by qPCR.

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Reference
O18
Risk factors for death and temporal trends in overall survival in patients with AIDS-associated primary central nervous system lymphoma (AIDS-PCNSL)
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Infectious Agents and Cancer 2012, 7(Suppl 1):O18

Background: AIDS-PCNSL is a rare EBV-associated B-cell neoplasm that continues to carry a poor prognosis, even in the highly active antiretroviral therapy (HAART) era. We hypothesized that overall survival (OS) is affected by prior diagnosis of central nervous system (CNS) infections as well as treatment approaches to both HIV and AIDS-PCNSL. We evaluated risk factors and temporal trends for OS in patients with AIDS-PCNSL.

Methods: Adults with AIDS-PCNSL were identified through a computer linkage that matched AIDS case diagnosed between 1990-2000 from the San Francisco adult AIDS case registry with the California Cancer Registry (1985-2002), with mortality follow-up through 12/31/2007. Patients with non-B-cell histology or history of systemic non-Hodgkin lymphoma diagnosed within 2 years prior to AIDS-PCNSL diagnosis were excluded. Prognostic factors evaluated include: diagnosis of CNS infection prior to AIDS-PCNSL, diagnosis of other common opportunistic infection (OI) prior to AIDS-PCNSL (pneumocystis pneumonia [PCP] or mycobacterium avium complex (MAC)), pathologic versus clinical diagnosis, receipt of cancer therapy, HAART prescribed prior to or within 30 days of AIDS-PCNSL diagnosis, and year of diagnosis (1990-1995, 1996-1998, 1999-2002). Survival analyses employed Kaplan-Meier methodology.

Results: A total of 207 patients were identified, 96% male and 4% female. Median age 39 (IQR 35-46), 68% white, 21% black, 20% Hispanic, 2% Asian. Median CD4 20 cells/µL (IQR 6-53). HIV risk group: 79% MSM, 8% IDU, 9% MS/IDU. CNS infections prior to AIDS-PCNSL: toxoplasmosis 8%, cryptococcus 9%, histoplasmosis 1%, extrapulmonary tuberculosis 1%. Treatment category: none 42%, radiation only 52%, chemotherapy 6% (5/13 chemotherapy only, 6/13 chemotherapy and radiation, 2/13 chemotherapy and immunotherapy). Risk factors for OS included prior CNS infection (p=0.0001), HAART (p=0.0023), AIDS-PCNSL treatment (p=0.0001), and calendar period of AIDS-PCNSL diagnosis (0.001), but not prior PCP or MAC (p=0.23). (Figures 1 A-D) OS was improved by HAART across treatment groups (p=0.0001).

Conclusions: Prior diagnosis of CNS infection, HAART, and cancer treatment are strong predictors of OS. OS improved over time in these patients. Earlier diagnosis of AIDS-PCNSL and/or CNS infection, treatment of CNS infections, and cancer treatment that includes HAART and concomitant chemotherapy may increase AIDS-PCNSL survival. Prospective evaluation of curative-intent chemotherapy-based approaches to AIDS-PCNSL is urgently needed. Additional analyses are ongoing.
was achieved, suggesting the need for earlier ART initiation. The IeDEA platform provides unique opportunities for prospective African KS research.

O20
Incidence of Kaposi Sarcoma in HIV-infected patients – a prospective multi-cohort study from Southern Africa
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Infectious Agents and Cancer 2012, 7(Suppl 1):O20

Background: The incidence of Kaposi Sarcoma (KS) is high in sub-Saharan Africa. Data on KS among HIV-infected patients receiving and not yet receiving antiretroviral therapy (ART) are, however, scarce in Africa. Within the framework of a large multi-cohort project, the International epidemiologic Database to Evaluate AIDS (IeDEA), we estimate the incidence and risk factors for the development of KS in HIV-infected patients in Southern Africa.

Methods: We analyzed prospectively collected data of HIV-infected children and adults participating in IeDEA-SA. We included all patients who were ART naive at start of observation, regardless of cancer history, with at least 30 days follow up. Prevalent KS cases were also excluded. Incidence rates and 95% confidence intervals (CI) were calculated based on the Poisson distribution; risk factors were estimated using crude and adjusted Cox proportional hazard models. Hazard ratios (HR) with 95% CI and medians with interquartile ranges (IQR) are presented.

Results: We included 184,592 patients from 10 cohort studies in Botswana, Mozambique, South Africa, Zambia and Zimbabwe. The median age was 34 years (IQR 28–41), the median CD4 count at first contact was 152 cells/μl (IQR 75–252) and 146 cells/μl (IQR 74–226) at start of ART. 61% of patients were female. During a total follow-time of 391,852 person-years, 349 patients developed KS before starting ART, 585 developed KS after starting ART and 183,658 remained KS-free. In patients not receiving ART the KS incidence rate was 624 (95% CI 562–692) per 100,000 person-years and in patients receiving ART the KS incidence rate was 174 (95% CI 161–189) per 100,000 person-years, rate ratio for ART versus no ART = 0.28 (95% CI 0.24–0.32). Univariate and multivariate analyses showed that men were more likely than women to develop KS and that the incidence rate for KS increased with increasing age and with decreasing CD4 cell counts. These effects were more pronounced in patients not receiving ART than in patients receiving ART.

Conclusions: In Southern African countries with a high prevalence of HHV-8 the risk of developing KS in HIV infected patients receiving ART increases steeply with age and immune-suppression. ART reduced the incidence of KS substantially.

Acknowledgement: This work was done on behalf of The International epidemiologic Database to Evaluate AIDS (IeDEA) Study Group. This study was funded by grants from NIAID, NICHD, NCI (number U01AI069924), PEPFAR (number 3U01AI069924-05S2) and the Swiss Bridge Foundation.

O21
Gene expression profiling using formalin-fixed paraffin-embedded primary specimens of AIDS-related Lymphomas
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Infectious Agents and Cancer 2012, 7(Suppl 1):O21

Background: Gene expression profiling has been useful for classification and prognostication of a variety of hematologic neoplasms occurring in the general population. This type of analysis of AIDS related lymphomas (ARLs) has been limited because of their rarity, heterogeneity and lack of frozen tissue for analysis, with the largest studies including 25 cases (Klein et al., Blood 2003, Deffenbacher et al., J AIDS 2010). To overcome this limitation, we employed a cDNA-based microarray technology, DNA-mediated Annealing, Selection, Ligation and Extension (DASL) for the analysis of formalin-fixed paraffin-embedded (FFPE) archival samples, allowing us to perform gene expression analysis of the largest cohort of ARLs thus far.

Material and methods: We performed expression profiling from FFPE samples of AIDS related lymphoma for using DASL (Illumina*), with modification of the cDNA and quality control (QC) steps. The following cases of ARL with confirmed diagnosis and sufficient RNA were used for evaluation in duplicate: Weill Cornell Medical College in New York, USA (36 cases), the AIDS Malignancy Consortium (AMC) (24 cases), University of Siena, Italy (20 cases), India (21 cases), Tata Memorial Hospital in Mumbai, India (35 cases), University of Ibadan, Nigeria (1 case). Non-AIDS lymphomas were included as controls (15 cases from India and 13 cases from Weill Cornell). A 1mm diameter core was obtained from each block and RNA extracted. Tissue microarrays were also prepared of the available specimens, and characterization of viral status and lymphoma subtype were determined by immunohistochemistry and in situ hybridization for Epstein-Barr encoded RNA (EBER). Universal and variate probes were used to evaluate for genetic deletions in A20, and translocations of cMYC, BCL-2 and BCL-6.

Results: Gene expression profiling of 126 cases was initially performed using DASL. Quality control assessment and data analysis revealed poor predictive ability of the QC method and poor quality of the cDNA resulting in data variability and lack of reproducibility. Therefore, we developed alternative methodologies for cDNA preparation and assessment of quality of the RNA, resulting in more than double the number of genes detected and good reproducibility in the majority of
the samples. Analysis of gene expression profiling of 116 cases of ARL and 28 matched non-AIDS lymphomas will be presented.

Conclusions: We have developed methods that allow gene expression analysis of large numbers of ARLs, which will pave the way of determining whether subtype specific signatures resemble those of lymphomas in immunocompetent individuals, and eventually if these have clinical implications.

Acknowledgement: The AIDS Malignancy consortium contributed cases. This project was funded by NCI grant R01CA068939 to EC.

O22
Decline in EBV-Specific IFN T cell responses in Kenyan infants from a malaria holoendemic region of Kenya
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Infectious Agents and Cancer 2012, 7(Suppl 1):O22

Background: Endemic Burkitt's lymphoma, the most prevalent childhood cancer in Equatorial Africa, is a rapidly growing B-cell malignancy that is ultimately fatal if untreated. Two co-factors are linked to the etiology of this pediatric cancer: Epstein-Barr virus (EBV) infection, and sustained and intense exposure to Plasmodium falciparum malaria (holoendemic malaria). In this study, we wanted to test the hypothesis that P. falciparum infections during early infancy results in elevated EBV viral load which results in diminished EBV-specific T cell immune responses over time.

Methods: Infants were enrolled from two rural sites in Kenya: Kisumu District where malaria transmission is holoendemic and risk for eBL is high and Nandi District where malaria transmission is limited and the risk for eBL is low. Finger prick blood samples were taken through 2 years of age to measure EBV viral load, EBV antibodies, and malaria parasitemia. Venous blood samples were collected at 12, 18 and 24 months of age and PBMC were isolated and stimulated with peptides for both EBV lytic and latent antigens. After 2.5 days of stimulation, IFNγ ELISPOTS enumerated EBV-specific T cell responses, and the number of SFU/10^6 PBMC was determined by scanning with ImmunoSpot Reader and Software.

Results: When we compared EBV lytic and latent IFNγ T cell responses at 12, 18 and 24 months of age, we saw that although children in Kisumu were able to mount an IFNγ response against EBV lytic peptides, the magnitude of that response declined significantly by 24 months of age. In contrast, the magnitude of the response did not decline in the Nandi cohort. We also observed higher overall viral loads in infants from Kisumu suggesting that the apparent loss of EBV-specific IFNγ response to lytic antigens in the Kisumu children may be associated with these higher viral loads.

Conclusions: We found that by 2 years of age, there was a significant difference in the capacity of children living in a malaria holoendemic region compared to malaria sporadic region to maintain a T cell response to EBV lytic antigens. This suggests that P. falciparum malaria contributes to loss of EBV-specific immunity by inducing the collapse of an antiviral IFN-γ mediated CD8+ T cell response.

Acknowledgement: Funding was provided by R01 CA120667 and D43 CA153701. ASA and EP contributed equally to this work.

O23
Identifying predictors of increased quantities of human Herpesvirus 8 DNA detection at oropharyngeal and plasma sites among Ugandan adults with and without HIV and Kaposi Sarcoma
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Infectious Agents and Cancer 2012, 7(Suppl 1):O23

Figure 1(abstract O23)
Background: Persons with KS and uncontrolled HIV infection have HHV-8 DNA detected more frequently at mucosal sites and plasma [1], but it remains unknown whether the quantity of HHV-8 detected is associated with KS development or HHV-8 transmission. We sought to characterize and determine the correlates of elevated HHV-8 DNA copy number in the oropharynx and plasma of Ugandan adults with and without HIV and KS.

Methods: Participants collected daily oral swabs and weekly plasma samples over 4 weeks to quantify HHV-8 DNA by polymerase chain reaction.

Results: 297 participants collected a total of 8,045 oral swabs and 1,392 plasma samples. HHV-8 DNA was detected in 1,561 (19%) oral swabs and 1,392 plasma samples. HHV-8 DNA was detected in the oropharynx of 70% (64/92) persons with KS vs. 27% (52/194) without KS (p<0.001) and in the plasma of 96% (88/92) persons with KS vs. 20% (38/194) without KS (p<0.001). The median amount of HHV-8 DNA detected in oral swabs was significantly lower in persons with KS (3.2 log copies/ml) than those without KS (3.8 log copies/ml; p<0.001) (Figure 1). HHV-8 quantities in the oropharynx did not differ by participants’ HIV status (p=0.13), but elevated HHV-8 quantities were associated with CD4 counts >500 (coef 0.59, CI 0.16-1.03, p=0.007). In multivariate analysis, factors associated with higher oral HHV-8 copy number included absence of KS (coef 0.45, CI 0.14-0.75, p<0.004) and poor dentition (coef 0.37, CI 0.08-0.65, p<0.01). The median amount of HHV-8 DNA in plasma was significantly higher in persons with KS (3.6 log copies/ml) than those without KS (2.4 log copies/ml; p<0.001). In contrast to oral detection, higher plasma HHV-8 quantities were associated with CD4 counts <500 (coef 0.73, CI 0.40-1.05, p<0.001). In multivariate analysis, higher plasma HHV-8 copy number was associated with KS (coef 0.99, CI 0.80-1.17, p<0.001) and HIV infection (coef 0.39, CI 0.15-0.63, p=0.002).

Conclusions: Increased quantities of HHV-8 DNA were detected in the oropharynx of persons with KS and those with poor dentition. The latter observation may be explained if higher CD4 counts allow for increased inflammation in the oropharynx, in turn leading to greater HHV-8 replication. Quantities of HHV-8 are higher in the plasma of persons with either HIV infection or KS, perhaps representing the propensity of HHV-8 to disseminate systemically in the absence of effective immune control or from foci of replication in KS tumors.

Reference

O24 Incidence and risk factors for lung cancer among women in the women’s interagency HIV study (WIHS) and men in the multicenter AIDS cohort study (MACS)
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Infectious Agents and Cancer 2012, 7(Suppl 1):O24

Background: Studies have reported an increased incidence of lung cancer among people with HIV/AIDS compared to population estimates [1], but it is unclear whether this increase is due to HIV or to other lung cancer risk factors such as cigarette smoking. One study found that HIV-infected adults with preexisting lung disease displayed trends for increased lung cancer risk [2]. Another study of people with AIDS reported that individuals with recurrent pneumonia were at significantly higher lung cancer risk than those without [3]. Our aims were to determine the incidence and risk factors for lung cancer among participants in two longitudinal studies of HIV infection in United States women and men.

Methods: Data from 3763 women in the WIHS and 6972 men in the MACS were analyzed and incidence rates (IR) per 100,000 person-years and rate ratios (RR) were calculated.

Results: We identified 44 lung cancer cases (33 HIV+ and 11 HIV-), 25 in the WIHS and 19 in the MACS, all with a history of smoking cigarettes. Among current and former smokers, the IR was significantly higher in the WIHS than in the MACS (WIHS IR=110.4 and MACS IR=35.8, p<0.001) but did not differ by HIV status. In multivariable analyses of the MACS participants, >30 pack-years of smoking (IRR=10.2) and a prior AIDS diagnosis (IRR=4.9) were significantly associated with an increased lung cancer rate. In multivariable analyses of the WIHS participants, age >49 (IRR=2.9 for 50-59; IRR=10.1 for 60+), Black race (IRR=4.6), >9 pack-years of smoking (IRR=14.7 for 10-30 pack-years; IRR=20.7 for >30 pack-years), and prior AIDS pneumonia (IRR=7.5) were significantly associated with and increased rate of lung cancer while more recent year of cohort enrollment (IRR=4.4 for 2001-2002) was associated with a lower rate.

Conclusions: HIV infection was not associated with lung cancer in the WIHS and was no longer significant in the MACS after adjustment for a prior AIDS diagnosis. A prior diagnosis of AIDS pneumonia was a risk factor for lung cancer in the WIHS. Pack-years of smoking was a strong risk factor for lung cancer in both cohorts but was twice as strong in the WIHS. A better understanding of the effect of HIV on lung cancer is needed but cessation of smoking is an ideal goal for HIV-infected individuals.

References

O25 Hepatobiliary cancers in persons With HIV/AIDS in the United States
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Infectious Agents and Cancer 2012, 7(Suppl 1):O25

Background: Cancers of the hepatobiliary tract (liver, bile duct and gall bladder) are characterized by relatively infrequent occurrence, aggressive growth, and recurrences after treatment. Hepatocellular carcinoma (HCC) is of special concern in the context of HIV/AIDS due to substantial associated morbidity and mortality. The overall burden of liver cancer may increase in people with AIDS as the combined effects of alcohol use, coinfection with hepatitis C virus (HCV) and hepatitis B virus (HBV), and other risk factors manifest as chronic liver disease.

Methods: Registry linkage data from the U.S. HIV/AIDS Cancer Match Study were used to estimate standardized incidence ratios (SIRs) to compare the risk of hepatobiliary cancers in people with HIV/AIDS to the general population. We also estimated rate ratios (RRs) of HCC by HIV risk group, calendar period and AIDS status. HIV risk groups were categorized by HCV prevalence [high prevalence: hemophiliacs, injection drug users (IDUs), and IDU-men who had sex with men (MSM); and low prevalence: heterosexuals, non-IDU MSM, and others].

Results: Compared to the general population, people with AIDS had higher risk for HCC (366 observed cases, SIR: 3.85; 95%CI: 3.47-4.27). SIRs were also elevated for other liver and intrahepatic bile duct tumors (27 cases, SIR: 3.26, 95%CI: 1.15-4.76), but not for cholangiocarcinomas (22 cases, SIR: 1.36; 95%CI: 0.85-2.05) or gallbladder tumors (11 cases, SIR: 1.39; 95%CI: 0.70-2.49). Adjusted for sex and age, people with high HCV prevalence were at higher risk for HCC than people with lower HCV prevalence (IRR: 2.12; 95%CI: 1.72-2.60). SIRs were elevated for all HIV risk groups, with the highest SIRs among hemophiliacs (40.4), IDUs (5.59), and IDU-MSM (4.39). Steadily increasing risk among persons with AIDS was observed across calendar time, including
This study reinforces the primary role of HCV coinfection in HCC pathogenesis in persons with AIDS in the United States. HCC risk is higher in people with AIDS than people with HIV infection without AIDS, consistent with a contribution from prolonged immunosuppression. Rising HCC incidence in the era of HAART suggests that HAART itself does not fully correct the negative impact of HIV on HCC-related cirrhosis, and that access to appropriate anti-HCV therapies in HIV-infected individuals is critical for prevention of progression to HCC.

**Conclusion:**

This study reinforces the primary role of HCV coinfection in HCC pathogenesis in persons with AIDS in the United States. HCC risk is higher in people with AIDS than people with HIV infection without AIDS, consistent with a contribution from prolonged immunosuppression. Rising HCC incidence in the era of HAART suggests that HAART itself does not fully correct the negative impact of HIV on HCC-related cirrhosis, and that access to appropriate anti-HCV therapies in HIV-infected individuals is critical for prevention of progression to HCC.

**References:**

Subjects and methods: Hospital records of patients with tissue diagnosis of lymphoma from January 2005-June 2011 were examined. Those with retroviral positive results were further followed up to treatment centre at the Presidential Emergency Programme for AIDS Relief (PEPFAR) clinic. Records were also reviewed at the Calabar Cancer Registry.

Results: Fifty-four patients with lymphoma were seen within the period (2005-2011). Non-Hodgkin’s lymphoma (NHL) was the most frequent (35/63%) Hodgkin’s lymphoma (HL) 8(18.2%), Burkitt’s lymphoma (BL) 7(15.9%) and nasopharyngeal lymphoma (NU) 1(2.3%). ARL was 12(18.2%) with NHL, HL and NL contributing 9(62%), 2(25%), 1(12.5%) respectively. Mortality was significantly higher in the ARL than in the non-ARL group.

Conclusions: ARL is not a rarity in our environment. A survey of all the HIV treatment centres will reveal a larger statistics. A greater understanding of the biology of this complex is needed. Training of ‘all care providers to effectively manage the disease is highly recommended. The cost of drugs for the treatment of the lymphoma is prohibitive to most of the indigent patients who present to our centre.

Material and methods: 20 cancer registries were trained and 5 have met criteria for population based cancer registries. Data for 2009 are presented in this report.

Results: The commonest cancer at all sites is Prostate in men and Breast in women. There was a gradient in the incidence that paralleled the socio-economic development of the regions of the country. The ASR for breast cancer ranged from 101.1 in Abuja to 7.5 in less cosmopolitan areas. For Prostate the ASR ranged from 73 in Abuja to 1.7. The other common cancers were Kaposi Sarcoma and Colo-Rectal in men, and cervix in women.

Conclusions: This study showed that breast and cervical cancer are the commonest in women while prostate is the commonest in men.

References

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P2

Changes in incidence and prognosis of malignancies in children with HIV
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Infectious Agents and Cancer 2012, 7(Suppl 1):P2

Aim: The aim of this study was to analyze the differences in patient demographics as well as in the relative incidence and outcome of childhood cancers, associated with the HIV infection.

Material and methods: A retrospective comparative study of two series of children with malignant disease, one with HIV one without, was carried out. The former series consisted of 99 African children with cancer and HIV, consecutively admitted at Tygerberg Children’s Hospital, Cape Town and Universitas Hospital, Bloemfontein, from 1995 to 2010. The latter series was formed of 570 African children with malignant diseases, not infected with HIV, consecutively admitted at the 2 hospitals, from January 2002 to December 2010. Variables studied were age, sex ratio, distribution of various malignancies, length of follow-up, treatment abandonment and mortality.

Results: The HIV positive children tended to be younger at diagnosis. The male/female ratio was slightly over 2 to 1 in the HIV positive group, while in the control group the sex ratio approached 1:1. Kaposi sarcoma was seen exclusively in the HIV positive series. The death rate was 50.3% in the HIV positive children (versus 40.8% in HIV negative) but the difference is not significant. When subgroups with matched cancers were compared, children infected with HIV had a significantly higher risk to die of drug-induced toxicity (relative risk 2.9, 95% confidence interval 3.7-225.8); only 26% of the HIV-positive children survived, compared with 51.2% in those not HIV infected (p=0.02).

Conclusions: The infection with HIV increases the risk for Kaposi sarcoma, for death due to cytostatic toxicity as well as the overall risk of death in children with cancer.

P3

Creating a nationwide cancer registration system to support AIDS-cancer match studies in Nigeria
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Infectious Agents and Cancer 2012, 7(Suppl 1):P3

Background: Cancer registration started in Nigeria in 1962 but after a very promising start, the momentum was lost [1,2]. Since 2009, Nigerian Health Ministry, IARC and IPRI, have given training, troubleshooting and mentoring.

Material and methods: 20 cancer registries were trained and 5 have

References

P4

Anal HIV DNA is associated with high-risk HPV genotypes in anal cytology specimens obtained for anal neoplasia screening
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Infectious Agents and Cancer 2012, 7(Suppl 1):P4

Purpose: High-grade anal neoplasia (AN) is associated with high-risk human papillomavirus (HPV) genotypes and is the precursor to anal cancer. Individuals infected with human immunodeficiency virus type 1 (HIV) continue to be at increased risk for AN even while on effective antiretroviral therapy (ART) with undetectable HIV RNA levels. The study was undertaken to assess HIV DNA from anal cytology specimens to determine if HIV DNA copy number was a factor for presence of high risk HPV genotypes.

Material and methods: Anal cytology specimens were obtained as part of an AN study according to guidelines approved by the local institutional review board. Anal HPV genotype, HIV DNA copy numbers, and cytology were obtained for each specimen. High-risk HPV genotypes included 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Analysis was performed by logistic regression model with high HPV risk as the response and HIV DNA copy numbers, anal Pap cytology results, and nadir CD4 cell counts as predictors. Lemeshow goodness-of-fit test was then performed to check model fit. Similar procedure was performed in sub-cohort of undetectable HIV RNA patients.

Results: 46 specimens were available from 38 males and 8 females. 52.2% of the specimens were negative for any HPV with 52.6% of males being HPV-positive compared to 25% of females. Of 46 specimens, 42 patients had undetectable HIV RNA level. The odds of having high-risk HPV genotypes among subjects with (x=100) copies of HIV DNA was 1.161 times the odds among subjects with x copies of HIV DNA (p=0.013). Inclusion of nadir CD4 count in the logistic regression model did not predict HPV risk. Distributions of HIV DNA were statistically different between normal and abnormal anal cytologies (p=0.009); median and interquartile HIV DNA copy numbers for normal and normal cytologies 792 (38-2100) and 27.5 (10-225), respectively. Figure 1. Anal cytology results were also associated with HPV risk (p=0.034). Patients with undetectable HIV RNA (n=42) had similar findings.

Conclusions: Individuals with higher HIV DNA copies in anal specimens were more likely to have high-risk HPV genotypes independent of nadir CD4 cell count. Abnormal anal cytologies were also associated with high-risk HPV. The association of HIV DNA copy number in anal specimens needs validation in future studies to determine the role in the pathogenesis of AN and high risk HPV. Grant support: RR011091, RR026136, CA121947, CA143727, CA096254.
In DC, despite high HIV/AIDS and cancer prevalence, only HIV-associated KS is the most common reported malignancy in sub-Saharan Africa, and appropriate therapy of KS requires continued monitoring of HIV-infected persons for cancers.

**Background:** Washington, DC (DC) has one of the highest HIV/AIDS rates in the U.S and cancer is the second leading cause of death among DC residents. This study sought to examine the demographic characteristics and survival of persons with AIDS defining cancers (ADCs) compared to those with non-AIDS defining cancers (NADCs) between the early HAART era (1996-2001) and the late HAART era (2002-2006) in DC.

**Methods:** Cases reported from 1996-2006 to the DC Cancer Registry and the AIDS Surveillance Registry were linked using a probabilistic matching algorithm. Cases were included if the cancer occurred from 4 months to 60 months post-AIDS diagnosis and were stratified into ADCs and NADCs for analyses. Cancer diagnoses were stratified into the early and late HAART eras to compare the availability of HAART on the distribution of cancer type. Kaplan-Meier survival analysis and adjusted Cox proportional hazards regression were used to assess survival time and risk of death by cancer type.

**Results:** From 1996-2006, among 8,800 AIDS cases, 300 (3.4%) cases had a cancer diagnosis. NADCs accounted for 51% of cancers and were significantly more likely to be diagnosed with AIDS (p<0.0001) and cancer (p<0.0001) at 40 years or older and had a significantly longer median time from AIDS to cancer diagnosis (2.46 vs. 1.75 years, p=0.01) compared to ADCs. The most common ADCs were Kaposi sarcoma (40%) and non-Hodgkin lymphoma (NHL) (44%); the most common NADC cases were lung (20%), Hodgkin lymphoma (8%) and anal (8%) cancer. ADCs accounted for 56% of cancer cases in the late-HAART era as compared to the early-HAART period (45%). Mortality within the first year of cancer diagnosis was similar (ADC 41% vs. NADC 37%) and no statistical difference in survival time was observed. In the adjusted model, NHL and lung cases were significantly more likely to die as compared to other cancers (NHL HR=3.06; Lung HR=3.44).

**Conclusions:** In DC, despite high HIV/AIDS and cancer prevalence, only a small proportion of AIDS cases also develop cancer with ADCs and NADCs being equally common. HAART availability does not seem to have altered survival among ADCs and NADCs. Survival among NHL cases was relatively low reflecting the need for increased access to care among HIV + persons. NADC cases are most likely developing cancers related to advancing age with higher proportions of lung cancers being observed. Public health efforts should focus on lung cancer prevention and continued monitoring of HIV-infected persons for cancers.

**Acknowledgement:** The authors would like to acknowledge the assistance of Drs. Aaron Adade, Joanne Lynn, Paul Levine, and Shannon Hader in the conduct of this study.

**P6 Diagnosing Kaposi’s Sarcoma (KS) in East Africa: how accurate are clinicians and pathologists?**

**Background:** KS was first described by Reed and Nevin in 1958 as red plaques in patients with AIDS. In East Africa, KS is the most common reported malignancy in sub-Saharan Africa, and appropriate therapy of KS requires accurate diagnosis. In much of the region, however, KS diagnosis is

**Methods:** From 1996-2006, among 8,800 AIDS cases, 300 (3.4%) cases had a cancer diagnosis. NADCs accounted for 51% of cancers and were significantly more likely to be diagnosed with AIDS (p<0.0001) and cancer (p<0.0001) at 40 years or older and had a significantly longer median time from AIDS to cancer diagnosis (2.46 vs. 1.75 years, p=0.01) compared to ADCs. The most common ADCs were Kaposi sarcoma (40%) and non-Hodgkin lymphoma (NHL) (44%); the most common NADC cases were lung (20%), Hodgkin lymphoma (8%) and anal (8%) cancer. ADCs accounted for 56% of cancer cases in the late-HAART era as compared to the early-HAART period (45%). Mortality within the first year of cancer diagnosis was similar (ADC 41% vs. NADC 37%) and no statistical difference in survival time was observed. In the adjusted model, NHL and lung cases were significantly more likely to die as compared to other cancers (NHL HR=3.06; Lung HR=3.44).

**Conclusions:** In DC, despite high HIV/AIDS and cancer prevalence, only a small proportion of AIDS cases also develop cancer with ADCs and NADCs being equally common. HAART availability does not seem to have altered survival among ADCs and NADCs. Survival among NHL cases was relatively low reflecting the need for increased access to care among HIV + persons. NADC cases are most likely developing cancers related to advancing age with higher proportions of lung cancers being observed. Public health efforts should focus on lung cancer prevention and continued monitoring of HIV-infected persons for cancers.

**Acknowledgement:** The authors would like to acknowledge the assistance of Drs. Aaron Adade, Joanne Lynn, Paul Levine, and Shannon Hader in the conduct of this study.
Limited to clinical suspicion without pathologic confirmation. Where pathology is available, specific anti-KSHV stains are rarely available and overall pathologic accuracy for KS has not been evaluated.

Methods: We introduced skin punch biopsy for KS at HIV/AIDS care clinics in Uganda and Kenya within the Eastern Africa IeDEA Consortium. Clinicians suspecting KS could obtain a biopsy sample without charge. After interpretation by local African pathologists who had access to routine H&E staining, biopsies were read by dermatopathologists at UCSF who could, at their discretion, recut and re-stain specimens or stain against different pathology services.

Results: Clinicians at 26 HIV/AIDS clinics in Uganda and Kenya referred 739 patients with clinically suspected KS for skin biopsy. Overall, 77% (95% CI: 74%–80%) of these clinically suspected cases were determined pathologically to be KS after U.S. review; 19% had another diagnosis and 4% were indeterminate. There was no significant difference in the percentage found to be KS between countries (p=0.20) or over time (p=0.11). When KS was not found, a wide variety of other diagnoses, both clinically significant and insignificant, were made by the U.S. dermatopathologists (Table 1). Two different pathology services, one in Uganda and one in Kenya, submitted biopsies for review by U.S. dermatopathologists. Overall concordance between African and U.S. interpretations was 71% (95% CI: 68%–74%). When the U.S. interpretation was considered gold standard, sensitivity of the African pathologic interpretation for KS was 72% and specificity 84%. Over time, sensitivity increased at one African center (p=0.04) but decreased in another (p=0.001); specificity increased at one center (p=0.001) and was unchanged in another (p=0.68).

Conclusions: Amongst clinicians at HIV/AIDS clinics in East Africa, clinical suspicion of KS alone is not optimally specific for KS diagnosis. Clinical suspicion alone often either misdiagnoses conditions which are less concerning than KS or misses other serious conditions that require different therapy than KS. Assuming the U.S. interpretation is the gold standard, pathologic determination of KS in East Africa is specific but not optimally sensitive. The findings urge for increased availability of skin punch biopsies for KS diagnosis in Africa and augmentation of pathology services.

Table 1 (abstract P6)
Sample of pathologic diagnoses made by U.S. dermatopathologists when KS was not present

<table>
<thead>
<tr>
<th>Scar (n=9)</th>
<th>Post-inflammatory pigmentation (9)</th>
<th>Psoriasis (8)</th>
<th>Lymphoma (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wart (5)</td>
<td>Bacillary angiomatosis (4)</td>
<td>Morphea (4)</td>
<td>Sarcoidosis (2)</td>
</tr>
<tr>
<td>Polyarteritis nodosa (2)</td>
<td>Pyogenic granuloma (2)</td>
<td>Mycobacterial dermatitis (2)</td>
<td>Lichen planus (2)</td>
</tr>
<tr>
<td>Dermatofibroma (2)</td>
<td>Castleman's Disease (1)</td>
<td>Squamous cell carcinoma (1)</td>
<td>Deep fungal infection (1)</td>
</tr>
<tr>
<td>Secondary syphilis (1)</td>
<td>Erythema multiforme (1)</td>
<td>Eccrine poroma (1)</td>
<td>Xanthoma (1)</td>
</tr>
</tbody>
</table>

Table P8
Evaluation of the AIDS clinical trials group staging criteria for Kaposis Sarcoma in a resource limited setting

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Background: Kaposis sarcoma (KS) is commonly staged using the AIDS Clinical Trials Group (ACTG) criteria. The three variables of the ACTG are dichotomized as good risk (0) and poor risk (1). Good risk immune status (I0) is defined as CD4 >200 cells/µl and poor risk (I1) as CD4 <200 cells/µl. Although validated in the US and Europe, no evaluation has been done in resource-limited settings during the HAART era. We sought to determine whether the ACTG staging criteria is predictive of overall survival among Ugandan patients with HIV-associated KS.

Methods: Data were abstracted from medical records of adult patients with HIV-associated KS seen at the Uganda Cancer Institute (UCI) from 2000-2006. The primary outcome was 2-year overall survival. Vital status at 2 years was determined from the medical chart, or by contacting the patient or next of kin using the phone contact provided in the chart or ART clinic. Survival was modeled using Kaplan-Meier methods. Factors associated with survival were evaluated using Cox proportional hazards.

Results: The median survival time was 468 days (range 0, 5411). At 2 years following KS diagnosis, 165 (40.8%) of participants were alive and...
F9 Factors associated with cancer pathogenesis among patients attending oncology clinic at Kamuzu Central Hospital in Malawi
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Introduction: Cancer is the cause of 13% of total worldwide mortality, and was the leading cause of in 2010 according to the World Health Organization. HIV prevalence in urban Malawi is approximately 20% and contributes to the pathogenesis of cancers, particularly AIDS-defining cancers. To evaluate risk factors for specific malignancies in Malawians, we designed an observational study to collect clinical data for cancer patients presenting at Kamuzu Central Hospital (KCH) in Lilongwe, Malawi.

Methods: This was an observational study enrolled patients with suspected or confirmed malignancies presenting to Kamuzu Central Hospital (KCH) in Lilongwe, Malawi. From June 2010 to July 2011, patients underwent interviews and medical chart reviews to complete database questionnaires. The questionnaire data were entered into a web-based database and extracted into Microsoft Excel. Descriptive statistics were performed.

Results: From June 2010 to July 2011 317 patients were enrolled into the study, 123 (39.3%) were male and 190 (60.7%) were female. 4 had missing information. Age ranged from 18 to 86. 227 (70.9%) tested negative for HIV and 90 (28.3%) tested positive for HIV. 3 (0.94%) had missing HIV test results. 38 (11.9%) had Kaposi’s sarcoma, 12 (7.74%) had lymphoma, 71 (22.4%) had cervical cancer, 26 (8.2%) had breast cancer, 96 (30.2%) had esophageal cancer and the other cancers were smaller categories. 232 (76.32%) and 216 (71%) had never taken alcohol. Only 8.5% had family history of primary cancers. On past medical history, only 1.5% never had malaria, and 295 (95.3%) reported to have past or present infection with malaria, TB, or schistosomiasis. Of note is that 89% of Kaposi’s sarcoma patients had concurrent HIV infection. Excluding the patients with KS, only 56 of the total were HIV positive.

Conclusions: AIDS related malignancies are common in Malawi. However, HIV rates in traditionally non-AIDS related malignancies appear to match general population HIV prevalence rates.

P10 Gender differences in HIV-infected and HIV-uninfected patients with lung cancer
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Clinical background: Lung cancer (LC) is the leading cause of cancer-related death among people living with HIV (PLWH) [1]. In the general population, adenocarcinoma is more common in women with LC while squamous cell carcinoma (SqCC) is more common in men. Survival after lung cancer is worse among PLWHA. We explore potential gender-related difference in lung cancer in HIV+ and HIV- patients.

Methods: A retrospective review of the hospital cancer registry from 2000-2010 was performed. HIV status of identified lung cancer patients was assessed. Demographics, stage of cancer, and outcome were recorded for HIV+ and HIV- patients. Data were analyzed using SAS 9.1.

Results: Over the 10-year period, 1250 lung cancer cases were identified (76 HIV+, 205 HIV-, and 970 unknown HIV status). There were 20 women (W+) and 55 men (M+) with HIV, and 85 women (W-) and 120 men (M-) who are HIV-. There were significantly more men tested for HIV at cancer diagnosis than women (p=0.0001). The distribution of lung cancer type is similar among the HIV+ and HIV-. Median age at cancer diagnosis is not significantly different with W+(50 years old), W-(55), M+(55), and M-(58). Presentation at stage IIB or IV occurred in 69%W+, 67% W, 68%M+ and 73%M-. There is no difference of median CD4 (W+=233, M+=159, p=0.1) or HAART use at cancer diagnosis among M+(53%) and W+(63%), p=0.4. The median survival time for W+(386 days), M+(192 days), W-(475 day) and M-(247 days). There is trend for longer survival for W+ versus M+ (log rank p=0.07), as well as W- versus M- (log rank p=0.06), but no difference for W+ vsW- (LR p=0.7) or M+ vs M- (LR p=0.8).

Conclusion: The experience in our hospital reveals that in the HAART era, there does not seem to be a difference in lung cancer presentation among HIV+ or HIV- patients, and that there is a trend for better survival among women compared to men whether HIV+ or HIV-.

Acknowledgement: This work was facilitated by the Center for AIDS Research at Emory University (P30 AI050409).

Reference

Table 1(abstract P8) Factors associated with death before 2 years from KS diagnosis

<table>
<thead>
<tr>
<th>FACTOR</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR  95% CI</td>
<td>HR  95% CI</td>
</tr>
<tr>
<td>T1 VS T0</td>
<td>4.13 (2.18-7.81)</td>
<td>4.33 (2.36-8.77)</td>
</tr>
<tr>
<td>I1 VS I0</td>
<td>1.25 (0.64-2.44)</td>
<td>1.69 (1.12-2.53)</td>
</tr>
<tr>
<td>S1 VS SO</td>
<td>1.71 (1.13-2.57)</td>
<td>0.98 (0.96-1.00)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>0.98 (0.96-1.00)</td>
<td>0.84 (0.76-0.91)</td>
</tr>
<tr>
<td>Nodular KS morphotype</td>
<td>1.50 (0.97-2.32)</td>
<td>1.34 (0.84-2.15)</td>
</tr>
<tr>
<td>Trunk edema</td>
<td>2.91 (1.53-5.53)</td>
<td>2.45 (1.27-4.75)</td>
</tr>
<tr>
<td>On HAART at diagnosis</td>
<td>0.75 (0.54-1.02)</td>
<td>0.62 (0.45-0.87)</td>
</tr>
<tr>
<td>Receipt of chemotherapy</td>
<td>0.46 (0.33-0.66)</td>
<td>0.29 (0.20-0.42)</td>
</tr>
</tbody>
</table>

a Multivariate analysis adjusted for T, S, age, nodular morphotype, trunk edema, HAART, and chemotherapy.
P11


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Infectious Agents and Cancer 2012, 7(Suppl 1):P11

Background: SSALC was established to characterize HIV/AIDS-related lymphoma and the indigenous background of malignant lymphomas (ML) in sub-Saharan Africa. Because WHO classified lymphoma subgroups can vary in prevalence African, Asian or European ancestry, we surveyed lymphoma heterogeneity in geographically diverse East, South and West sub-Saharan populations, particularly for HIV/AIDS associated immunophenotypes.

Methods: A consortium of African pathologists, hematologist/oncologists and oncologic surgeons contributed ML cases and participated in subgrouping according to WHO classification criteria after appropriate Institutional Review Board (IRB) approvals, Memorandum of Understanding and Material Transfer Agreements were obtained. Paraffin blocks were examined for tissue morphology (H&E), immunophenotype (34 antibodies IHC), EBER, kappa and lambda light chains (CISH) and bcl2 translocations (FISH). HIV/AIDS diversity controls were contributed from Europe by consortium and USA by ACSR.

Results: Consortium members contributed 46 - 368 cases each with 1408 total cases at date: 246 diffuse large B-cell lymphoma (DLBCL), 296 Burkitt lymphoma, 163 Hodgkin disease, 69 plasma cell proliferative disorders and 644 others. Aggressive DLBCL, plasmacytoma/plasmablastic lymphoma, KSHV disease and lymphoid hyperplasia will be highlighted.

Conclusions: Sub-Saharan Africa has a variety of ML subgroups; true incidence altered by: 1) Aspiration vs. biopsy for diagnosis; 2) HIV status not communicated to pathologist; 3) known HIV/AIDS patients not biopsied; 4) initial diagnosis by morphology alone; 5) tissue preservation/processing variable. General observations: HIV/AIDS-related lymphoma is more likely EBER+, has higher cell proliferation rates, and unfavorable immunophenotypes; regions differ in HIV clades with South (clade C) having the most “immunosuppression” associated lymphoma subgroups; East region has more pre-T lymphoblastic lymphomas and West region has more follicular lymphomas. Ongoing studies: infectious lymphadenopathies (EBV+ lymphoproliferations), undifferentiated neuroblastomas, neuroectodermal tumors (PNETs), metastatic carcinomas and malignant melanoma (amelanotic).

Acknowledgement: AIDS and Cancer Specimen Resource (ACSR) NCI U01-CA66531-s Sub-Saharan Africa Lymphoma Consortium (SSALC).

P12

HIV-associated non-Hodgkin’s lymphoma- experience from a tertiary referral cancer center

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Infectious Agents and Cancer 2012, 7(Suppl 1):P12

Background: Infection with human immunodeficiency virus infection (HIV) is associated with an increased risk of Non Hodgkin’s lymphoma (NHL).

There is limited data on the treatment and outcome of these lymphomas in India. We describe a retrospective study of 277 HIV infected patients with NHL at a tertiary referral cancer center in Mumbai.

Material and methods: All patients included in this study were registered at the HIV cancer clinic of the hospital during 2001-2010. All patients were diagnosed to have NHL by tissue biopsy and were confirmed by immunohistochemical tests. Patients were staged with the Ann Arbor staging system. Data of their demographic profiles, immune status, NHL stage, treatment received, response and outcomes were analyzed. We used the gender and age-specific proportion of NHL of the year 2002 that was recorded in the Hospital Cancer Registry to estimate an expected number of NHL among HIV positive cancer patients during the period 2001-2010 (n=770) and the proportional incidence ratio (PIR) was calculated.

Results: There were 277 patients during the ten year study period. In males the PIR for NHL was 12.6 (95% CI 1.2-14.6) and in females it was 22.1 (95%CI 17.1-28.3). Among the 277 patients there were 69 females (24.9%) and 208 males (75.1%). The median age of males was 38 years. In females the median age was 37 years.100 Patients (36.1%) were previously known to be HIV positive (range 6 mths-15 years). The CD4 count was less than 200 per cumm in 127/192 (66.14%) patients.76/277 (27.43%) had current or past history of tuberculosis. 172/277 (62%) patients had extranodal involvement. 168/277 (60.64%) received cancer directed treatment. The data of the 168 patients who received treatment was analyzed. 91/134 (67.91%) had CD4 counts less than 200. 115/168 (68.45%) received antiretroviral therapy. 60% had extranodal involvement. 72 (42.9%) had DLCL, 42 (25%) plasmablastic, 21 (12.5%) Burkitt’s type and 31 (18.5%) other9.0/168 (53.6%) had advanced disease at presentation. All patients were treated with chemotherapy. 54 patients also received RT. The response was evaluated in 96 patients. There was complete response in 46 (47.9%), partial in 13 (13.6%), stable in 6 (6.3%) and 29 (30.2%) patients had progressive disease. The median survival was 25.3 months (range 0-56 months). ART affected survival significantly; however age, sex, CD4 counts at presentation, histopathology, and presence of extranodal involvement and stage of disease did not affect the survival.

Conclusions: In our study the PIR for NHL was high in HIV-infected patients. The proportion of plasmablastic lymphomas is high. The use of antiretroviral therapy has impacted the overall survival.

P13

Making the case for better integration of cervical cancer screening and treatment for HIV-infected women attending care and treatment clinics in Dar es Salaam, Tanzania

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Infectious Agents and Cancer 2012, 7(Suppl 1):P13

Background: HIV infected women are more likely to have persistent oncogenic human papillomavirus infections that lead to precancerous cervical lesions and cervical cancer. Of the incident cases of cervical cancer in Africa, 40% occur in East Africa (5). Tanzanian women bear the highest burden of cervical cancer in the region with age adjusted standardized incidence and mortality rates of 50.9 and 37.5 cases per 100,000 women. Cervical cytology was performed to estimate the prevalence of squamous intraepithelial lesions (SIL) and determine patient follow up for treatment of histologically confirmed SIL.

Methods: Between December 2006 and August 2009, physicians in HIV care and treatment clinics in Dar es Salaam, Tanzania performed conventional PAP smears on 1440 women who voluntarily accepted a cervical screening. Slides were prepared and sent to the histopathology lab at the Muhimbili National Hospital, Dar es Salaam, for examination. Positive smears included the detection of low-grade SIL (LSIL) and high-grade SIL (HSIL). Negative smears were defined by the detection of atypical squamous cells of undetermined significance and normal results.
Results: A total of 1440 smears were examined, and 124 (8.61%) of women had SIL. On cytology, 72 (5%) had LSIL and 49 (3.4%) had HSIL. On histology, 889 (61.74%) of all women screened had cervicitis or inflammation. Of those who were positive for SIL, 95 (76.61%) had cervicitis or inflammation. None of the women were found to have invasive cancer. Of the 124 women with SIL, 5 (4%) presented for follow up and treatment at the national cancer center in Dar es Salaam. The remaining 119 women had to be tracked using a district tracking mechanism comprised of trained lay health workers. 

Conclusion: The findings indicate a need for better integration of cervical cancer prevention for women attending HIV care and treatment clinics. Even in highly efficient HIV clinics with good health services, cervical screening programs based on cytology do not provide adequate screening coverage and timely access to treatment. Single visit models including immediate treatment with cryotherapy are more effective and context appropriate. However, innovative patient retention approaches will likely be necessary for treatment procedures that do not meet the criteria for cryotherapy and require follow up at the national cancer center. Implementation research will be needed to identify novel and sustainable approaches for comprehensive service delivery of cervical cancer screening and treatment in the context of HIV care and treatment clinics.

Acknowledgement: This work was supported by the National Institutes of Health Office of the Director, Fogarty International Center, Office of AIDS Research, National Cancer Center, National Eye Institute, National Heart, Lung, and Blood Institute, National Institute of Dental and Craniofacial Research, National Institute on Drug Abuse, National Institute of Mental Health, National Institute of Allergy and Infectious Diseases, and NIH Office of Research on Women's Health through the International Clinical Research Scholars and Fellows Program at Vanderbilt University (R24 TW007988) and the American Relief and Recovery Act.

P14
Digital cervicography and cold coagulation for cervical cancer screening in Nigeria
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Infectious Agents and Cancer 2012, 7(Suppl 1):P14

Background: Cervical cancer (CC) is most common cancer among women in Africa and in women living with HIV [1,2]. Its prevalence has remained stable or increasing with introduction of HAART suggesting complex interactions with HIV [3,4]. Current screening programs can substantially reduce all-cause mortality of CC but implementation in LMIC is hobbled by poor infrastructure, cost and lack of personnel. Nurse provider led, minimal visit, screen and treat programs offer an opportunity to reduce CC morbidity and mortality in LMIC [5]. In this study we evaluate the implementation of cervical cancer screen and treat programs at 2 HIV treatment and prevention sites in Nigeria.

Material and methods: CC screening programs using nurse providers, VIA, off the shelf camera for digital cervicography, treatment of eligible lesions by cold coagulation and referral as required was implemented at 2 PEPFAR supported sites in Abuja, Central Nigeria. QA was provided by Gynecologist and based on weekly review of digital cervicographs and client recall as required.

Results: From July 2010 to July 2011, 2002 HIV+ women had been screened for CC at the 2 sites, but only data on 925 is reported in this abstract. Mean (SD) age was 35.2 (7.0) years; mean (sd) age at sexual debut was 19.0 (3.9) years; range, mean, sd of pregnancies was 0 – 16, 3.4, 2.5; range, mean, sd of most recent cd4 count before screening was 11 – 1197, 466.7, 239.0; 6.8% were VIA positive; 0.2% had invasive CC and 0.2% were uncertain. Concordance between the clinical review and nursing diagnosis was 65% at the beginning of the program but reached 100% after 3 months.

Conclusions: This study showed nurse provider led CC screening and treatment program is a viable public health intervention among PLWHIV in Nigeria.

Acknowledgement: This study is supported by the IHV-UM Capacity Development for Research into AIDS Associated Malignancies (NIH/NCI D43CA153792-01 Pl, Adebamowo) and IHV-UM AIDS International Training and Research Program (NIH/FIC D43TW001041-11 Pl, Blattner).

References

Table 1 (abstract P15) Prevalence of HPV genotypes in renal transplant patients

<table>
<thead>
<tr>
<th>HPV genotype</th>
<th>HPV positive, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV positive</td>
<td>49 (47.5)</td>
</tr>
<tr>
<td>HPV negative</td>
<td>54 (52.4)</td>
</tr>
</tbody>
</table>

Single infections

<table>
<thead>
<tr>
<th>HPV genotype</th>
<th>HPV positive, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>25 (51.0)</td>
</tr>
<tr>
<td>56</td>
<td>2 (4.0)</td>
</tr>
<tr>
<td>58</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>54</td>
<td>5 (10.2)</td>
</tr>
<tr>
<td>6</td>
<td>2 (4.0)</td>
</tr>
<tr>
<td>11</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>12</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>20</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>53</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>62</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>66</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>71</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>Total single infections</td>
<td>42 (85.7)</td>
</tr>
<tr>
<td>Total multiple infections</td>
<td>4 (8.2)</td>
</tr>
<tr>
<td>Undetermined</td>
<td>3 (6.1)</td>
</tr>
</tbody>
</table>

*Gray shadow indicates HPV genotypes defined by IARC working group as class I carcinogens for humans (Bouvard et al., Lancet Oncol 10:321–322, 2009).
to determine the spectrum of viral genotypes in urine samples of men with renal transplants.

**Material and methods:** The study included 103 patients who underwent kidney transplantation between 1999 and 2008. HPV sequences were detected by nested PCR, using the broad-spectrum consensus-primer pairs MY09/MY11 and the new MGP system, and characterized by nucleotide sequence analysis.

**Results:** Overall, 49 (47.5%) samples were found positive for HPV sequences and the most common genotypes were HPV 16 (51.0%) and HPV 54 (10.2%) followed by HPV56, 57, 58, 66, 11, 12, 20, 45, 62, and 71, in descending order of prevalence (Table 1). The majority of HPV 16 isolates were classified as European and only two as African-1 variant on the basis of nucleotide signature present within the MGP L1 region.

**Conclusion:** The high prevalence of HPV 16 among renal allograft recipients suggests that an HPV-16-based preventive or therapeutic vaccine may be effective for prevention or treatment of HPV-related neoplasia in this group of immune compromised patients.

**Reference**

**P16**

**Knowledge and practice of malignancies among PLWHIV in Nigeria**

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**Material and methods:** Random sample of HIV+ and HIV- persons in Nigeria, were consented, and asked to participate in FGD on AIDS Associated Malignancies. Each FGD consisted of 10 persons, managed by a researcher and a note-taker using a discussion guide. FGD was culturally appropriate health education awareness of cancers in LMIC and there is need to provide contextual, evidence-based health education [3].

**Results:** Most participants had heard about cancer and considered it a fatal disease, but they had poor knowledge of the causes. None had heard of any of the common cancers that occur in PLWHIV. When asked about specific cancer like Kaposi Sarcoma, Lymphoma and Cervical Cancer, only cervical cancer was mentioned and while they know that it occurs in female reproductive tract, they did not associate it with HIV. Most respondents did not believe that it is possible to have HIV and cancer though some opined that it may be possible since both are caused by viruses.

**Conclusion:** AIDS associated malignancies appear to be very common in this environment and perhaps non AIDS associated malignancies may be on the increase. Under-reporting and lack of capacity may account for the few numbers reported in this environment.

**Reference**

**Material and methods:** A five year retrospective study was carried out to review the frequency of diagnosis of three tumours classified as AIDS defining malignancies (Kaposi sarcoma, non Hodgkin lymphoma, cervical cancer) and one non AIDS defining malignancy (squamous cell carcinoma of the conjunctiva), also commonly diagnosed in these patients. Recorded of the patients which are histologically confirmed and diagnosed between 1st January 2005 and 31st January 2009 were sorted out and their retroviral status classified.

**Results:** A total of 4123 histologically confirmed biopsies were received, 852 (21%) were cancers, 24 (2.8%) were Kaposi sarcoma (KS), 8 (3%) KS occurred in females, range 21-60 years (y), 16 (67%) in males, range 19-60 y and 17 (71%) of KS were AIDS associated, 6 (35%) females and 11 (65%) males. Thirty five 35 (4.1%) of cancers were Non Hodgkin lymphomas including Burkitt’s lymphomas, 8 (23%) in females, range 6-60 y and males 27 (77%), range 6-71 y. Two 5.7% were AIDS associated 2 (100%) were males on long standing antiretroviral treatment. Cervical cancers accounted for 84 (9.9%) all cancers and 14 (17%) occurred in HIV positive patients age range. Conjunctival squamous cell carcinomas were 13 (1.5%) of all cancers, 6 (46%) females 7 (54%) males. Two 2 (15%) occurred in HIV positive patients.

**Conclusion:** AIDS associated malignancies appear to be very common in this environment and perhaps non AIDS associated malignancies may be on the increase. Under-reporting and lack of capacity may account for the few numbers reported in this environment.

**Reference**
P19
Penile cancers without the AIDS epidemic in Cameroon
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Infectious Agents and Cancer 2012, 7(Suppl 1):P19
Background: Cancer of the penis is a uncommon malignancy in developed countries. But the incidence is as high as 17% of all male cancers in some undeveloped countries. The most important aetiologic factor is the presence of an intact foreskin but this is still unknown. Cameroon is a blank area on the world cancer map because medical facilities necessary for recording cancer cases and the population data necessary for the calculation of rate are scarce or inexistent. Only 10% of malignant neoplasms are confirmed by histology.
Methods: We described the pathological aspects of 10 cases of penile cancers observed in Cameroon, a developing country of 20.000.000 inhabitants, within a period of twenty seven years (1984-2011). Human Immunodeficiend Virus (HIV) serology test was done for nine patients of this series. Human Papilloma Virus (HPV) DNA detection and typing were carried out on paraffin-embedded specimens of our cases by Polymerase Chain Reaction.
Results: The patients aged 43 to 75 years and were circumcised. Four of the ten cases were diagnosed in 2004. HIV serology test done on 3 cases before 2004 were negative. After 2004, six patients were registered and out of these six, three came down with HIV/AIDS. One patient has type II diabetes mellitus. All patients consulted late with metastatic disease. The pathological type was squamous cell carcinoma for nine patients while one other has a Diffuse large B cell lymphoma. HPV DNA was detected in six cases.
Conclusions: Ten cases of penile cancer were observed in Cameroon within the AIDS epidemic. These are cases which are confirmed by history as only 10% of the patients with cancer can have histology performed. The aetiology is unclear. The HIV should be investigated as an etiological factor.

P20
Multicentric Castleman's disease in HIV/AIDS patients at an urban HIV clinic in Atlanta, Georgia, in the combined antiretroviral therapy era
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Infectious Agents and Cancer 2012, 7(Suppl 1):P20
Background: Multicentric Castleman’s disease (MCD), which has been associated with human herpesvirus-8 (HHV-8), is a lymphoproliferative disorder with an increased prevalence in HIV positive patients [1]. We describe our experience with MCD in a group of patients with HIV/AIDS in an urban HIV clinic.
Methods: Our clinic serves annually 5,000 patients diagnosed with AIDS. Patients with a diagnosis of multicentric Castleman’s disease between 2006 and 2010 were identified from the pathology database at Grady Memorial Hospital or referrals to the clinic. Clinic charts and medical records were abstracted. Patients’ demographics, CD4 counts, HIV viral load, HIV and MCD treatment and outcomes were recorded.
Results: Nine patients diagnosed with MCD were identified in our HIV/AIDS population. All patients were male and reported sex with men (MSM) as their risk for HIV infection. The mean age at MCD diagnosis was 39.22 ± 11.40; the mean CD4 cell count nadir was 68.33 ± 62.2 cells/mm³, 85% (7/9) were on cART (combined antiretroviral therapy) at the time of MCD diagnosis with a mean CD4 count of 233.67±157.44 cells/mm³. MCD was of the hyaline vascular variant in 3 patients, plasma cell variant in 2, transitional in 1 patient, and unspecified in 2 patients. Symptomatic lesions were present in three patients. Five patients had both Kaposi sarcoma (KS) and MCD (2 with KS occurring after MCD diagnosis, 1 with KS before MCD, 2 with KS and MCD diagnosed simultaneously). Most of the patients were anemic with mean hemoglobin of 8.99±0.44 g/dL and hypoalbuminemic (2.31±0.96), 85% had anaemia, hepatosplenomegaly, and low albumin at diagnosis. Treatment consisted of valganciclovir, chemotherapy and/or rituximab. In the 5 patients who died, the mean time from MCD diagnosis was 425±447 days.
Conclusions: HIV-associated MCD is characterized by lymphadenopathy, splenomegaly, anemia and hypoalbuminemia. Among the diseases associated with HHV8 (KS, primary effusion lymphoma, and MCD), MCD appears to be the least affected by cART use or degree of immune-suppression [2]. In our cohort, 85% of patients had a CD4 count above 200 at MCD diagnosis. The survival with cART is still dismal, with one year survival of 50%. Larger multicenter study is needed to better understand the pathogenesis of HIV-associated MCD and its treatment.
References
Prevalence of HIV in Medicare beneficiaries with lung cancer

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Infectious Agents and Cancer 2012, 7(Suppl 1):P21

Background: Human immunodeficiency virus (HIV) patients are at higher risk for lung cancer than the general population [1]. The impact of HIV on the lung cancer population is unclear. In this study, we estimate the prevalence of HIV among Medicare beneficiaries diagnosed with lung cancer.

Material and methods: This study used the SEER-Medicare database which links Medicare claims data with patients identified through cancer registries as part of the Surveillance Epidemiology and End Results (SEER) program. There were 250,500 patients who were diagnosed with malignant lung cancer between 1998 and 2007: 225,233 qualified for Medicare based on age and were 65 years or older at diagnosis and 25,267 qualified for Medicare based on disability and were less than 65 years old at diagnosis. Demographic information was taken at the time of the initial lung cancer diagnosis. Patients were classified as prevalent HIV cases if their first Medicare claim with a diagnosis of HIV preceded the diagnosis of lung cancer or occurred within one year after lung cancer was diagnosed. Relative risk (RR) was used to assess risk factors.

Results: The prevalence of HIV in lung cancer cases was 180.6 (95% CI: 163.8 to 199.0) and 1,646.0 (95% CI: 1,495.1 to 1,812.3) per 100,000 among elderly and disabled beneficiaries, respectively. It doubled from 1998 to 2007 for elderly beneficiaries and increased by 33% for disabled beneficiaries. Risk factors for HIV were male gender, non-white race, never having been married, and residence in a metropolitan area (Table 1).

Elderly HIV and non-HIV patients were comparable with respect to stage of lung cancer at diagnosis, but HIV-infected disabled beneficiaries were more likely to present with distant metastases than their non-HIV counterparts.

Conclusions: The prevalence of HIV among elderly Medicare beneficiaries with lung cancer was 2.6 higher than in the general population 65 years and older [2]. For disabled beneficiaries, the prevalence of HIV among lung cancer cases was higher than for those without lung cancer [3]. The increasing prevalence of HIV in lung cancer cases may result in a commensurate increase in demand for health care services for Medicare beneficiaries.

References

Table 1(abstract P21)

<table>
<thead>
<tr>
<th></th>
<th>Elderly Beneficiaries RR</th>
<th>Disabled Beneficiaries RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male vs. Female</td>
<td>1.9*</td>
<td>3.5*</td>
</tr>
<tr>
<td>African-American vs. White</td>
<td>6.4*</td>
<td>3.1*</td>
</tr>
<tr>
<td>Other race vs. White</td>
<td>2.0*</td>
<td>0.7</td>
</tr>
<tr>
<td>Never married vs. Other (Men)</td>
<td>4.8*</td>
<td>7.3*</td>
</tr>
<tr>
<td>Never married vs. Other (Women)</td>
<td>2.2*</td>
<td>4.6*</td>
</tr>
<tr>
<td>Big Metro vs Other</td>
<td>5.5*</td>
<td>5.2*</td>
</tr>
<tr>
<td>Metro vs. Other</td>
<td>2.9*</td>
<td>2.9*</td>
</tr>
</tbody>
</table>

*RR significantly > 1.0
P24

The AIDS malignancy clinical trials consortium (AMC) patient navigator (PN) initiative

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Infectious Agents and Cancer 2012, 7(Suppl 1):P24

Background: Cancer remains a major health concern in the management of HIV infection in areas of the world with and without access to highly active antiretroviral therapy. In the USA alone, approximately 56,000 people were newly diagnosed with HIV infection in 2006; 53% of these new diagnosis occurred in gay and bisexual men but Black/African American men (45%) women (27%), and Hispanics (17%) were also strongly affected [1]. Recruitment of members of these groups into cancer clinical trials has traditionally been challenging [2]. Among domestic AMC studies, a relatively small percentage of all participants have included women (8%), African-Americans (29%), and Hispanics (21%). Patient Navigation has been identified as an effective strategy to reduce barriers to care as well as to increase access to cancer clinical trials [3]. In an effort to bolster opportunities for HIV-infected minorities, women, and medically underserved populations to become involved in AMC clinical trials, a PN initiative was implemented in seven AMC sites located in Boston, Los Angeles, San Diego, Houston, Columbus, and Honolulu. The main objectives of the PN initiative were to provide greater opportunity for minority groups and women to participate in AMC-sponsored cancer trials and to increase awareness of HIV/AIDS malignancies in the local communities where the PNs worked. From January 2010 to April 2011, PNs implemented multi-strategy activities to increase the enrollment of women and minorities in AMC trials. PNs reported 466 activities in 58 programmatic areas of recruitment and retention, community outreach and education and awareness. Recruitment and retention refers activities to recruit new participants and increase retention in AMC trials. Community outreach was targeted to the medical community or the general population to increase their awareness of AIDS-related malignancies. Education and awareness were activities to educate the community on HIV-related malignancies in general and AMC-sponsored clinical trials in specific. PNs efforts were concentrated on community outreach (54%, n=251), followed by recruitment and retention (28%, n=129) and education and awareness 18% (n=86).

Conclusion: AMC-PNs conducted activities that raised awareness in their local communities of AIDS-related malignancies, developed partnerships with local health community organizations and identified areas where further communication was needed. PNs took the lead in developing a PN brochure and in the design of several tailored recruitment strategies. The PN program is making important inroads into behavioral interventions to increase participation of minorities and underserved populations in AMC trials.

Acknowledgement: The AMC PNs Participating Principal Investigators and Patient Navigators and a supplemental grant from NIH/NCI U01CA121947.

References:

P25

Ultraviolet radiation exposure and HIV-associated non-Hodgkin lymphoma risk

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Infectious Agents and Cancer 2012, 7(Suppl 1):P25

Introduction: Although the role of sun exposure to risk of non-Hodgkin lymphoma (NHL) has been controversial, recent studies have suggested a protective effect rather than a promotive effect. The impact of HIV infection on this relationship is unknown, thus we sought to explore the association between sun exposure and sun sensitivity and NHL in the setting of HIV.

Methods: The study population consisted of a subset of the Multicenter AIDS Cohort Study: 573 HIV+ men who responded to a special ultraviolet radiation exposure questionnaire administered between October 1993 and April 1994, including 33 men who were subsequently diagnosed with pathologically confirmed NHL. The questionnaire elicited information on skin color, natural hair color, eye color, sunburn tendency, average daily sun exposure, occupational sun exposure, recreational sun seeking behaviors, vacationing in sunny locations, sun screen usage, and use of sun lamps or light therapy. Cox proportional hazards regression models were used to obtain hazard ratios (HR) and 95% confidence intervals (CI) for the association between exposures of interest and NHL risk. HIV positive participants entered the analysis on the date of their UVE questionnaire and were followed until an NHL diagnosis, death, or loss to follow-up. Models were adjusted for race, MACS study site, and CD4+ T cell count.

Results: Men who reported a high frequency of going to the beach or pool on summer weekends over the last five years had a significantly reduced risk of NHL: HR=0.31 (95% CI =0.15-0.86) for ≥2 times versus never, and HR=0.45 (95% CI =0.20-1.0) for 1-4 times versus never. Compared to men who have rarely taken a beach vacation in the last five years, men who occasionally have were at a significantly reduced risk of NHL (HR = 0.36, 95% CI =0.15-0.86). With respect to sunburn tendency, men who never
blister were at reduced risk of NHL compared to men who occasionally blister, although this did not reach statistical significance (HR=0.45, 95% CI=0.19-1.06). Men with green or hazel eyes were at reduced risk of NHL compared to men with blue eyes, although this did not reach statistical significance (HR=0.38, 95% CI=0.14-1.05).

Conclusions: Consistent with the NHL literature on HIV uninfected populations, we found that a high level of recreational sun exposure and a low level of sun sensitivity are associated with a decreased risk of NHL in the setting of HIV. Studies are currently underway to elucidate possible mechanisms for these associations, including a possible role of vitamin D.

Table 1 (abstract P26) Characteristics of HL in HIV- and HIV+ patients

<table>
<thead>
<tr>
<th></th>
<th>HIV-</th>
<th>HIV+</th>
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<tbody>
<tr>
<td>N</td>
<td>25</td>
<td>29</td>
</tr>
<tr>
<td>M/F</td>
<td>16:9</td>
<td>22:7</td>
</tr>
<tr>
<td>Race (black/other)</td>
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<td>24:5</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>33(19-52)</td>
<td>40(22-54)</td>
</tr>
<tr>
<td>Stage (I-II versus III-IV)</td>
<td>5.9</td>
<td>2.19</td>
</tr>
<tr>
<td>B symptoms</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Diagnosis made solely by bone marrow biopsy</td>
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<td>3</td>
</tr>
<tr>
<td>Morphology NS/LR versus MC/LD</td>
<td>12.5</td>
<td>9.2</td>
</tr>
<tr>
<td>One year survival</td>
<td>76%</td>
<td>45%</td>
</tr>
</tbody>
</table>

Reference


P26

Hodgkin’s lymphoma characteristics in HIV-infected and uninfected patients at an urban hospital in the late combined antiretroviral era

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Infectious Agents and Cancer 2012, 7(Suppl 1):P26

Background: As combined antiretroviral therapy has allowed patients infected with HIV to survive longer due to improved immunity, increasing incidences of non-AIDS associated malignancies as well as chronic comorbidities are reported. One of the more commonly reported non-AIDS associated cancers is Hodgkin’s lymphoma (HL) [1]. We report our experience of HL among HIV-infected and uninfected patients.

Methods: Grady Health System (GHS) provides care to the majority of the urban indigent population of Atlanta. Patients who were diagnosed with HL between January 2000 and June 2011 were identified from the GHS pathology records and the GHS cancer registry. Clinic charts and medical records were reviewed. Patients’ demographics, CD4 counts, HIV viral load, HIV and HL treatment and outcomes were recorded.

Results: During the study period, 95 patients were diagnosed with HL. Among the HIV+ patients, 95 were diagnosed with HL (26% HIV-, 30% HIV+ and 43% HIV status unknown). The characteristics are displayed in Table 1.

Among the HIV+ patients, at time of HL diagnosis, the median CD4 at time of HL diagnosis was 95(8-865) cells/mmu, and 3 (10%) are on cART. The median time from HIV diagnosis to HL diagnosis was 2 years (0-20).

Conclusions: In the current cART era, in our institution, HL in HIV+ patients is more likely to present with advanced disease (65% with stage III/IV). Interestingly, in 3 HIV+ patients, HL was diagnosed solely by bone marrow biopsy. Despite the availability of cART, patients are not accessing care. This may account for the poor one-year survival among HIV+ patients with HL.

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P27

A core laboratory for the generation of quality-controlled g-herpesvirus bacmids: generation of KSHV microRNA mutants

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Kaposis’s sarcoma-associated herpesvirus (KSHV) encodes 12 viral microRNAs that are expressed during latency. Research into the function of these microRNAs has suffered from the lack of an experimental system that allows for the systematic removal of individual microRNAs. Here we have used the E. coli Red recombination system in conjunction with a new bacmid background, 210BAC, generated in the Jung Lab to create mutants for every known KSHV microRNA. The specific microRNA deletions or mutations and the integrity of the viruses has been strictly quality controlled using PCR, restriction digestion and sequencing based assays. In addition, stable viral producer cell lines for wildtype, Δmir-K12-1, Δmir-K12-3, and Δmir-K12-11 have been created in ISKL cells generously provided by Don Ganem. Deep sequencing was employed to sequence verify all of the current producer cell line mutants and a qPCR assay was used to verify the expression of the remaining viral microRNAs. Creation of producer cell lines for all of the microRNA mutants is ongoing and these viruses will be made available to the research community for further study.

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P28

A regulatory circuit between Kaposi sarcoma-associated herpesvirus and host innate immune system

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Kaposi sarcoma-associated herpesvirus (KSHV) is a human γ-herpesvirus associated with several human malignancies. The replication and transcription activator (RTA) is necessary and sufficient for the switch from KSHV latency to lytic replication. Toll-interleukin-1 receptor (TIR) domain-containing adaptor-inducing β-interferon (TRIF, also called TIR-domain-containing adaptor molecule-1 (TICAM-1)) is a signaling adaptor molecule that is critically involved in the Toll-like receptor 3 (TLR-3) and TLR-4 signaling pathways for type I interferon (IFN) production, a key component of innate immunity against microbial infection. Previously we have identified that RTA blocks TLR3 signaling activation by degrading cellular TRIF, and this RTA-mediated degradation is at least partially mediated through the ubiquitin-proteosome pathway. In this report, we have identified a new mechanism that innate immunity regulates KSHV replication. We find that TRIF increases the expression of KSHV RTA. The enhancement of RTA expression and the degradation of TRIF are two independent pathways. TRIF specifically enhances the translation efficiency of RTA mRNA. Because RTA may not directly interact with TRIF, the functional interactions between TRIF and RTA may be indirect through unknown mediators. Taken together, these data suggest that KSHV employs a novel mechanism to block the innate immunity by degrading TRIF protein, and at the same time, use the innate immune system to boost viral replication by increasing the expression of KSHV RTA. This regulatory circuit may be an important part of the KSHV-host interactions for the initial infections. This work may contribute to our understandings on how KSHV interacts with the host immune system for its survival in vivo.

P29

CpG methylation as a tool to characterize cell-free Epstein-Barr virus DNA

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CpG methylation as a tool to characterize cell-free Epstein-Barr virus DNA
In order to differentiate Epstein-Barr virus (EBV) virion DNA from viral DNA isolated from latently infected cell lines, we have taken advantage of the observation that viral episomal genomes of herpesviruses are methylated in latently infected cells whereas unmethylated genomes are synthesized and packaged into virions during the lytic phase. We used paramagnetic beads linked to methylCpG binding protein to separate virion and cell-derived viral DNA. DNA isolated from EBV (Figure 1A) virions failed to bind to the methylCpG binding protein and were detected only in the non-captured (NC) fractions, while DNA isolated from latently infected cell lines were detected predominantly in the bound fractions ($E_{2000}$, high salt elute). Unmethylated EBV DNA, presumably virion DNA, was detected in the plasma of 3 AIDS patients without lymphoma, while methylated DNA was detected in the blood of 3 patients with EBV-associated Hodgkin lymphoma (HL) (without HIV infection) (Figure 1B).

**Background:** In order to differentiate Epstein-Barr virus (EBV) virion DNA versus viral DNA released from tumor cells, we have taken advantage of the observation that viral episomal genomes of herpesviruses are methylated in latently infected cells whereas unmethylated genomes are synthesized and packaged into virions during the lytic phase. We used paramagnetic beads linked to methylCpG binding protein to separate virion and cell-derived viral DNA. DNA isolated from EBV (Figure 1A) virions failed to bind to the methylCpG binding protein and were detected only in the non-captured (NC) fractions, while DNA isolated from latently infected cell lines were detected predominantly in the bound fractions ($E_{2000}$, high salt elute). Unmethylated EBV DNA, presumably virion DNA, was detected in the plasma of 3 AIDS patients without lymphoma, while methylated DNA was detected in the blood of 3 patients with EBV-associated Hodgkin lymphoma (HL) (without HIV infection) (Figure 1B).

**Conclusions:** Tumor derived viral DNA can be distinguished from virion associated viral DNA based on preferential binding to methylCpG binding protein. Tumor derived viral DNA was predominantly present in the blood from patients with Hodgkin-Lymphoma, but not in patients without EBV associated malignancy. This technique may be applied to detect tumor derived viral DNA in the blood of patients with EBV associated malignancies.

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**Development of multiplex serological assays to detect oncoviral infections**

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Serological markers of infection (antibodies or antigens) of viruses that cause cancer are most often detected using ELISA-based methodologies. In many cases, multiple markers of infection must be assessed to determine a final sero-status. Volume requirements and costs of reagents for single analyte ELISAs are high and studies which include multiple viruses can require milliliters of plasma, often not available from archived cohorts. Thus, we sought to develop a Luminex® bead-based customizable panel initially including Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Epstein-Barr Virus (EBV) and often the concomitant infection, Human Immunodeficiency Virus (HIV) to reduce sample volume requirements, overall cost and increase flexibility. Peptides, antigens and antibodies were sourced from multiple manufacturers and tested for suitability in this platform. Where necessary, suitable reagents were designed and produced in-house. The HCV assay multiplexes four HCV peptides designed to detect antibodies raised to HCV Core (2), HCV NS5, HCV NS5 gene regions. The HBV assay multiplexes a HBV early antigen peptide, a recombinant HBV core protein and either a recombinant HBV surface antigen or an antibody specific for HBV surface antigen to assess HBV infection. The EBV assay multiplexes peptides specific to viral capsid antigen, EBNA-1 and early antigen (Cyto-Barr, Zuidhorn, The Netherlands). HIV-1 assay development is ongoing and the list of antigens to be included in the assay has not been finalized. These assays can be run singly or with any combination (multi-plex) of the above listed targets. Each target has been independently validated using samples of known molecular and serological status to determine specificity (false positive versus false negative) and re-evaluated under multiplex conditions to confirm assay performance. In addition, where possible, samples were assayed on commercial testing platforms as well as our multiplex assay to assess concordance (94-99%). Dependent on the panel selected and the expected antibody titers in a particular population, plasma or serum volumes in the range of 10 μL to 125 μL per subject would be required to determine the HBV, HCV, EBV and/or HIV serostatus of a subject. This assay platform is inherently flexible and the benefits include amenability to expansion to include other oncogenic viruses as well as screening large epidemiological cohorts or smaller subsets of samples in an economical and high throughput manner.

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**Epstein-Barr virus induces adhesion molecule CD226 (DNAM-1) expression during primary B cell transformation into lymphoblastoid cell lines**

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Epstein-Barr virus (EBV), an oncogenic herpesvirus associated with Burkitt’s lymphoma and other AIDS-related B cell malignancies, transforms primary human B cells into lymphoblastoid cell lines (LCLs) ex vivo. As LCLs express viral gene products similar to those found in EBV-mediated cancers, LCLs provide a practical model for tumorigensis. Previous unpublished findings from our lab indicate that LCLs constitutively express the adhesion molecule CD226 (DNAM-1), found on virtually all peripheral blood NK cells,
T cells, and monocytes, but only a small subset (~3%) of B cells. Although CD226 is known to mediate T-cell differentiation and cytotoxicity, NK cell cytotoxicity, NK cell apoptosis, and monocyte extravasation, CD226 function in B cells remains relatively unstudied. Biochemically, CD226 functions to support the interaction between the intracellular adhesion molecules LFA-1 and ICAM-1. Here, we demonstrate that EBV specifically induces CD226 expression in primary human B cells and EBV-negative B lymphoblasts during viral-mediated proliferation and outgrowth. EBV infection of primary B cells increased CD226 surface expression 5-fold during early proliferation and approximately 30-fold upon transformation into LCLs. EBV-converted Burkitt’s lymphoma cells constitutively express CD226, while EBV-negative B cell lymphomas do not. Additionally, we demonstrate that LMP-1, an EBV latency III membrane oncoprotein, induces CD226 expression in EBV-negative Burkitt’s lymphoma cells. Finally, we demonstrate that the NF-κB pathway regulates CD226 expression. Indeed, B cell lymphomas with high NF-κB activity (activated B cell-like diffuse large B-cell lymphomas) express CD226 at higher levels than B cell lymphomas with low NF-κB activity (germinal center B cell-like diffuse large B cell lymphomas). As CD226 supports the interaction between LFA-1 and ICAM-1, which is critical to maintain the constitutive aggregation of EBV-transformed B cells, we propose that EBV-mediated induction of CD226 drives cell-cell contact ensuring B cell survival. These data suggest that CD226, a newly identified EBV-induced cell adhesion molecule, may play a key role in the pathogenesis of AIDS-associated and other B cell lymphomas.

P32
Epstein-Barr virus lytic gene expression is tightly linked to ER stress but not cytotoxicity with bortezomib or nelfinavir
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Epstein-Barr virus (EBV) is associated with AIDS-related lymphomas and other malignancies. We have previously shown that the proteasome inhibitor bortezomib is an activator of EBV lytic gene expression and that these effects are mediated by ER stress and the unfolded protein response (UPR) [1]. We investigated the relationship between the induction of UPR and EBV lytic gene expression with a variety of UPR inducers, as well as the association of their viral and antitumor effects in a variety of tumor cell lines. Bortezomib, thapsigargin, and tunicamycin activate the UPR and EBV lytic cycle. Recently, nelfinavir has also been reported to lead to ER-stress and the UPR. We found a dose-dependent relationship with bortezomib and nelfinavir the induction of ER stress markers (Bip, ATF-4, XBP1(s), and CHOP10), EBV lytic gene expression (measured as ZTA RNA), and cell toxicity in Burkitt’s lymphoma cell lines. Blocking ER stress and UPR activation, by cycloheximide (CHX) treatment or by Bip knockdown, diminished ZTA induction but had no effect on cellular toxicity. We also studied EBV lymphoblastoid cell lines (LCLs). In contrast to the BL cell lines, bortezomib did not induce ER stress, activate the UPR or lead to EBV lytic gene expression but was nonetheless toxic to LCLs. These results indicate that bortezomib and nelfinavir both induce ER stress and UPR leading to EBV lytic reactivation in BL cells. UPR induction corresponds with EBV lytic gene induction but appears to be distinct from cellular toxicity. Our findings suggest that ER stress, UPR and viral activation are closely linked but may be separable from the cytotoxic effects of some pharmacologic inducers. Bortezomib and nelfinavir may serve as laboratory and clinical tools for manipulating viral gene expression in EBV associated malignancies.

Reference

P33
ER stress activates lytic gene expression in KSHV-associated tumor cell lines
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Infectious Agents and Cancer 2012, 7(Suppl 1) P33

Background: Activating the herpesvirus lytic replication cycle presents an opportunity for targeted therapy. We explored the effects of endoplasmic reticulum (ER) stress inducers on Kaposi’s sarcoma herpesvirus (KSHV) lytic activation in primary effusion lymphoma (PEL) cell lines. We included nelfinavir (an HIV-1 protease inhibitor) in our investigations because it has been reported to induce ER stress in various tumor cell lines [1]. Treatment with bortezomib, thapsigargin or nelfinavir resulted in increased expression of ER stress markers such as activating transcription factor 4 (ATF-4) and the spliced form of X-box binding protein 1 (XBP-1(s) (see figure 1). Treatment was also associated with an increase in RNA expression of the KSHV immediate early “replication and transcriptional activator” (RTA) (see figure 2). To determine whether ER stress mediated KSHV lytic reactivation associated with these agents, we prepared doxycycline-activated short hairpin RNA knockdowns of ER stress genes (Grp78 and XBP-1(s)). Treatment of these knockdowns with doxycycline for 72 hours resulted in inhibition of ER stress and inhibition of viral lytic gene expression.

Conclusion: These results demonstrate that in KSHV-infected cell lines, induction of ER stress is associated with activation of KSHV lytic genes and raises the possibility that nelfinavir might be incorporated into future treatment strategies for KSHV-associated malignancies.

Figure 1 (abstract P33) Treatment of BC-3 cells leads to induction of ER stress markers. KSHV PEL cells (BC-3) were treated with DMSO, 20nM bortezomib (20nm BZ), 1μM thapsigargin (1μM TG) or 72μM nelfinavir (72μM NFV). RNA was isolated and RT-PCR was performed using primers for ATF-4 and XBP-1 (s). Primers for β-actin were used as an internal control. Error bars indicate SEM.
Nelfinavir, a lead HIV protease inhibitor, attenuates the immune clearance of EBV in vitro and in vivo. EBV also plays a role in the pathogenesis of endemic African Burkitt lymphoma and other lymphomas in HIV-infected individuals. EBV infection of primary human B cells results in proliferation and outgrowth of indefinitely proliferating lymphoblastoid cell lines, or LCLs, which represent a viable model for the pathogenesis of EBV-associated malignancies. Ongoing studies in our group have shown that the earliest EBV-infected proliferating B cells differ greatly from LCLs phenotypically. Using CFSE staining and flow cytometry-based sorting, we have isolated these early proliferating B cells and analyzed genome-wide exon level mRNA expression relative to uninfected resting B cells and LCLs. Gene ontology analysis of these expression data identified enrichment of genes associated with proliferation and the DNA damage response in early proliferation. Furthermore, c-Myc mRNA and activity, as inferred from its genome-wide expression signature, were also highly induced early. Most interestingly, however, analysis of changes from early proliferating to final LCL outgrowth revealed striking attenuation of proliferative gene sets and c-Myc, along with delayed induction kinetics of NF-κB activation. Specifically, genes with NF-κB motifs in their promoters were highly expressed from early proliferating B cells to LCL and many canonical NF-κB targets and pathway components were induced at late times after infection. These results suggest a novel, dynamic EBV-driven growth pattern and expression program that relies on mutually exclusive signals from c-Myc and NF-κB. Furthermore, our data suggest that the earliest stages of EBV-driven B cell immortalization may provide unique insight into the pathogenesis of EBV-associated malignancies.

**P35**

**HPV+ cancer cell lactate production attenuates immune response during treatment: lactate production inhibition leads to improved long-term cures**

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**Background:** Normal cellular metabolism is altered in cancer cells, shifting away from the TCA cycle towards glycolysis, increasing glucose consumption and lactate production. This key characteristic change in metabolism is termed the Warburg effect. Importantly, HPV 16 E7 oncoprotein alters the function pyruvate kinase type M2, increasing glucose consumption and lactate production. Clinically, increased lactate production in head and neck cancers is associated with a decreased response to therapy. Cancers with high lactate production have a poor five year survival, approximately 40% worse than similar tumors with low lactate production. Lactate has also recently been shown to disrupt functions of key immune cells (CD8 and DCs) in vitro. We have recently shown that an immune response is required to clear HPV+ head and neck squamous cell carcinomas (HNSCC) in vivo. In this project we tested the hypothesis that lactate within the tumor microenvironment inhibits immune mediated clearance of HPV+ cancers.

**Material and methods:** Experiments were completed in culture on human and mice HPV+ cancer lines, in a preclinical mouse model of HPV+ cancer and a human phase 2 clinical trial initiated.

**Results:** We show that human and mouse HPV+ HNSCC’s have enhanced lactate production. Inhibition of lactate with either Dichloroacetate (DCA) or Oxamate decreases tumor cell growth in colony forming assays. DCA-mediated lactate inhibition in vivo was well tolerated, decreased tumor lactate levels, increased tumor pH. DCA treatment by itself did not alter tumor growth significantly. However, to test whether it would enhance immune related clearance during cisplatin/radiation, studies in immune competent mice were completed and compared to identical studies in immune deficient (RAG1) mice. The studies show that inhibition of lactate production resulted in enhanced immune mediated clearance during treatment with cisplatin and radiation therapy. Furthermore, siRNA-mediated knock down of lactate dehydrogenase (LDH) confirmed the role of LDH and epithelial cell lactate production in this response. These findings show that altered metabolism and decreasing lactate in the tumor microenvironment not enhances immune clearance during therapy. Due to these finding a phase 2 clinical trial has been initiated which combines DCA with cisplatin/radiation. The initial results from the trial will be presented.

**Conclusion:** Tumor produced lactate attenuates the immune clearance of HPV+ cancers. Decreasing this lactate and thus enhancing immune clearance may be very relevant for immune suppressed HPV+ individuals during therapy.

**P36**

**Human herpesvirus 8 replicates in primary B lymphocytes and induces polyfunctional cytokine and chemokine responses**

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**Objectives:** We have previously shown that DC-SIGN expressing, activated B cells support human herpesvirus 8 (HHV-8; KSHV) replication. Cytokines and chemokines play an important role in KS, including tumor-cell proliferation, angiogenesis and vascular permeability. We therefore examined virus replication in relation to production of soluble immune mediators by HHV-8 infected B cells.

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**Reference**

**P37**

**Induction of KSHV latency-associated nuclear antigen (LANA) by hypoxia and hypoxia-inducible factors (HIF)**

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Hypoxia activates KSHV lytic replication in primary effusion lymphoma (PEL) cells. In the current study, we show that LANA mRNA levels were upregulated in BC-3 PEL cells in hypoxia. Further, the total levels of LANA protein were elevated in BC-3 PEL cells after 24 h hypoxia and increased through 72 h. Also, infection of BC3 cells with a retroviral vector encoding LANA mRNA levels were upregulated in hypoxia. Computer analysis of a 1.2-kb sequence upstream of the LANA translational start site revealed six potential hypoxia-responsive elements (HRE). Reporter assays in Hep3B cells utilizing this region resulted in moderate activation by hypoxia and CoCl2 (a hypoxia mimic) and greater activation by co-transfection with degradation-resistant HIF-1a or HIF-2a. Greater induction was seen with HIF-2a than HIF-1a. Subsequent transfection studies revealed that much of this activity was mediated by one of these HREs (HRE 4R) oriented in the 3' to 5' direction located between the constitutive (LTc) and RTA-inducible (LTi) mRNA start sites. Site-directed mutation of this HRE substantially reduced the response to both HIF-1a and HIF-2a in reporter assays. Electrophoretic mobility shift assays (EMSA) and chromatin immunoprecipitation (CHIP) assays demonstrated binding of both HIF-1a and HIF-2a to this region. Consistent with the reporter assays, CHIP revealed greater binding of HIF-2a than HIF-1a. These observations suggest that hypoxia induces the transcriptional activation of LANA by the interaction of HIF through at least one HRE in the LANA promoter region and that this activity is preferentially responsive to HIF-2a. Computer analysis of LTi promoter revealed the presence of RTA-responsive elements adjacent to HRE 4R and SR. Cotransfection assays in Hep3B cells revealed that RTA cooperates with HIF to induce LTi promoter activity. Hypoxia or CoCl2 treatment of Hep3B cells transfected with RTA confirmed this cooperative effect on LTi promoter activity. Immunoprecipitation assays using the nuclear extract of PEL cells exposed to hypoxia revealed that RTA associates with HIF-1a and HIF-2a to activate the inducible LANA promoter. Taken together with previous studies, these results provide evidence that hypoxia and HIFs activate both latent and lytic KSHV replication and play a central role in the life cycle of this virus.

**P38**

**Kaposi sarcoma associated herpesvirus infection of primary human endothelial cells activates the proto-oncogene STAT3**

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Kaposi’s sarcoma associated herpesvirus (KSHV) in the etiological agent for 3 AIDS-related cancers: Kaposi’s sarcoma (KS), primary effusion lymphoma, and multicentric Castleman’s disease. The molecular mechanisms used by KSHV to induce cancer are incompletely understood. KS lesions harbor proliferating late-infected endothelial cells (ECs), large numbers of inflammatory cells, and marked neangiogenesis. Considered the major driving force in the development of KS, these KSHV-infected ECs elaborate a variety of pro-inflammatory and angiogenic factors that contribute to tumourigenesis. Considerable evidence has accumulated suggesting a critical role for activated signal transducer and activator of transcription-3 (STAT3) in malignant transformation. STAT3 is a latent transcription factor that upon activation, drives the expression of a number of genes involved in cell proliferation, survival, and immune responses. Canonical STAT3 activation occurs via phosphorylation of Y705, dimerization, and nuclear translocation, followed by phosphorylation of Y727 for maximal transcriptional activity. Activated STAT3 has been observed in a variety of malignancies and has been shown to induce fibroblast transformation in vitro suggesting that STAT3 is a proto-oncogene. Interestingly, evidence has accumulated suggesting a role for Y727 mono-phosphorylated STAT3.

Here we show that latent KSHV infection of primary human endothelial cells (ECs) in vitro activates STAT3, and identifies a key latency protein, kaposin B, that contributes to this activation. Kaposin B expression in ECs causes STAT3 phosphorylation at Y727, in the absence of significant Y705 phosphorylation, and enhanced expression of a subset of STAT3 target genes including CCL5. Recent work shows that the tripartite motif-containing protein 28 (TRIM28, a.k.a. TIF-1, KAP-1) negatively regulates STAT3 by recruiting transcriptional silencing complexes. The repressive activity of TRIM28 is mediated by post-translational modifications and a key site in the regulation of repressor activity maps to Y547. Phosphorylation of this residue disrupts the recruitment of transcriptional silencing complexes effectively deactivating the co-repressive function of TRIM28. Confocal microscopy and western blot analysis demonstrate phosphorylation of TRIM28 at Y547 in KSHV latently infected and kaposin B expressing ECs. Taken together, our studies suggest kaposin B may contribute to tumourigenesis via constitutive activation of STAT3.

**P39**

**Kaposi sarcoma herpesvirus (KSHV)-associated lymphomas are associated with markedly elevated serum IL-10, elevated IL-6, IL-17 and circulating KSHV**

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Background: KSHV, also called human herpesvirus-8 (HHV8), is the etiological agent of primary effusion lymphoma (PEL) (including extracavitary variant), and large B-cell lymphoma arising in HHV8-associated multicentric
Castelman disease (together, KSHV-associated non-Hodgkin lymphoma (KSHV-NHL). Additional KSHV-associated diseases include: Kaposi sarcoma (KS), a form of multicentric Castelman disease (KSHV-MCD), and a proposed KSHV-associated inflammatory cytokine syndrome (KICS). Like KSHV-MCD, inflammatory syndromes are common in KSHV-NHL. We compared an array of inflammatory and angiogenic cytokines, chemokines, growth factors, and select clinical laboratory values between HIV-infected patients with KSHV-NHL and other lymphomas (HIV-lymphoma).

**Methods:** 
Patients were enrolled in HAMBI and/or NIAID protocols. Cases had KSHV-NHL; controls other HIV-lymphoma. Clonality of PEL from effusions was confirmed by PCR for immunoglobulin rearrangements. Serum was evaluated by ELISA for IFN-γ, IL-1β, IL-6, IL-8, IL-10, IL-12p70, TNF-α, IL-17, VEGF-A, (Meso-Scale Discovery, Gathersberg, MD), CXCL1, VEGF-C (R&D Systems, Minneapolis, MN). In patients with KSHV-NHL, peripheral blood mononuclear cell associated KSHV viral load was measured. Clinical data included demographics, CD4 count, albumin, platelets, hemoglobin, and c-reactive protein (CRP). Comparison of each parameter between patients with KSHV-NHL and other HIV-lymphoma employed an exact form of the Wilcoxon rank-sum test. P-values are 2-sided, with p ≤ 0.01 considered statistically significant, and 0.01 < p ≤ 0.05 considered strong trends.

**Results:** Subjects:13 KSHV-NHL cases: 12 men, 1 woman. Median age 44, IQR 35-46. 17 white, 4 Hispanic, 6 African-American, 1 African. Histologies: primary central nervous system lymphoma (13), diffuse large B-cell lymphoma (DLBCL) (10), Hodgkin disease (1), Burkitt lymphoma (2), plasmablastic lymphoma (1), EBV+ large B-cell lymphoma NOS (1). KSHV-NHL subjects had elevated KSHV viral load, [median 2812 copies/10^6 cells] (KSHV-IQR 186-115,789) and CRP [median 51 mg/L (IQR 45-67)]. Compared to other HIV-lymphomas, patients with KSHV-NHL have higher CD4 counts (median CD4 133 vs. 29 cells/μL, p=0.002), hypoaalbuminemia (median albumin 1.9 vs. 3.5 mg/dL, p=0.0034), and trend towards more severe anemia, thrombocytopenia, and hyponatremia. KSHV-NHL is associated with elevated circulating KSHV, marked elevations in IL-10 (313 vs. 12.2 mg/L, p <0.0001), elevations in IL-6 (29 vs. 4.1 mg/L, p=0.0013), IL-17 (1.6 vs. 0.5 mg/L, p=0.0074), and trends towards increased IFN-γ and IL-1β.

**Conclusions:** Inflammatory cytokines are important in KSHV-NHL pathogenesis and symptomatology. Clinical and translational studies evaluating these abnormalities in KSHV-associated malignancies are ongoing.

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**References**

**P41**
KSHV induces rapid release of angiopoietin-2 from endothelial cells to promote angiogenesis and inflammation

The development of Kaposi’s sarcoma (KS), the most common malignancy in AIDS patients, results from Kaposi’s sarcoma-associated herpesvirus (KSHV) infection of endothelial cells and subsequent induction of proliferation, angiogenesis, and inflammation. Previously, we demonstrated that KSHV infection of human umbilical vein endothelial cells (HUVEC) induced a transcriptional induction of Angiopoietin-2 (Ang-2), a pro-angiogenic and pro-inflammatory cytokine that is highly present in KS tumors. This transcriptional up-regulation of Ang-2 started at 12 h and peaked at 54 h post-infection. In difference to our previous data, here we demonstrate that KSHV infection of HUVEC induces rapid release of Ang-2 within minutes of viral contact. Pre-made Ang-2 is stored in the Weibel-Palade body of endothelial cells and is released through regulated exocytosis. KSHV binding to HUVEC is responsible for rapid Ang-2 release because blockade of viral binding inhibits this cytokine exocytosis. We find that KSHV binding to its integrin receptors on endothelial cells activates the integrin tyrosine kinase receptors signaling pathways, including tyrosine phosphorylation of the kinases FAK and Src, and triggers rapid calcium mobilization. This mobilization likely plays a key role in mediating Ang-2 release, as its inhibition by various calcium chelators and calcium channel blockers substantially reduces Ang-2 release. We also demonstrate a direct interaction and association of the kinase Src with the alpha1C subunit of L-type calcium channel. Indeed, specific inhibitors of protein tyrosine phosphorylation not only disrupt this interaction but also abolish Ang-2...
release. Finally, preliminary data from in vitro cell adhesion assays suggest that this rapidly released Ang-2 enhances migration and adhesion of monocytes to the infected endothelial cells. To our knowledge, this is the first demonstration of interaction between KSHV and its integrins receptors in regulating rapid cytokine release. This study also uncovers a novel mechanism of KSHV induction of angiogenesis and inflammation, which is much faster, and could likely play important roles in the early event of KS tumor development.

**P42**

**Signal transducer and activator of transcription 3 (STAT3) controls susceptibility to Epstein-Barr virus reactivation in B cells**

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Epstein-Barr Virus (EBV) is known to cause malignancies in immunocompromised individuals, including lymphomas and lymphoproliferative disorders. Reactivation of EBV from latency is important in the pathogenesis of such malignancies. Therapeutically, oncology of tumors harboring lytic EBV holds promise. However, exposure of latently infected cultured B cells to lytic cycle inducing stimuli results in virus reactivation within only 20% to 50% of cells. Host cell determinants, that govern this susceptibility to lytic reactivation within latently infected B cells, are not well understood. Our research has previously identified that higher levels of signal transducer and activator of transcription 3 (STAT3) within cells correlate with resistance to EBV lytic reactivation. We now investigate whether inhibiting function of STAT3 promotes susceptibility of latently infected B cells to lytic cycle inducing stimuli or results in induction of EBV lytic cycle. We found that pharmacological inhibition of STAT3 phosphorylation by Janus Kinase 2 (JAK2) inhibitors (AG490 or WP1066) in latently infected cells (HHs14-17 Burkitt lymphoma and B95-8 lymphoblastoid cells) increased lytic reactivation by chemical stimuli that function by distinct mechanisms. Moreover, functional inhibition of STAT3 alone resulted in lytic reactivation in these cells. EBV-Lymphoblastoid cell lines (LCL) newly generated from healthy individuals also demonstrated increased susceptibility to lytic cycle inducing stimuli in the presence of AG490 but variable susceptibility to lytic reactivation when exposed to AG490 alone. Since this lack of uniform response to AG490 alone could be due to inadequate functional suppression of STAT3, we examined EBV-LCLs derived from patients with Autosomal Dominant Hyperimmunoglobulin-E syndrome (AD-HIES or Job syndrome). Patients with AD-HIES carry a dominant negative mutation in their STK3 gene resulting in lower basal levels of functional STAT3. When LCLs from AD-HIES patients were exposed to a JAK2 inhibitor alone, we observed a strong increase in lytic reactivation by expression of early and late lytic antigens over LCLs derived from healthy individuals. Lytic reactivation in the presence of AG490 occurred in these cells despite lack of discernable increase from basal levels of expression of ZEBRA, the viral lytic switch protein, when compared to cells not exposed to AG490. Thus, STAT3 is important in determining susceptibility to EBV reactivation. Fully understanding how STAT3 governs such susceptibility can lead to novel therapeutic strategies for EBV-related diseases.

**P43**

**The prevalence of HIV-1 DNA in AIDS-related lymphoma and Kaposi Sarcoma throughout the AIDS epidemic**

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Background: Chronic inflammation is linked to tumorigenesis for many cancer types and likely contributes to tumor development in the HIV-infected patient population. AIDS-related lymphoma (ARL) and Kaposi Sarcoma (KS), two AIDS-defining cancers, are associated with the tumor viruses EBV and KSHV, respectively. However, EBV is only detectable in ~40% of ARLs and KSHV alone is not sufficient for KS development. Recent studies have shown that HIV is localized to tumor associated macrophages (TAM), not malignant B cells, in a portion of EBV-negative ARLs suggesting, that HIV infected TAM may play a role in tumorigenesis. The goal of this research was to determine the prevalence of HIV+ ARL and KS throughout the AIDS epidemic and examine tumor associated HIV for unique genetic signatures.

**Material and methods:** Whole genomic amplified DNA from ARL and KS biopsies was used for quantitative HIV gag gene amplification. The 3′ envLTR segment of HIV-1 gag gene from tumor and tumor-matched skin samples from two patients that died of ARL were sequenced and Bayesian phylogenies were inferred using BEAST. All specimens were provided by the AIDS and Cancer Specimen Resource.

**Results:** Of the 119 ARL and 91 KS biopsies, 45% and 40% contained detectable HIV-1 DNA, respectively. There was a significant decrease in the prevalence of HIV-1 DNA positive ARL and KS cases in the post-HAART era. A subset of ARL contained extremely high levels of HIV-1 DNA (~1 copy/cell). In addition, visceral KS had a higher prevalence of HIV-1 DNA (51.9%) as compared to skin KS (30.7%).

**Conclusions:** The prevalence of HIV-1 DNA positive ARLs declined in the post-HAART era, but not to the same extent as KS, consistent with the incidence of both tumor types in the post-HAART era. Higher prevalence of HIV-1 DNA in visceral sites of KS and lymphoma-specific HIV sequences in sites of metastatic lymphoma suggests that HIV, especially HIV infected macrophages, may play a role in the pathogenesis of KS and ARL disease progression. Additionally, HIV-infected macrophages are a source of chronic inflammation that may further enhance tumorigenesis. Our data suggest a tumor specific form of HIV may be evolving within individuals who develop ARL.

**P44**

**Viral FLICE inhibitory protein of Rhesus monkey rhadinovirus inhibits apoptosis by enhancing autophagosome formation**

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**Infectious Agents and Cancer 2012, 7(Suppl 1):P44**

Rhesus monkey rhadinovirus (RRV) is a gamma-2 herpesvirus closely related to human herpesvirus 8 (HHV8). RRV encodes viral FLICE inhibitory protein (vFLIP), which has death effector domains. Little is known about RRV vFLIP. This study intended to examine its function in apoptosis. Here we found that RRV vFLIP inhibits apoptosis induced by tumor necrosis factor-α (TNF-α) and cycloheximide. In HeLa cells with vFLIP expression, the cleavage of poly [ADP-ribose] polymerase 1 (PARP-1) and activities of caspase 3, 7, and 9 were much lower than those in controls. Cell viability of HeLa cells with vFLIP expression was significantly higher than control cells after apoptosis induction. However, RRV vFLIP appears unable to induce NF-κB signaling when tested using NF-κB reporter assay. RRV vFLIP was able to enhance cell survival under starved conditions or apoptosis induction. At early time points after apoptosis induction, autophagosome formation was enhanced and LC3-II level was elevated in cells with vFLIP and, when autophagy was blocked with chemical inhibitors, these cells underwent increased apoptosis. Full length of vFLIP is needed for the function against apoptosis as truncation variants of vFLIP were unable to block apoptosis induction. Moreover, RRV latent infection of BJAB B-lymphoblastoid cells protects the cells against apoptosis by enhancing autophagy to maintain cell survival. Knockdown of vFLIP expression in the RRV-infected BJAB cells with siRNA abolished the protection against apoptosis. These findings indicate that vFLIP from cells against apoptosis by enhancing autophagosome formation to extend cell survival. The finding of vFLIP’s inhibition of apoptosis via the autophagy pathway provides insights of vFLIP in RRV pathogenesis.
**P45**

**Pathogen discovery in AIDS-related lymphoma by high-throughput sequencing**

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**Background:** Approximately 30% of AIDS-related lymphomas (ARL) are associated with infection by the EBV, and about 4% by the KSHV/HHV-8. It is likely that if other lymphomagenic pathogens exist, these associations would occur in the context of ARL. The advent of high throughput sequencing provides a unique opportunity to address this question. High throughput sequencing, followed by computational subtraction of human sequences was used for enrichment of candidate pathogenic sequences.

**Methods:** Eleven primary tissues of ARL have been used to generate cDNA libraries. Out of these six were frozen specimens and five were formalin-fixed paraffin-embedded (FFPE). The libraries were subjected to high throughput Illumina sequencing, followed by computational subtraction of human transcriptome sequence databases; ii) human transcriptome sequence databases; and iii) other vertebrate sequence databases. Residual sequence reads were then compared with microbial databases, either individually or as part of de novo assembled contigs.

**Results:** Using both frozen and formalin-fixed paraffin-embedded (FFPE) tissues, we have identified unique sequences previously unassociated with hematological cancers and inflammatory diseases. In addition, our pathogen discovery pipeline works with both transcriptome and whole genome sequencing (WGS) data, and it is applicable to data across all high throughput sequencing platforms. Most notably, we are able to detect as low as 1 viral sequence per billion total sequence reads for WGS data, a sign of the sensitivity of our method. Among the known pathogens, we found 12423 sequences corresponding to EBV in the one case where the presence of this virus was also documented by EBER in situ hybridization. Three additional cases that were EBER-negative revealed EBV sequences, in the range of 3 to 351 reads, suggesting that the virus was present in tumor-infiltrating cells, rather than in the lymphoma. Eight cases had HIV sequences ranging from 1 to 403 reads, and one case had a single read corresponding to KSHV.

**Conclusions:** We have developed an integrated pipeline, PathSeq, for pathogen discovery in both frozen and FFPE tissues using a high throughput sequencing-based computational subtraction process. The presence of >10,000 reads in the known EBV-positive case confirms the effectiveness of the method. Specific EBV and HIV sequences were seen emanating from tumor-infiltrating cells that will shed light on expression patterns of these viruses in this cellular compartment.

**Acknowledgement:** This project was funded by the Starr Cancer Consortium and NCI grant RC2CA148317 to MM and EC.

**P46**

**Dysregulated cytokine and growth factor expression in OSSN HIV-1 patients from Botswana with multiple infections**

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**Background:** Ocular surface squamous neoplasia (OSSN) is a conjunctival or corneal neoplastic tumor that is becoming prevalent in HIV-1 infected patients. Prior to the HIV pandemic, OSSN was noted to occur predominantly in the elderly for whom it is the third most common ocular orbital tumor after melanoma and lymphoma. In Africa OSSN is becoming more common, more aggressive, and affects young people, especially females. In parallel with the dramatic increase of HIV in Africa, several countries have noted a sharp rise in the incidence of OSSN in HIV infected individuals such that OSSN is currently the most common ocular tumor among adults. The underlying cause of this cancer in HIV-infected patients from Botswana is not well defined.

**Method:** Diluted sera from OSSN, pterygia, and control samples were used in Ray Biotech Assay kit for determination of expression of several cytokines and growth factors. We extracted RNA from tissue samples and used designed type specific primers for cytokines and growth factors to analyze expression. The samples were further analyzed for the expression of other pathogens using pyrosequencing technology.

**Results:** Cytokine array results from OSSN and pterygia cases indicated expression of some inflammatory cytokines and growth factors associated with tumor development and growth. Further, quantitative RT-PCR showed the expression of similar inflammatory cytokines and growth factors by a panel of OSSN and pterygia tissues. The expression of the factors were not different in the two conditions of OSSN and pterygia respectively. Additional analysis utilizing pyrosequencing technique identified a number of bacterial, viral, parasitic, and fungal sequences in the patient samples.

**Conclusion:** We identified anti-inflammatory cytokines and growth factors associated with cancer pathogenesis in OSSN and pterygia tissues. We also showed sequences of bacterial, parasitic, fungal, and other viral pathogens in the samples that may contribute to immunosuppression. Further studies are necessary to characterize the molecular mechanisms associated with cytokines and growth factors elicited by oncogenic viral proteins and the development of OSSN. Studies to elucidate the significance of other infectious pathogens in OSSN pathology will be necessary.

**P47**

**Effect of immunodeficiency and tumor marker expression on HIV-related diffuse large B-cell lymphoma prognosis**

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**Background:** Several tumor markers may predict survival in HIV+ patients with diffuse large B-cell lymphoma (DLBCL). Here, we evaluate the association of immunodeficiency (CD4<200) on expression of prognostic tumor markers and survival.

**Methods:** HIV+ DLBCL cases diagnosed between 1996-2007 within Kaiser Permanente California were identified. H&E slides were reviewed to identify representative tumor blocks for tissue microarray (TMA) construction. Immunohistochemistry staining of TMA cores was used to detect the expression of selected markers in the categories of (1) cell cycle regulators, (2) B-cell activators, (3) anti-apoptotic proteins, and (4) others, including Epstein Barr Virus (EBV). Percent of DLBCL cells with visible marker staining was scored on a scale from 0-4 (0-0%, 1-24%, 25-49%, 50-74% and ≥75%). EBV infection was determined by in situ hybridization of EBV RNA. We also considered high vs. low expression levels based on previously established cut-offs. Of the 20 markers previously examined, three had emerged as significant predictors of survival, including EBV, cMYC and BLIMP1. Here, we evaluated the association between CD4 and expression of these three markers by t-test for mean levels and chi-square for % high levels. We also evaluated the combined effect of immunodeficiency and marker expression on 2-year survival in unadjusted Cox models.

**Results:** We identified 194 HIV+ DLBCL cases; 80 patients had adequate tissue for the marker analyses. Of the three markers, only EBV was associated with CD4 level (Table 1). Survival was lowest in cases with high levels of EBV or cMYC in combination with low CD4 (Table 2). Survival was not evaluated for BLIMP1 given the low prevalence.

**Table 1 (abstract P47) Tumor marker levels by CD4**

<table>
<thead>
<tr>
<th>Marker</th>
<th>CD4 ≤200</th>
<th>CD4 ≥200</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBV</td>
<td>&lt;200</td>
<td>≥200</td>
</tr>
<tr>
<td>cMYC</td>
<td>&lt;200</td>
<td>≥200</td>
</tr>
<tr>
<td>BLIMP1</td>
<td>&lt;200</td>
<td>≥200</td>
</tr>
</tbody>
</table>

Mean levels: EBV 19.9 0.6 0.009; cMYC 1.6 1.7 0.90; BLIMP1 0.3 0.2 0.05
% high levels: EBV 45.7 16.0 0.016; cMYC 68.6 64.0 0.71; BLIMP1 11.1 8.0 0.69
Conclusion: Immunodeficiency was associated with EBV+ DLBCL. Cases with low CD4 and high levels of EBV or cMYC had worse survival. Risk stratification may consider both CD4 and tumor marker expression, although confirmation is needed in larger studies.

**Table 2 (abstract P47)** Two-year survival by CD4 and marker levels

<table>
<thead>
<tr>
<th></th>
<th>EBV</th>
<th>cMYC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Low CD4/high marker</td>
<td>4.0</td>
<td>1.6-10.2</td>
</tr>
<tr>
<td>Low CD4/low marker</td>
<td>1.8</td>
<td>0.7-4.9</td>
</tr>
<tr>
<td>High CD4/high marker</td>
<td>1.6</td>
<td>0.3-7.7</td>
</tr>
<tr>
<td>High CD4/low marker (ref)</td>
<td>1.0</td>
<td>-</td>
</tr>
</tbody>
</table>

**Conclusion:** Immunodeficiency was associated with EBV+ DLBCL. Cases with low CD4 and high levels of EBV or cMYC had worse survival. Risk stratification may consider both CD4 and tumor marker expression, although confirmation is needed in larger studies.

**P48**

**[18F]-Fluoro-D-deoxyglucose positron emission tomography findings in Kaposi sarcoma herpes virus associated multicentric Castleman disease: correlation with clinical, inflammatory, and virologic parameters**

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**Background:** KSHV-associated multicentric Castleman disease (KSHV-MCD) is a lymphoproliferative disorder associated with severe inflammatory symptoms, cytopenias and biochemical abnormalities. Improved techniques to assist diagnosis and aid monitoring are required. We prospectively assessed [18F]-FDG-PET/CT findings in KSHV-MCD in relation to clinical symptoms and markers of disease activity.

**Methods:** Patients enrolled on a natural history study of KSHV-MCD underwent [18F]-FDG-PET/CT at disease activity, except where unstable, and at complete clinical and biochemical remission. [18F]-FDG-PET/CT was evaluated blind to clinical status. Symptoms, C-reactive protein (CRP), HIV viral load (VL) in plasma and KSHV VL in peripheral blood mononuclear cells were assessed. Associations with [18F]-FDG-PET/CT maximal standardized uptake value (SUV_{max}) were explored using Spearman correlations (CRP, symptoms, log_{10}(KSHV VL)) or exact Wilcoxon rank sum (HIV VL, detectable or not).

**Results:** 26 patients (24 male, median age 43 [range 34-56], all with HIV) were studied. In 3, we identified intercurrent lymphoma; these were excluded from the primary analysis. The remaining 23 underwent 19 studies during disease activity (16 symptomatic, 3 with laboratory manifestations only), and 21 studies at remission. In symptomatic patients, [18F]-FDG-PET showed symmetrical hypermetabolic adenopathy (diffuse in 15 [94%), focal in 1 [6%]) and increased splenic metabolic activity with splenomegaly (abnormal in 14 [93%]) of the 15 with intact spleens). Marrow and hepatic abnormalities were less common and mild, in patients with laboratory manifestations only, 2 (6%) had mild splenomegaly and limited adenopathy and 1 (3%) isolated adenopathy. During disease activity, median SUV_{max} was 6 (2-8), and was associated with symptom severity (R=0.61, p=0.005), CRP (R=0.54, p=0.017) and KSHV VL (R=0.56, p=0.013), but not HIV VL (p=0.69). Intercurrent lymphomas (2 PET and 1 diffuse large B-cell) demonstrated intensely hypermetabolic abnormalities involving restricted asymmetrical sites, with median SUV_{max} 11 (range 7-38). At remission, 11 (53%) had normal [18F]-FDG-PET/CT; 10 (47%) had minor nodal abnormalities and 4 (19%) mildly increased splenic metabolism without splenomegaly. Intercurrent pathologies contributed to some abnormalities. One had progressive increase in splenic and nodal SUV_{max} over 3 scans (not included in primary analysis) before relapse.

**Conclusion:** [18F]-FDG-PET/CT demonstrated widespread nodal and splenic abnormalities during disease activity, improving with remission. Subclinical disease may also be detectable. Findings were distinguishable from suppressed HIV or intercurrent lymphoma by intermediate metabolic intensity and diffuse anatomic distribution. SUV_{max} was associated with symptom severity, systemic inflammation, and KSHV burden. [18F]-FDG-PET/CT may be a useful non-invasive adjunct in the diagnosis and monitoring of KSHV-MCD.

**P49**

**Nylon-flocked swab collection method better predicts high-grade AIN than does dacron swab method**

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**Background:** Invasive anal cancer (IAC) is a health crisis for gay, bisexual, transgender, and other men who have sex with men (MSM) who show a 20-40 fold higher risk for disease, especially if infected by HIV despite the introduction of HAART. Human papillomaviruses (HPV) that cause invasive cervical cancers (ICC) in women appear responsible for the majority of IAC. Although cervical cytology using Pap test has reduced ICC incidence by ~70%, anal Pap test only shows modest sensitivity and poor-to-modest specificity for detecting high-grade anal intraepithelial neoplasias (HG-AIN). Currently anal Pap testing using Dacron swab is recommended annually and biennially for HIV-infected and unaffected MSM, respectively. Swabs are inserted blindly through the anus, and ASCUS, low- and high-grade dysplasias (LG-, HG-SIL) are evaluated using high resolution anoscopy (HRA).

**Material and methods:** Dacron-swab cytology specimens were collected first using standard procedures; subsequently, Nylon Flocked (NF)-swabs were collected through an anoscope inserted just beyond the verge. Swabs were approximated to the canal, rotated slowly while withdrawn, and placed into preservative. HRA, with medical biopsy, where indicated, was performed by experienced clinicians. Pathologists evaluated cytology using the Bethesda Classification System, and histology using the International Classification of Diseases for Oncology. HPV genotypes were assessed from cytology specimens using Linear Array (Roche Diagnostic Laboratories, Pleasanton, CA).

**Results:** Among 69 specimens obtained, 10 Dacron and 8 NF-specimens were inadequate for cytological evaluation: 14.5% and 11.6%. Sensitivity for HG-AIN and specificity were higher for cytology using NF- than Dacron swabs: 82% (66-98%) and 59% (44-74%), versus 55% (34-76%) and 49% (33-65%), respectively. Multivariate analyses showed NF-swab specimens more accurately predicted HG-AIN than Dacron swabs. Specimens showing either ASCUS/LG-SIL or HG-SIL on NF-swab were 10 (1.9, 52.0) and 5.3 (0.4, 74) times more likely than unaffected specimens similarly collected to predict HG-AIN; whereas, Dacron-swab specimens using these cut-points showed no statistically greater risk for HG-AIN on histology, OR=0.4 (0.1, 2.3) and OR=4.7 (0.4, 61.5), respectively. These relationships persisted after controlling for age, HIV-infection, duration of infection, and multiple observations (n=7).

**Conclusions:** Cytology specimens using Dacron swab blindly inserted through the anus less often predicted HG-AIN than did NF-swab specimen used in conjunction with an anoscope to guide placement.

**Cite abstracts in this supplement using the relevant abstract number, e.g.: Wiley et al. Nylon-flocked swab collection method better predicts high-grade AIN than does dacron swab method. Infectious Agents and Cancer 2012, 7(Suppl 1) P49**