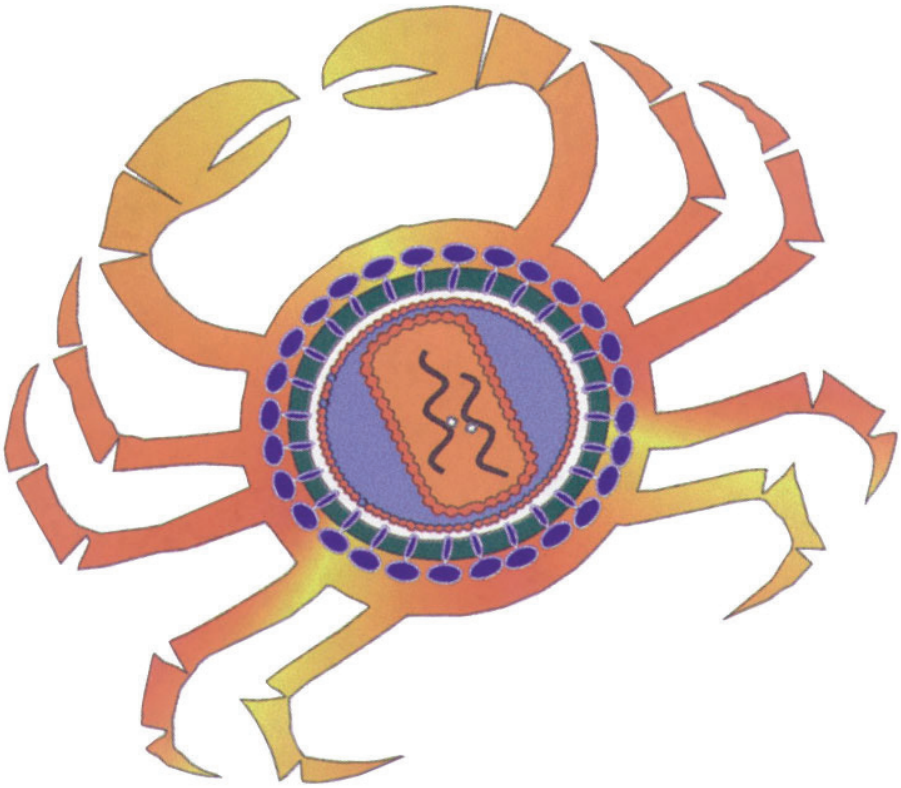
NIH NATIONAL CANCER INSTITUTE

15th International Conference on Malignancies in AIDS and Other Acquired Immunodeficiencies

October 26-27, 2015

Lister Hill Auditorium
NIH Main Campus
Bethesda, Maryland



Office of HIV and AIDS Malignancy | National Cancer Institute
National Institutes of Health | U.S. Department of Health and Human Services

Acknowledgments

Program Committee for Scientific Input
and
National Institute of Dental and Craniofacial
Research for Financial Support

PROGRAM

October 26
Day 1 Poster Setup
Highlighted Poster Setup (will stay up for the entire meeting)
Day 1 Abstracts 1-14 and 15-58

October 27
8:00 a.m. - 8:15 a.m. Poster Viewing
Day 2 Abstracts 1-14 and 59-98

October 26

8:00 a.m. - 8:30 a.m. **Day 1 Poster Setup**
Highlighted Poster Setup (will stay up for the entire meeting)

8:30 a.m. - 8:45 a.m. **Welcome**
Robert Yarchoan, M.D.
Director
Office of HIV and AIDS Malignancy
National Cancer Institute, NIH

8:45 a.m. - 9:00 a.m. **Opening Remarks**
Douglas R. Lowy, M.D., FRACP
NCI Acting Director
National Cancer Institute, NIH

9:00 a.m. - 10:30 a.m. **Session 1: HPV-Associated Diseases and HIV**
Moderator: Joel Palefsky, M.D., FRCP(C)
University of California, San Francisco

9:00 a.m. - 9:30 a.m. ***P1. Incorporating HPV Testing in Cervical Cancer Screening for HIV+ Women***
Howard Strickler, M.D., M.P.H.
Albert Einstein College of Medicine

9:30 a.m. - 10:00 a.m. ***P2. CpG Methylation of HPV16 and Other Oncogenic Human Papillomaviruses (HPVs) Is Associated With High-Grade Cervical Intraepithelial Neoplasia (CIN)***
Robert D. Burk, M.D.
Albert Einstein College of Medicine

10:00 a.m. - 10:30 a.m. ***P3. Synthetic DNA Vaccines for Difficult Immune Targets Including HPV***
Matthew P. Morrow, Ph.D.
Inovio Pharmaceuticals

10:30 a.m. - 11:00 a.m. **Break and Poster Viewing**

11:00 a.m. - 12:15 p.m. **Session 2: EBV and Lymphomagenesis**
Moderator: Richard F. Ambinder, M.D., Ph.D.
Johns Hopkins University School of Medicine

11:00 a.m. - 11:30 a.m. ***P4. Epstein-Barr Virus Oncoprotein Super-Enhancers Control B Cell Growth***
Elliott D. Kieff, M.D., Ph.D.
Harvard Medical School

11:30 a.m. - 11:45 a.m. ***O1. EBV-Mediated B-cell Transformation Is Suppressed by Oncogene-Induced Senescence Due to Depletion of Nucleotide Pools***
Micah A. Luftig, Ph.D.
Duke University

11:45 a.m. - 12 noon ***O2. Increased Levels of Bregs Are Seen Prior to AIDS Non-Hodgkin Lymphoma Diagnosis***
Marta Epeldegui, Ph.D.
University of California, Los Angeles

12 noon - 12:15 p.m. ***O3. Exquisite Sensitivity of KSHV-Associated Lymphomas to a New Nucleoside Analog Activated by Overexpressed Adenosine Kinase***
Jouliana Sadek, Ph.D.
Weill Cornell Medical College

12:15 p.m. - 1:00 p.m. **Lunch**

1:00 p.m. - 2:00 p.m. **Poster Viewing** (presenters to stand by their posters)

2:00 p.m. - 3:00 p.m. **Session 3: Immune Response to Viral Infections**
Moderator: Otoniel Martinez-Maza, Ph.D.
University of California, Los Angeles

2:00 p.m. - 2:30 p.m. ***P5. The Innate Immune Response to KSHV***
Blossom Damania, Ph.D.
The University of North Carolina at Chapel Hill

2:30 p.m. - 3:00 p.m. ***P6. IFNL4 and HCV Infection***
Thomas O'Brien, M.D., M.P.H.
National Cancer Institute, NIH

3:00 p.m. - 3:30 p.m. **Break and Poster Viewing**

3:30 p.m. - 5:15 p.m. **Session 4: Epidemiology**
Moderator: Corey Casper, M.D., M.P.H.
University of Washington and
Fred Hutchinson Cancer Research Center

3:30 p.m. - 4:00 p.m. ***P7. Cancer in HIV-Infected People and Solid Organ Transplant Recipients: Lessons Learned and Open Questions***
Eric A. Engels, M.D., M.P.H.
National Cancer Institute, NIH

4:00 p.m. - 4:15 p.m.	***O4. Risk of Both AIDS-Related and Non-AIDS-Related Cancers Decreased by Early Initiation of Antiretroviral Therapy: Results From the START Trial (INSIGHT 001)*** Karen L. Klingman, M.D. National Institute of Allergy and Infectious Diseases, NIH
4:15 p.m. - 4:30 p.m.	***O5. Long-Term Viral Suppression Predicts Lower Cancer Incidence Among HIV-Infected Veterans, but Higher Than Among Uninfected*** Lesley S. Park, Ph.D., M.P.H. Stanford University School of Medicine
4:30 p.m. - 4:45 p.m.	***O6. Risk of Kaposi Sarcoma in HIV-Positive Adults on ART: A Global Analysis*** Julia Bohlius, M.D., M.Sc.P.H. University of Bern
4:45 p.m. - 5:00 p.m.	***O7. Determinants of Shedding of Kaposi Sarcoma-Associated Herpesvirus in Saliva Among Ugandan Mothers and Children*** Vickie Marshall, M.S. Leidos Biomedical Research, Inc. Frederick National Laboratory for Cancer Research
5:00 p.m. - 5:15 p.m.	***O8. Kaposi Sarcoma in HIV-Infected Children and Adolescents in Central Malawi: A Novel Clinical Staging Classification Determines Risk Stratification*** Nader Kim El-Mallawany, M.D. New York Medical College
5:15 p.m.	**End of Day 1**

October 27

8:00 a.m. - 8:15 a.m. **Day 2 Poster Setup and Viewing**

8:15 a.m. - 8:25 a.m. **Opening Comments**

8:25 a.m. - 10:00 a.m. **Session 5: Round Table Discussion of KSHV Transmission: Implications for Public Health Approaches to Reduce Transmission**
Each speaker has 15 minutes.

8:25 a.m. - 8:30 a.m. ***Session Overview***
Robert Yarchoan, M.D.
National Cancer Institute, NIH

8:30 a.m. - 8:45 a.m. ***T1. KSHV Transmission Globally and Lifestyle Factors That Play a Role in Acquisition***
Jeffrey Martin, M.D., M.P.H.
University of California, San Francisco

8:45 a.m. - 9:00 a.m. ***T2. KSHV Risk Factors for Early Childhood Infection***
Charles Wood, Ph.D.
University of Nebraska, Lincoln

9:00 a.m. - 9:15 a.m. ***T3. Comparison of Kaposi Sarcoma-Associated Herpesvirus Transmission With Epstein-Barr Virus and Other Human Herpesviruses in Ugandan Households***
Soren Gantt, M.D., Ph.D.
University of British Columbia

9:15 a.m. - 9:30 a.m. ***T4. Prevalence and Incidence in the United States in the cART Era***
Denise Whitby, Ph.D.
Leidos Biomedical Research, Inc.
Frederick National Laboratory for Cancer Research

9:30 a.m. - 10:00 a.m. ***Panel Discussion***

10:00 a.m. - 10:30 a.m. **Break and Poster Viewing**

10:30 a.m. - 12 noon **Session 6: Kaposi's Sarcoma-Associated Herpesvirus (KSHV)/Human Herpesvirus 8 (HHV8) Biology**
Moderator: Dirk Dittmer, Ph.D.
The University of North Carolina at Chapel Hill

10:30 a.m. - 11:00 a.m. ***P8. Deregulation of Host Cellular Long Noncoding RNAs by Kaposi Sarcoma-Associated Herpesvirus***
Rolf Renne, Ph.D.
University of Florida

11:00 a.m. - 11:15 a.m. ***O9. Comprehensive RNAseq Analysis of KSHV Gene Expression in Kaposi Sarcoma Tumors and Correlation With In Vitro Infection Models***
Timothy Rose, Ph.D.
Center for Global Infectious Disease Research

11:15 a.m. - 11:30 a.m. ***O10. KSHV Induces Revision of B Cell Receptors***
Jennifer E. Totonchy, Ph.D.
Weill Cornell Medical College

11:30 a.m. - 11:45 a.m. ***O11. The cryoEM Structure and Structure-Guided Mutagenesis of KSHV Capsid***
Xinghong Dai, Ph.D.
University of California, Los Angeles

11:45 a.m. - 12 noon ***O12. Modeling of the KSHV Episome-Host Chromatin Synapse by Super-Resolution Localization Microscopy***
Margaret J. Grant, M.S., B.S.
University of Virginia, Charlottesville

12 noon - 12:45 p.m. **Lunch**

12:45 p.m. - 1:45 p.m. **Poster Viewing** (presenters to stand by their posters)

1:45 p.m. - 3:00 p.m. **Session 7: Lung Cancer**
Moderator: Alexandra M. Levine, M.D., MACP
City of Hope

1:45 p.m. - 2:15 p.m. **P9. Screening for Lung Cancer in HIV+ Persons in the Context of General Lung Cancer Screening Guidelines**
Kristina Crothers, M.D.
University of Washington

2:15 p.m. - 2:30 p.m. ***O13. Benefits and Harms of Lung Cancer Screening in HIV-Infected Individuals: A Simulation Study***
Chung Yin Kong, Ph.D.
Massachusetts General Hospital

2:30 p.m. - 2:45 p.m. ***O14. Lung Cancer Screening and Smoking Behavior in HIV+ Smokers***
Keith M. Sigel, M.D.
Mount Sinai School of Medicine

2:45 p.m. - 3:00 p.m. ***O15. Characteristics of HIV+ Lung Cancer Patients Undergoing Surgical Resection at an Urban Center With High HIV Prevalence: Implications for Lung Cancer Screening***
Vipul Pareek, M.D.
Montefiore Medical Center

3:00 p.m. - 3:30 p.m. **Break and Poster Viewing**

3:30 p.m. - 4:30 p.m. **Session 8: HPV Pathogenesis**
Moderator: Erle S. Robertson, Ph.D.
University of Pennsylvania

3:30 p.m. - 4:00 p.m. ***P10. Effect of HIV on Oral HPV Infection and Oropharyngeal Cancer***
Gypsyamber D'Souza, Ph.D.
Johns Hopkins Bloomberg School of Public Health

4:00 p.m. - 4:15 p.m. ***O16. Homologous Prime-Boost With an HPV-E6/E7 Immunotherapy Plus PD-1 Checkpoint Inhibition Results in Tumor Regression and Reduction in PD-L1 Expression***
Adrian E. Rice, Ph.D.
Etubics Corporation

4:15 p.m. - 4:30 p.m. ***O17. High-Risk Human Papillomavirus Oncoprotein E6 Induces FoxM1 in Human Keratinocytes Through Transcriptional Regulation by Grainyhead-Like 2 (GRHL2)***
Mo K. Kang, D.D.S., Ph.D.
University of California, Los Angeles, School of Dentistry

4:30 p.m. - 5:30 p.m. **Session 9: Clinical Trials in HIV-Associated Malignancies**
Moderator: Richard Little, M.D.
National Cancer Institute, NIH

4:30 p.m. - 4:45 p.m. ***O18. Lenalidomide and Pomalidomide Inhibit KSHV-Induced Downregulation of MHC Class I Expression in Primary Effusion Lymphoma Cells***
David A. Davis, Ph.D., M.S.
National Cancer Institute, NIH

4:45 p.m. - 5:00 p.m. ***O19. Radiation-Sparing Treatment of Acquired Immune Deficiency Syndrome (AIDS)-Related Primary Central Nervous System Lymphoma (PCNSL) With Highly Active Antiretroviral Therapy (HAART), Rituximab (R), and High-Dose Methotrexate With Leucovorin Rescue (HD-MTX)***
Thomas S. Uldrick, M.D., M.S.
National Cancer Institute, NIH

5:00 p.m. - 5:15 p.m. ***O20. AMC-085: A Pilot Trial of AVD and Brentuximab Vedotin in the Upfront Treatment of Stage II-IV HIV-Associated Hodgkin Lymphoma. A Trial of the AIDS Malignancy Consortium***
Paul G. Rubinstein, M.D.
John H. Stroger, Jr. Hospital of Cook County

5:15 p.m. - 5:30 p.m. ***O21. Non-Myeloablative Haploidentical Allogeneic Bone Marrow Transplantation in HIV-Infected Individuals***
Richard F. Ambinder, M.D., Ph.D.
Johns Hopkins University School of Medicine

5:30 p.m. **Meeting Adjourned**

PROGRAM COMMITTEE

Program Co-Chairs

Geraldina Dominguez, Ph.D.
Program Director
Office of HIV and AIDS Malignancy
National Cancer Institute
National Institutes of Health
Building 31, Room 33A-33
31 Center Drive
Bethesda, MD 20892-2440
(301) 496-4995
domingug@mail.nih.gov

Robert Yarchoan, M.D.
Director
Office of HIV and AIDS Malignancy
Chief
HIV and AIDS Malignancy Branch
Center for Cancer Research
National Cancer Institute
National Institutes of Health
Building 10, Room 6N-106
10 Center Drive
Bethesda MD 20892-1868
(301) 496-0328
robert.yarchoan@nih.gov

Program Committee

Richard F. Ambinder, M.D., Ph.D.
Director
Division of Hematologic Malignancies
Professor of Oncology
Johns Hopkins University School of Medicine
CRB1, Room 389
1650 Orleans Street
Baltimore, MD 21287
(410) 955-8839
ambinri@jhmi.edu

Kishor Bhatia, Ph.D., FRCPath
Director
AIDS Malignancy Program
Office of HIV and AIDS Malignancy
National Cancer Institute
National Institutes of Health
Building 31, Suite 3A-33
31 Center Drive
Bethesda, MD 20892-2440
(301) 496-4995
bhatiak@mail.nih.gov

Corey Casper, M.D., M.P.H.
Head, Global Oncology
Member
Vaccine and Infectious Disease Division
Fred Hutchinson Cancer Research Center
Professor of Medicine, Epidemiology and Global Health
University of Washington
M1-B140
1100 Fairview Avenue, North
Seattle, WA 98109
(206) 667-4600
ccasper@fhcrc.org

Ethel Cesarman, M.D., Ph.D.
Professor
Pathology and Laboratory Medicine
Weill Cornell Medical College
1300 York Avenue
New York, NY 10065
(212) 746-8838
ecesarm@med.cornell.edu

Dirk Dittmer, Ph.D.
Professor
Department of Microbiology and Immunology
Lineberger Comprehensive Cancer Center
Center for AIDS Research
The University of North Carolina at Chapel Hill
Chapel Hill, NC 27599-7290
(919) 966-7960
ddittmer@med.unc.edu

Gypsyamber D'Souza, Ph.D.
Associate Professor
Department of Epidemiology
Johns Hopkins Bloomberg School of Public Health
Room E6132B
615 North Wolfe Street
Baltimore, MD 21205
(410) 502-2583
gdsouza2@jhu.edu

Eric A. Engels, M.D., M.P.H
Senior Investigator
Division of Cancer Epidemiology and Genetics
National Cancer Institute
National Institutes of Health
Room 6E226
9609 Medical Center Drive
Rockville, MD 20850
(240) 276-7186
engelse@exchange.nih.gov

Thomas Gross, M.D., Ph.D.
Deputy Director of Science
Center for Global Health
National Cancer Institute
National Institutes of Health
Room 3W534
9609 Medical Center Drive
Rockville, MD 20850
(240) 276-6984
thomas.gross@nih.gov

Missak Haigentz, M.D.
Associate Professor of Clinical Medicine
Albert Einstein College of Medicine
Hofheimer Building, Room 100
111 East 210th Street
Bronx, NY 10467
(718) 920-4826
mhaigent@montefiore.org

Rebecca Liddell Huppi, Ph.D.
Program Director
Office of HIV and AIDS Malignancy
National Cancer Institute
National Institutes of Health
Building 31, Room 3A-33
31 Center Drive
Bethesda, MD 20892-2440
(301) 496-4995
liddellr@exchange.nih.gov

Elliott D. Kieff, M.D., Ph.D.
Harriet Ryan Albee Professor of Microbiology and Molecular Genetics
Harvard Medical School
Director of Infectious Diseases
Brigham and Women's Hospital
181 Longwood Avenue
Boston, MA 02115
(617) 525-4252
ekieff@partners.org

Alexandra M. Levine, M.D., MACP
Chief Medical Officer
Professor of Hematology/HCT
City of Hope National Medical Center
1500 East Duarte Road
Duarte, CA 91010
(626) 471-7213
alevine@coh.org

Richard Little, M.D.
Head
Hematologic, HIV, and Stem Cell Therapeutics
Clinical Investigations Branch
National Cancer Institute
National Institutes of Health
Room 5W426
9609 Medical Center Drive
Rockville, MD 20850
(240) 276-6560
littler@mail.nih.gov

Otoniel Martinez-Maza, Ph.D.
Professor
David Geffen School of Medicine
UCLA AIDS Institute
University of California, Los Angeles
BSRB 173
615 Charles E. Young Drive, South
Los Angeles, CA 90095-7363
(310) 825-2542
omartinez@mednet.ucla.edu

Sam Mbulaiteye, M.D.
Senior Investigator
Division of Cancer Epidemiology and Genetics
National Cancer Institute
National Institutes of Health
Room 6E118
9609 Medical Center Drive
Rockville, MD 20850
(240) 276-7108
mbulaits@mail.nih.gov

Ashlee Moses, Ph.D.
Associate Professor
Oregon Health & Science University
Vaccine and Gene Therapy Institute
505 NW 185th Avenue
Beaverton, OR 97006
(503) 418-2712
mosesa@ohsu.edu

Mostafa Nokta, M.D., Ph.D.
Director
AIDS Cancer Clinical Program
Office of HIV and AIDS Malignancy
National Cancer Institute
National Institutes of Health
Building 31, Room 3A-33
31 Center Drive
Bethesda, MD 20892-2440
(301) 496-4995
noktam@mail.nih.gov

Joel Palefsky, M.D., FRCP(C)
Professor
Department of Medicine
University of California, San Francisco
Box 0126
505 Parnassus, M-1203
San Francisco, CA 94118
(415) 476-1574
joelp@medicine.ucsf.edu

C. David Pauza, Ph.D.
Professor and Associate Director
Institute of Human Virology
University of Maryland School of Medicine
725 West Lombard Street
Baltimore, MD 21201
(410) 706-1367
cdpauza@ihv.umaryland.edu

Elizabeth Read-Connole, Ph.D.
Program Director, Viral Oncogenesis
Cancer Etiology Section Chief
Cancer Immunology, Hematology, and Etiology Branch
Division of Cancer Biology
National Cancer Institute
National Institutes of Health
Room 6W534
9609 Medical Center Drive
Rockville, MD 20850-9748
(240) 276-6226
bconnole@mail.nih.gov

Erle S. Robertson, Ph.D.
Professor
Department of Microbiology
Comprehensive Cancer Center
University of Pennsylvania Medical School
201E Johnson Pavilion
3610 Hamilton Walk
Philadelphia, PA 19104-6076
(215) 746-0114
erle@mail.med.upenn.edu

Isaac R. Rodriguez-Chavez, Ph.D., M.S., M.H.S.
Director
AIDS and Immunosuppression Program
IBIDB
DER
National Institute of Dental and Craniofacial Research
National Institutes of Health
Room 614
6701 Democracy Boulevard
Bethesda, MD 20892-4878
(301) 594-7985
isaac@nidcr.nih.gov

Michael J. Silverberg, Ph.D., M.P.H.
Research Scientist
Division of Research
Kaiser Permanente
5th Floor
2000 Broadway
Oakland, CA 94612
(510) 891-3801
michael.j.silverberg@kp.org

Ren Sun, Ph.D.
Professor
University of California, Los Angeles
Factor Building, Room 10-155
650 Charles E. Young Drive, South
Los Angeles, CA 90095
(310) 794-5557
rsun@mednet.ucla.edu

Denise Whitby, Ph.D.
Principal Investigator
Viral Oncology Section
AIDS and Cancer Virus Program
Leidos Biomedical Research, Inc.
Frederick National Laboratory for Cancer Research
P.O. Box B
Frederick, MD 21702
(301) 846-1714
whitbyd@mail.nih.gov

Charles Wood, Ph.D.
Professor
School of Biological Sciences
Director
Nebraska Center for Virology
University of Nebraska, Lincoln
102 C Morrison Center
Lincoln, NE 68583-0900
(402) 472-4550
cwood1@unl.edu

PLENARY ABSTRACTS

P1. Incorporating HPV Testing in Cervical Cancer Screening for HIV+ Women

Howard Strickler

Albert Einstein College of Medicine, Bronx, New York, USA

This talk will provide an overview of current recommendations for cervical cancer screening in HIV+ women, and how these recommendations may change in part due to recently reported cohort data. HIV+ women have several-fold increased risk of invasive cervical cancer and precancerous cervical lesions, relative to the general population, as well as increased prevalence, incidence, and persistence of oncogenic human papillomavirus (oncHPV), the viral cause of cervical cancer. Each of these risks increases with diminishing CD4+. According to data from the Women's Interagency HIV Study (WIHS), the largest prospective U.S. cohort of HIV+ and at-risk HIV- women, >25% of HIV+ women at each clinic visit have abnormal Pap tests (i.e., atypical squamous cells of undetermined significance or more severe [ASC-US+]). Most of these abnormal Pap tests do not, however, reflect clinically relevant disease, i.e., cervical intraepithelial neoplasia grade 2 or more severe (CIN-2+) by histology. USPHS guidelines currently recommend aggressive cervical cancer screening of HIV-infected women: two Pap tests at 6-month intervals in the first year following diagnosis of HIV and, if normal, then on an annual basis. However, WIHS data have shown that HIV+ women with normal Pap tests who additionally co-test negative for oncHPV have a similar low 5-year risk of CIN-2+ and CIN-3+ as in HIV- women. Conversely, HIV+ women who tested positive for oncHPV despite a normal Pap had a high 5-year cumulative risk of CIN-3+. In multivariable Cox models, testing non16-oncHPV+ was associated with a 3-fold increased risk of CIN-3+ relative to oncHPV-negative, whereas it was 13-fold for HPV16+, and 9-fold for those with LSIL by Pap (a benchmark for immediate colposcopy). Overall, HIV+ women with a normal Pap who test oncHPV- may not require screening for several years, whereas those who are HPV16+ may warrant immediate colposcopy, and those with other oncHPV have intermediate risk.

The recent findings are also interesting for another reason: they provide new insight into the special character of HPV16. We have shown that the prevalence of HPV16 is the least affected of any oncHPV type by changes in immune status in HIV+ women. Consistent with this, we recently reported that HPV16 is present in a significantly lower percentage of CIN-3+ in HIV+ (25%) than in HIV- (>60%) women in the WIHS cohort. The relative independence of HPV16 from immune status has been interpreted as evidence that HPV16 may have a greater innate ability to avoid the effects of immune surveillance than other oncHPV. Taken together, the data suggest that while HPV16 prevalence is less affected by CD4 count, when HPV16 occurs it is strongly associated with precancer risk in HIV+ women. Pilot data suggesting a role for HPV testing in triage of ASC-US in HIV+ women will also be presented.

P2. CpG Methylation of HPV16 and Other Oncogenic Human Papillomaviruses (HPVs) Is Associated with High-Grade Cervical Intraepithelial Neoplasia (CIN)

Robert D. Burk

Albert Einstein College of Medicine, Bronx, New York, USA

Human papillomaviruses cause cervical cancer by actions of the E6 and E7 open reading frames (ORFs). These proteins act to disrupt differentiation of the cervical epithelium, particularly in the squamocolumnar junction region. One of the results of de-differentiation is the change in the biochemical milieu of the infected epithelial cells resulting in the decreased or lack of expression of the L1 and L2 ORFs. The L1 and L2 are essential for the production of infectious viral particles. Thus, de-differentiated lesions (i.e., HG-CIN) and cancer do not contribute to the viral life cycle and can be considered "collateral damage" of long-term persistent oncogenic HPV infections [1]. The host cellular machinery is responsible for the marking of DNA not involved in transcription by methylating the "C" of CpG sites. The exact mechanisms and signals of these events are not clearly known. Moreover, exfoliated cells from the cervix are collected during routine cytologic screening and include cells from lesions that contain oncogenic HPV. This serves to enrich the signal from dysplastic cell amongst the large number of host cells collected.

To interrogate the methylation of specific CpG sites and patterns of CpG methylation (i.e., methyl-haplotypes) in oncogenic HPV infections, we developed a next-generation sequencing (NGS) assay utilizing barcoded primers to identify each sample [2,3]. Total DNA from exfoliated cervical cells is extracted and treated with bisulfite. PCR primer design is complicated by the reduction in DNA complexity. After PCR of bisulfite treated DNA using specially designed primers, unmethylated "C's" are converted to "T's", whereas methylated "C's" are protected and remain as "C's" in the DNA sequence. This biochemical difference can be leveraged to quantitatively assess the percent methylation of CpG sites in the oncogenic HPV genome. To employ bisulfite-sequencing using NGS technology, we have also developed a bioinformatics pipeline to demultiplex each sample (i.e., sort the NGS reads of each sample into a folder based on the unique barcode) and characterize the percent methylation (i.e., ratio of C/T+C) [2,3]. Using this technology, we have developed assays for all the oncogenic HPV types.

We have utilized the basic understanding that CpG methylation of the L1 and L2 ORFs of oncogenic HPV is coupled with de-differentiated lesions to evaluate the association with HG-CIN in properly designed epidemiological studies [3-6]. Updated data will be presented on the use of this technology to stratify women with oncogenic HPV infections for risk of HG CIN.

P3. Synthetic DNA Vaccines for Difficult Immune Targets Including HPV

David B. Weiner

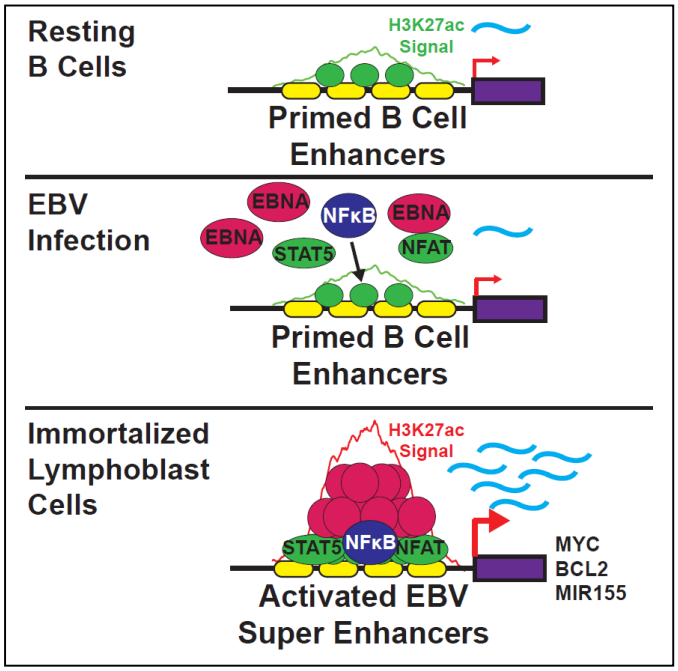
University of Pennsylvania, Philadelphia, Pennsylvania, USA

DNA vaccines represent an important vaccine technology, which has specific conceptual advantages over traditional vaccine platforms. However, in humans, prior generations of this technology were poorly immunogenic. Through multiple improvements including synthetic plasmid optimization, genetic adjuvant technology further combined with enhanced EP delivery, this platform now generates significant immune responses of relevance to human disease settings. These combined (synthetic) DNA approaches drive immune responses similar or superior to live viral vector protocols in diverse important model systems. We will review this important technology with a particular focus on clinical evaluation of both the immune responses as well as the possible impact of this technology in the clinic. A specific application to treatment of HPV infection will be highlighted where we report that this synthetic approach can induce strong CTL in humans that can result in CD8T cells increasing at the mucosal site of underlying HPV infection. We will also show that this immune phenotype can result in disease regression and immune clearance of infection in 49.5% of treated women in a double blind placebo controlled study. The implications of these studies for broader approaches to HPV disease including cancer immune therapy will be discussed.

P4. Epstein-Barr Virus Oncoprotein Super-Enhancers Control B Cell Growth

Elliott D. Kieff

Harvard Medical School, Boston, Massachusetts, USA



Super-enhancers are clusters of gene-regulatory sites bound by multiple transcription factors that govern cell transcription, development, phenotype, and oncogenesis. By examining Epstein-Barr Virus (EBV)-transformed lymphoblastoid cell lines (LCLs), we identified four EBV oncoproteins and five EBV-activated NF-κB subunits co-occupying ~1,800 enhancer sites. Of these, 187 had markedly higher and broader histone H3K27ac signals, characteristic of super-enhancers, and were designated "EBV super-enhancers." EBV super-enhancer-associated genes included the MYC and BCL2 oncogenes, which respectively mediate LCL proliferation and survival. MYC and BCL2 super-enhancers looped to MYC and BCL2 as shown by ChIA-PET and Hi-C. EBV super-enhancers were enriched for B cell transcription factor motifs and had high STAT5 and NFAT transcription factor (TFs) co-occupancy. EBV super-enhancer-associated genes were more highly expressed than other LCL genes. EBV super-enhancer disruption by the bromodomain inhibitor JQ1 or through conditional inactivation of an EBV oncoprotein or NFkB decreased MYC or BCL2 expression and arrested LCL growth. EBV super-enhancers more frequently interacted with other EBV super-enhancers than with EBV typical enhancers by ChIA-PET. These findings provide new insights into the mechanisms of EBV-induced lymphoproliferation and identify potential therapeutic interventions.

P5. The Innate Immune Response to KSHV

Blossom Damania

Lineberger Comprehensive Cancer Center and Department of Microbiology & Immunology, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA.

Innate immunity is the primordial defense against invading viruses. Pattern recognition receptors (PRRs) recognize pathogen-associated molecular patterns (PAMPs) on viruses resulting in the activation of an anti-viral response through the induction of interferon and inflammatory cytokines. Kaposi's sarcoma-associated herpesvirus (KSHV) is a herpesvirus that is linked to several human malignancies including Kaposi's sarcoma (KS), primary effusion lymphoma, and multicentric Castleman's disease. KSHV is detected by multiple PRRs during primary infection and reactivation from latency. This talk will describe cellular sensors that are activated by KSHV and elicit an innate immune response against the virus. KSHV-encoded viral proteins that counteract host defenses and help the virus evade the innate immune response will also be discussed.

P6. IFNL4 and HCV Infection

Thomas O'Brien

Infections and Immunoepidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland, USA

Work by our collaborative group led to the discovery of the interferon lambda 4 (*IFNL4)* gene, *IFNL4*-ΔG/TT polymorphism, and IFN-λ4 protein. Like other interferons, IFN-λ4 has antiviral activity; however, this cytokine is unique in that it is genetically restricted such that it is generated only by individuals who carry the *IFNL4*-ΔG allele of *IFNL4*-ΔG/TT. Paradoxically, the protein generating *IFNL4*-ΔG variant is associated with failure to clear hepatitis C virus (HCV) infection either spontaneously or in response to treatment. In the ION-4 trial of ledipasvir/sofosbuvir among HIV/HCV co-infected patients, all of the patients who failed to clear HCV had an unfavorable *IFNL4* genotype. *IFNL4*-ΔG is the ancestral allele for the *IFNL4*-ΔG/TT polymorphism and the major variant in Africans, whereas the *IFNL4*-TT allele, which abrogates the IFN-λ4 protein, is the major variant in Europeans and Asians. Those findings suggest strong negative genetic selection against *IFNL4*-ΔG by an as yet unidentified infectious agent. Studies examining a possible association between the *IFNL4*-ΔG/TT polymorphism and HIV infection have yielded mixed results.

P7. Cancer in HIV-Infected People and Solid Organ Transplant Recipients: Lessons Learned and Open Questions

Eric A. Engels

Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland, USA

Solid organ transplant recipients, because of the need for lifelong immunosuppressive therapy to prevent rejection of their organ graft, have impaired T-cell immune function that is similar to that seen in people with AIDS. Studies that link HIV and transplant registries to cancer registries provide valuable population-level information on cancer risk in these immunosuppressed populations. In this presentation, data will be reviewed from large registry linkage studies and other sources, with a special focus on recent results from NCI's Transplant Cancer Match Study.

Both HIV-infected people and transplant recipients have elevated risks for virus-related cancers, including non-Hodgkin lymphoma, Kaposi sarcoma, HPV-related cancers (most notably cervical and anal cancers), Hodgkin lymphoma, liver cancer, and Merkel cell carcinoma. These increases arise, to a variable degree, due to loss of immune control of oncogenic viruses, while differences in risk between the two groups may reflect differences in the magnitude or quality of immune dysfunction, differing prevalence of viral infections, or cancer screening. Lung cancer risk is elevated in both groups, although it is higher in HIV-infected people than in transplant recipients, probably because of higher rates of smoking. Risk of non-melanoma skin cancers, especially squamous cell carcinoma, is markedly increased in transplant recipients, while it is only modestly increased in HIV-infected individuals. Melanoma risk is also higher in transplant recipients than in HIV-infected people. These differences in skin cancer risk may reflect photosensitizing or DNA damaging effects of some immunosuppressant medications. Transplant recipients also have elevated risks of kidney, colon, and thyroid cancers. A number of factors may contribute to high risks for these three cancers, including underlying medical conditions, kidney damage from medications, and over-detection from medical surveillance; these cancers are not elevated in HIV-infected people. Risks of prostate and breast cancers are not increased in HIV-infected individuals and transplant recipients, and for unclear reasons may actually be decreased compared with the general population.

A comparison of cancer risk in HIV-infected people and transplant recipients identifies a number of cancers that are elevated in both populations, but others that are elevated to a greater extent (or only) in transplant recipients. These observations may suggest avenues for further research on cancer etiology.

P8. Deregulation of Host Cellular Long Noncoding RNAs by Kaposi Sarcoma-Associated Herpesvirus

Rolf Renne

University of Florida, Gainesville, Florida, USA

Kaposi's sarcoma-associated herpesvirus (KSHV), a human gamma-herpesvirus, is the causative agent of AIDS malignancies like KS and primary effusion lymphomas (PEL). In recent years it became clear that pathogenic herpesviruses including EBV, MHV68, and KSHV express numerous microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), many of which are in antisense orientation to protein coding transcripts. Additionally, KSHV and EBV express EBERs and PAN RNAs, which play pleiotropic roles in the regulation of viral gene expression. The function and structure of the newly identified lncRNAs is largely unknown. While characterizing the KSHV miRNA targetome, we identified several hundred host cellular lncRNAs as putative miRNA targets. Furthermore, infection of endothelial cells with wtBAC16 KSHV induced dramatic deregulation of host lncRNAs including the down-regulation of 533 lncRNAs. Of these, 126 were rescued when cells were infected with a KSHV recombinant that lacked 10 of 12 KSHV miRNAs. Using cell fractionation as well as confocal microscopy, we demonstrate the presence of both Ago protein and mature miRNAs within the nucleus of PEL cells and KSHV-infected endothelial cells. Together these data strongly suggest that both KSHV encoded proteins and miRNAs contribute to dysregulation of host lncRNAs. Importantly, aberrant expression of 10 lncRNAs that are perturbed following KSHV infection, including HOTTIP, ANRIL, and UCA1, is reported to be associated with human cancers. We demonstrate that up-regulation of UCA1 affects proliferation and migration of KSHV-infected endothelial cells. Additionally, we provide experimental evidence that the anti-sense LANA transcript is bound by EZH2/PRC2 complexes and may contribute to the regulation of viral latency. These data suggest that both viral lncRNAs as well as virally perturbed host lncRNAs are important regulators of viral gene expression and hence may be important for viral pathogenesis and/or tumorigenesis.

P9. Screening for Lung Cancer in HIV+ Persons in the Context of General Lung Cancer Screening Guidelines

Kristina Crothers

Department of Medicine, Harborview Medical Center, University of Washington, Seattle, Washington, USA

Lung cancer is the most common non-AIDS defining cancer (NADC) and leading source of NADC mortality among HIV-infected (HIV+) individuals. HIV+ persons have a greater burden of lung cancer due to higher smoking rates combined with an independent HIV-related increased risk of lung cancer. Unfortunately, most lung cancers are clinically diagnosed at an advanced stage and have 5-year survival rates <15%. Earlier detection strategies to improve lung cancer mortality among HIV+ persons are urgently needed. The National Lung Screening Trial (NLST) demonstrated that low-dose screening chest computed tomography (LDCT) led to a 20% reduction in lung cancer mortality among high-risk, HIV uninfected (uninfected) smokers; however, the impact of LDCT screening on mortality of HIV+ individuals remains uncertain. While the increased risk and burden of lung cancer in this aging population would seem to make HIV+ persons excellent candidates for screening, HIV+ persons also experience considerable multimorbidity and can have higher mortality from competing risks, and could experience greater morbidity from the work-up of positive LDCT screening tests. This talk will discuss the overall balance of these benefits and harms that should be considered in comprehensively assessing the net impact of LDCT lung cancer screening on mortality in HIV+ persons. Results regarding these benefits and harms that are being used to inform a mathematical model estimating the impact of lung cancer screening on mortality among HIV+ persons will be highlighted. These include data on lung cancer incidence with age and smoking history; clinical consequences that result from performing screening chest CT scans among HIV+ individuals; and a comparison of lung cancer treatment and outcomes in HIV+ to uninfected patients. Future work will be outlined, including the need to determine the optimal candidates, regimen, and age for screening; the impact of LDCT screening on other outcomes such as smoking behavior; and issues surrounding the implementation of an LDCT screening program.

P10. Effect of HIV on Oral HPV Infection and Oropharyngeal Cancer

Gypsyamber D'Souza

Johns Hopkin University, Baltimore, Maryland, USA

Oral human papillomavirus (HPV) infection is now the major cause of oropharyngeal cancer, and the incidence of this cancer is increasing in the United States. People with HIV are at increased risk of HPV infection and HPV-related oropharyngeal cancer, due to increased sexual exposure to HPV and the effects of immunosuppression.

This talk will review existing data on the effect of HIV on oral HPV prevalence, natural history, and oropharyngeal cancer. This includes recent oral rinse data collection in the MACS and WIHS showing multi-year oral HPV natural history. The effects of HIV and CD4 cell count on oral HPV incidence and clearance are discussed. Differences in risk factors for oral HPV incidence and clearance in HIV-infected and HIV-uninfected individuals are reviewed. Characteristics of HIV-infected oropharyngeal cancer cases are also compared to oropharyngeal cancer cases in the general population.

PANEL DISCUSSION TOPICS

T1. KSHV Transmission Globally and Lifestyle Factors That Play a Role in Acquisition

Jeffrey N. Martin

University of California, San Francisco, San Francisco, California, USA

From a global perspective, infection with Kaposi's sarcoma-associated herpesvirus (KSHV) is most concentrated and has the most relevant disease consequences in two socio-geographic groups. The first is men who have sex with men (MSM, predominantly, although not exclusively, in resource-rich regions such as the U.S. and Europe), in whom seroprevalence is 20% to 30% in HIV-uninfected MSM and 30% to 60% in HIV-infected MSM. This compares to approximately 5% in the rest of the population. The second is the entire general population of sub-Saharan Africa where KSHV infection is endemic with seroprevalences varying between 15% to 65%.

Among MSM, there is strong evidence that KSHV transmission is facilitated by some form of intimate contact with other men and that saliva is the accessible body fluid that most commonly harbors the virus. This naturally led to the belief by some that kissing was the culprit route of spread, but this does not easily explain why KSHV seroprevalence is so low in ambient heterosexual populations. Instead, we have hypothesized that under-recognized uses of saliva during sexual activity that are predominantly practiced by MSM — namely as a lubricant during anal intercourse or fisting/fingering — deserve equal attention as potential routes of spread. While this hypothesis awaits rigorous scientific testing, what is indisputable is that KSHV is heavily concentrated in MSM in resource-rich regions and that, among the readily exchangeable body fluids, saliva carries it most often. Despite this, there is scant awareness of KSHV in the MSM community, let alone its linkage to saliva. This is not surprising because no clinical care guideline recommends any mention about KSHV to MSM. While there are insufficient data to inform MSM about exactly which intimate practices spread KSHV, we feel that educating them about the unique concentration of KSHV among MSM and the unique role of saliva is long overdue. Even without specific recommendations for behavioral modification, the medical community owes this education to the affected group, which can then decide how to use the information. We note that this form of educational message, which could be focused in primary care settings, is different than a widespread public health campaign.

In sub-Saharan Africa, the situation is more complex. There are at least two patterns of KSHV spread, one featuring widespread KSHV transmission during childhood (in equatorial Africa) and one featuring spread mainly during the adult years (at least in South Africa and perhaps in other locations). Focusing on equatorial Africa, in which the disease state manifestations of KSHV infection (i.e., KS) are most profound, it is again saliva which harbors KSHV most commonly and is very likely the conduit for transmission. Like the situation with MSM, we again believe it is important to look beyond the obvious ways by which saliva is passed to children, and, indeed, there are several heretofore under-recognized behavioral practices. These include use by caregivers of their saliva on the skin of children to soothe insect bites, in the anus to relieve constipation, and on the face as a ritualistic healing practice. Thus, unlike the situation with MSM in resource-rich countries, the plethora of ways that saliva is passed to children in sub-Saharan Africa and the fact that the entire general population is affected precludes at this time any meaningful educational message to the community or public health campaign about KSHV. Instead, until more research pinpoints the specific culprit route of spread, work towards a vaccine seems to be a more realistic option for primary prevention of KSHV.

T2. KSHV Risk Factors for Early Childhood Infection

Charles Wood

University of Nebraska, Lincoln, Nebraska, USA

The Kaposi sarcoma-associated herpesvirus (KSHV) is now known to be associated with Kaposi's sarcoma (KS), primary effusion lymphoma, and multicentric Castleman's disease. Many studies have been conducted to understand its epidemiology and pathogenesis and their results clearly show that the worldwide distribution of KSHV is uneven. Some geographical areas like sub-Saharan Africa and Xinjiang region of China are endemic but Western Europe and United States have low prevalence in the general population. This makes it imperative to understand the risk factors associated with acquisition of infection.

KSHV can be transmitted via sexual contact and non-sexual routes, such as transfusion of contaminated blood and tissues transplants, or via saliva contact. Our group has long standing interest in understanding whether vertical transmission of KSHV can occur, what are the risk factors associated with early childhood infection in Zambia, a sub-Saharan African country considered as part of the "KS belt" where endemic KS was prevalent, and where significant increase in KS incidence in adults and children has coincided with the emergence of the HIV-1 epidemic. Currently, KS remains one of most prevalent cancers in the setting. There is now a general consensus that salivary transmission is the main route in children residing in endemic areas; however, there is a need to better understand the sources of transmission to young children. Also, lack of animal models to study transmission, gold standard serological assay, and the lack of emphasis on endemic KS research have hampered the efforts to further delineate KSHV transmission in order to design effective prevention strategies.

Our ongoing studies of KS in Zambia have focused on investigating the transmission of KSHV during early childhood, the risk factors involved, the potential source of infection, and whether KSHV itself plays a role in the prevalence of infection in the region. We have shown that early childhood infection by KSHV is common, indicating that this contributes to the high prevalence rate in the adult population. KSHV can be detected in saliva but not in breast milk, suggesting that the major mode of KSHV transmission to children is via saliva contact. We also found that HIV-1 infected children have a five-fold higher risk for KSHV infection compared to uninfected children, which is most likely due to immune suppression as a result of HIV-1 infection. This was confirmed by characterizing 151 children who underwent KSHV seroconversion; we found that among HIV-infected children, ART-naïve children had significantly increased risk of KSHV acquisition. Time-updated $CD4^+$ T-cell percentage was also significantly associated with risk of KSHV acquisition such that each 5% increase of $CD4^+$ T-cells represented an 18% decrease in risk of acquiring KSHV. Our data suggest that early ART and prevention of immune suppression reduce the risk of KSHV acquisition among HIV-infected children in an area that is highly endemic for both viruses. Our study highlights the importance of programs in Africa to provide children with ART immediately after HIV infection is diagnosed.

T3. Comparison of Kaposi Sarcoma-Associated Herpesvirus Transmission With Epstein-Barr Virus and Other Human Herpesviruses in Ugandan Households

Soren Gantt

University of British Columbia, Vancouver, British Columbia, Canada

KSHV is transmitted primarily through saliva, similar to most other HHVs including EBV, a related oncogenic Ɣ-HHV. However, the prevalence of KSHV is much more variable across different regions than other HHVs. Seroepidemiologic studies largely agree that in much of Africa KSHV infection begins during early childhood and most adults are infected. In contrast, KSHV infection appears rare in North America, especially in children, and is primarily limited to men who have sex with men and groups with risk factors for sexually transmitted infections. The basis for these patterns of KSHV transmission is unknown.

A household-based transmission study was conducted in Kampala, Uganda, in order to compare the risk factors for primary KSHV infection with other HHVs. Detailed measures of exposure to infection were performed. Quantitative viral shedding and behavioral data were collected each week from a cohort of newborns, their mothers, and siblings in the home. Primary infection with KSHV, EBV, CMV, HHV-6, and HSV in infants was defined by the onset of viremia or persistent high-level oral viral shedding detected by quantitative PCR. Congenital infection with KSHV was found in one infant and with CMV in two others. The cumulative incidence of post-natal infection at 12 months was 47% for EBV, 76% for HHV-6, 59% for CMV, and 11% for HSV; no primary KSHV infections were observed.

The infrequency of primary KSHV infection is attributable at least in part to low contact shedding. Associations were found between the level at which a virus was shed by contacts and the risk of acquisition by infants. KSHV shedding by contacts was less frequent and in lower quantities than other HHVs. However, contact shedding of KSHV occurred in over half of the households, and at levels that were comparable to that of HSV. Furthermore, low levels of KSHV DNA were occasionally detected on oral swabs from a large proportion of uninfected infants, as was seen with the other HHVs, indicating that infants were directly exposed to KSHV even though productive infection did not occur. Interestingly, mathematical modeling of the clinical data suggests that natural HHV acquisition is inefficient, and infection is frequently aborted following exposure to a small number of infectious particles.

We speculate that compared to other EBV and other HHVs, KSHV transmission may be particularly inefficient due to lower levels of exposure as well as low infectivity. This paradigm would suggest that KSHV transmission typically requires large numbers of exposures and/or large inocula (e.g., via sexual saliva exposures). The implications of this paradigm for the epidemiology and prevention of KSHV and EBV will be discussed.

T4. Prevalence and Incidence of KSHV in the United States in the cART Era

Denise Whitby

Viral Oncology Section, AIDS and Cancer Virus Program, Frederick National Laboratory for Cancer Research, Frederick, Maryland, USA

The incidence of Kaposi's Sarcoma in the United States has decreased dramatically since the introduction of effective anti-retroviral therapy in the mid-1990s. Few studies have addressed the epidemiology of KSHV in individuals with or at risk of HIV infection since the introduction of cART. We have studied KSHV prevalence and incidence in two complementary United States cohort studies. The AIDS Clinical Trials Group (ATCG) longitudinal linked randomized trials (ALLRT) study is a longitudinal cohort of HIV-1 infected subjects who had been enrolled into multicenter clinical trial of cART. We selected 5,140 naïve participants enrolled between 1997 and 2007 and tested for antibodies to KSHV in the first and last available sample. The Multicenter AIDS Cohort Study (MACS) is an ongoing prospective longitudinal cohort investigating HIV infection established in 1984. Subjects are all men and recruited in Baltimore, Chicago, Pittsburgh, and Los Angeles. MACS participants are mostly MSM and include HIV seropositive and seronegative participants. We tested >11,000 samples from study entry to 2009.

In the ALLRT KSHV prevalence was 38.1% overall and was higher in men in their third and fourth decades. There was significant geographical variation in KSHV prevalence, which was highest in California, Washington, DC, Massachusetts, New York, Colorado, and Washington. Prevalence correlated with the estimated proportion of men who have sex with men (MSM) in the source population. Incidence of KSHV infection was 4.07/100 person years and was higher in younger men and those who enrolled after 2001. In the MACS, prevalence of KSHV has not changed significantly during the study period and was higher in HIV-positive men (76-80%) than in HIV-negative men (22-24%). In HIV-positive individuals, incidence of KSHV infection declined from 1984 to 1998 but has since increased. In HIV-negative men, KSHV incidence was stable from 1984 to 1998 but has increased in the most recent period.

The data from these two cohort studies suggest that KSHV is prevalent in those with or at risk for HIV infection in the United States and that incidence is increasing since the introduction of cART in both HIV-infected and uninfected MSM.

ORAL PRESENTATIONS

O1. EBV-Mediated B-cell Transformation Is Suppressed by Oncogene-Induced Senescence Due to Depletion of Nucleotide Pools

Amy Y. Hafez[1], Karyn McFadden[1], Baek Kim[2], Micah A. Luftig[1]

[1]Department of Molecular Genetics and Microbiology, Center for Virology, Duke University, Durham, North Carolina, USA; [2]Center for Drug Discovery, Emory Center for AIDS Research, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia, USA

Epstein Barr virus (EBV) is an oncogenic γ-herpesvirus that is associated with B cell lymphomas, particularly in HIV immunocompromised patients. Approximately half of all cases of AIDS-related lymphomas, including AIDS Non-Hodgkin's Lymphomas (AIDS NHL), are associated with EBV. Since the beginning of HAART therapy, NHL accounts for a greater percentage of AIDS-related illness with a nearly 200-fold increased incidence rate in HIV-positive patients. EBV is ubiquitous in the human population and healthy individuals can prevent EBV-induced malignancies by a strong cytotoxic T-cell response. Furthermore, we have identified the DNA damage response (DDR) as an intrinsic pathway that suppresses EBV-mediated transformation.

Early after EBV infection in vitro, primary human B cells undergo a transient period of hyper-proliferation. The hyper-proliferative period can result in replicative stress and formation of double stranded DNA breaks, which could lead to genomic instability. However, this DNA damage is sensed by a DDR kinase, ATM, which triggers a signaling cascade ultimately resulting in the inhibition of EBV-mediated transformation.

In our recent studies we have used a novel cell sorting method to isolate EBV-infected B cells that undergo hyper-proliferation and subsequently arrest from those that continue to proliferate. The arrested population fails to proliferate upon re-culturing, whereas the proliferating population becomes immortalized lymphoblastoid cells. The cells that arrest are enriched for markers of the DDR and senescence-associated heterochromatic foci. Additionally, we observe that the arrested cells senesce following activation of a G1/S checkpoint. We compared the differential expression of genes between the arrested and proliferating populations and found that the arrested population is enriched for an activated p53-mediated cell cycle checkpoint including the cell cycle regulators p21 and p16. Importantly, EBV-infected B cells that proliferate initially and then arrest exhibit depleted nucleotide pools. We find that supplementation with exogenous nucleosides rescues EBV-induced senescence and enhances EBV-mediated transformation. Notably, nucleotide metabolism plays an important role in cancer by controlling genomic integrity and transformation. Depleted nucleotide pools can lead to replicative stress and trigger oncogene-induced senescence, a tumor suppressor pathway. The work presented has facilitated our understanding of the key effectors responsible for inducing senescence and suppressing EBV-associated malignancies.

O2. Increased Levels of Bregs Are Seen Prior to AIDS Non-Hodgkin Lymphoma Diagnosis

Marta Epeldegui, Y. Guo, M.L. Penichet, O. Martínez-Maza

UCLA AIDS Institute, Jonsson Comprehensive Cancer Center, University of California, Los Angeles, Los Angeles, Calfiornia, USA

Background: AIDS-associated non-Hodgkin lymphoma (AIDS-NHL) remains a significant problem, even after the introduction of effective combined anti-retroviral therapy (cART). A population of B cells that have regulatory functions, termed "regulatory B cells" (Bregs), has recently been described. Breg cells are analogous to regulatory T cells, or Tregs, which are T cells that can dampen adaptive immune responses via the secretion of inhibitory cytokines, such as IL-10 and TGF-β. Similarly, Bregs can dampen T-cell function by producing and secreting IL-10 and TGF-β and via co-stimulatory signals (CD80 and CD86). However, Breg's regulatory function is mainly associated with their capacity to secrete IL-10. We have previously seen that serum IL-10 levels are elevated in HIV infection, and further elevated over a period of years preceding AIDS-NHL diagnosis, as well as after AIDS-NHL diagnosis. In addition to this, we have shown that an IL-10 genotype that is associated with enhanced IL-10 production was associated with risk for AIDS-NHL. Also, others have described the important role of IL-10 in the modulation of T-cell function in HIV infection, showing recently that HIV-positive persons, even those who are on cART, display high levels of IL-10 expressing B cells. Such IL-10 secreting Bregs have the potential to promote lymphomagenesis in a dual fashion, by (1) enhancing B-cell activation and (2) by impairing/inhibiting T-cell function, including the activity of cytotoxic T lymphocytes (CTL) that are involved in the immunoregulation of Epstein-Barr virus (EBV) infected B cells and/or HIV-infected CD4 T cells. Hence, we hypothesized that levels of Bregs may be elevated prior to the development of AIDS-NHL, and may play an important role in lymphomagenesis.

Methods: To determine whether Bregs are elevated prior to AIDS-NHL diagnosis, we used viably frozen peripheral blood mononuclear cells (PBMC) from (1) HIV-positive persons, collected 1 to 4 years prior to AIDS-NHL diagnosis (n=31), (2) HIV-positive persons who did not develop lymphoma (n=29), and (3) persons who were HIV-negative (n=15), obtained from the UCLA Multicenter AIDS Cohort Study (MACS), and performed multi-color flow cytometry. We defined Breg cells as cells that were positive for CD19+, CD24+, and CD38+, a Breg phenotype that has been described by others.

Results/Conclusions: We observed that Bregs were significantly elevated prior to AIDS-NHL diagnosis (p=0.0078, Mann-Whitney test); the median absolute number of CD19+CD24+CD38+ cells was 19.8 cells/mm^3 for the AIDS-NHL group, 10.6 cells/mm^3 for the HIV positive group, and 9.25 cells/mm^3 for the HIV negative group. These results suggest that Bregs may be playing an important factor in AIDS-NHL development.

O3. Exquisite Sensitivity of KSHV-Associated Lymphomas to a New Nucleoside Analog Activated by Overexpressed Adenosine Kinase

Utthara Nayar[1], Jouliana Sadek[1], Jonathan Reichel[1], Denise Hernandez-Hopkins[1], Gunkut Akar[1], Peter J. Barelli[1], Michelle A. Sahai[1], Jennifer Totonchy[1], Shizuko Sei[2], Robert H. Shoemaker[3], J. David Warren[1], Olivier Elemento[1], Kenneth M. Kaye[4], Ethel Cesarman[1]

[1]Weill Cornell Medical College, New York, New York, USA; [2]Viral Vector Toxicology Section, LHTP, SAIC-Frederick, National Cancer Institute at Frederick, Frederick, Maryland, USA; [3]Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute at Frederick, Frederick, Maryland, USA; [4]Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, Massachusetts, USA

Background: Primary effusion lymphoma (PEL) and plasmablastic lymphoma (PBL) are both AIDS-related B cell malignancies, with marked plasma cell differentiation. PEL and PBL carry an extremely poor prognosis with a median survival time of less than a year, and are largely resistant to conventional chemotherapy. Thus, there is a need for new therapeutic approach for these malignancies.

Methods: We conducted a high throughput screen using an NF-kB reporter cell line to search for molecules that inhibit NF-kB in PEL but not in KSHV-negative cell lines. We validated the hit compounds in secondary assays and used cell viability, EMSA, cell cycle assays, western blotting, flow cytometry, immunohistochemistry and genomic analysis of resistant clones to investigate the mechanism of action and selectivity of the primary hit. Furthermore, we examined the phosphorylation of the primary hit by adenosine kinase (ADK) using an in vitro kinase assay and mass spectrometry analysis and tested the efficacy of the compound in PEL xenograft mouse models. We tested ADK expression as a biomarker for sensitivity to our lead compound by immunohistochemistry, and extended our findings to other tumors with plasma cell differentiation. We also compared sensitivity of other nucleoside analogs that are further along in clinical development.

Results: We identified 6-ETI, a nucleoside analog, as a novel specific inhibitor with dramatic in vivo effectiveness for KSHV-associated primary effusion lymphoma (PEL). This compound induced necrosis accompanied by S-phase arrest without affecting known oncogenic viral protein levels. To understand the selectivity towards PEL, we performed unbiased genomic analysis of 6-ETI-resistant subclones using RNASeq, and found inactivating mutations or loss of ADK, the mechanisms of resistance. Sensitive PELs have higher basal levels of cellular adenosine kinase (ADK) than resistant lymphoma cell lines including those with EBV infection; the latter could be sensitized by cell crowding-induced ADK upregulation. Furthermore, ADK directly activated 6-ETI through phosphorylation, implicating this enzyme as a new player and potential biomarker in anti-cancer therapeutics. Immunohistochemistry for ADK showed that this enzyme is expressed by plasma cells in reactive tonsils, as well as by other plasma cell malignancies, including multiple myeloma and PBL. Multiple myeloma cell lines were also shown to be sensitive to 6-ETI in vitro. We compared 6-ETI to a panel of other nucleoside analogs that are in clinical use, and failed to identify other compounds with similar effectiveness and/or selectivity towards PEL.

Conclusions: We report the discovery and characterization of a new nucleoside analog, 6-ethylthioinosine, as a drug for the treatment of PEL. We effectively used RNASeq-based "resistome" analysis to identify the mechanism of specificity and based on these findings, we developed a biomarker for sensitivity. This compound may also be useful for the treatment of other plasma cell malignancies, including PBL.

O4. Risk of Both AIDS-Related and Non-AIDS-Related Cancers Decreased by Early Initiation of Antiretroviral Therapy: Results From the START Trial (INSIGHT 001)

Karin Klingman[1], Jens Lundgren[2], Abdel Babiker[3], Fred Gordin[4], Sean Emery[5], Birgit Grund[6], Shweta Sharma[6], James Neaton[6] for the INSIGHT START Study Group.

[1]NIAID, Bethesda, Maryland, USA; [2]Rigshospitalet, Copenhagen, Denmark; [3]University College London, London, United Kingdom; [4]Washington Veterans Affairs Medical Center, Washington, DC, USA; [5]University of New South Wales, Sydney, Australia; [6]University of Minnesota, Minneapolis, Minnesota, USA

Background: It remains uncertain whether antiretroviral therapy (ART) initiated at higher CD4s is able to reduce the risk of cancer. The START trial was performed to provide randomized data to inform patients and providers on the safety and efficacy of ART at higher CD4s.

Methods: 4,865 participants at 215 sites in 35 countries with CD4 counts above 500 cells/μL were randomized 1:1 to either initiate ART immediately (Immediate Group) or wait until a CD4 threshold of 350 cells/μL, or another reason (e.g., an AIDS-defining event, pregnancy) for starting ART occurred (Deferred Group). The primary endpoint was a composite of all-cause mortality, serious AIDS-defining events (using the CDC 1993 definition, but including Hodgkin's lymphoma and excluding non-fatal herpes simplex infection and esophageal candidiasis), serious non-AIDS-related events (non-AIDS-defining cancers, cardiovascular disease (myocardial infarction, stroke, coronary revascularization, decompensated liver disease, and end-stage renal disease (initiation of dialysis or renal transplant).

Results: Mean follow-up time was 3.0 years; 23% of participants were followed >4 years. Selected baseline characteristics were 26.8% female, 30.1% Black, 8.3% Asian, 44.5% White, 13.6% Hispanic, 31.9% current cigarette smokers; 54% of participants are from low-middle income countries; median (IQR) for age 36 (29-44), entry CD4 651 (584-765), entry HIV viral load 12,759 (3019-43,391). The composite primary end point occurred in 42 participants in the Immediate Group and in 96 in the Deferred Group hazard ratio [immediate vs. deferred] = 0.43 (95% CI, 0.30–0.62; p <0.001). Of the 138 primary endpoints, 53 were cancer (26 AIDS-related and 27 non-AIDS-related). Those with cancer had a median baseline age of 46 [IQR 34-53]. The hazard ratio for cancer (combining AIDS and non-AIDS cancers) in the Immediate vs. Deferred Group was 0.36 (95% CI, 0.19 to 0.66; P = 0.001); more pronounced for Kaposi's sarcoma and malignant lymphoma than for the non-AIDS cancers (Table 1). Forty of 53 (75.5%) of cancers occurred in participants with CD4 >500 cells/μL (73.1% and 77.8% of AIDS- and Non-AIDS-defining, respectively). Twenty-three of 53 (43.3%) occurred in current smokers (at baseline). Thirty-four of 53 (64.1%) of all cancer events and 22 of the 27 (81.5%) non-AIDS cancers occurred in high-income countries and regions.

The non-AIDS cancers are in Table 2.

Conclusions: Early initiation of ART decreased both AIDS and non-AIDS-related cancers, with a larger effect seen in AIDS-related cancers. Immediate initiation of ART reduces the risk of cancers that are thought to and not thought to be induced by pro-oncogenic viruses.

Table 1. Cancers as Part of Primary Endpoint

	# of events Immediate Group N=2326	# of events Deferred Group N=2359	Hazard Ratio (95% Confidence Interval)	P-value
Cancer, total[1]	14	39	0.36 (0.19–0.66)	0.001
Non-AIDS cancer	9	18	0.50 (0.22–1.11)	0.09
Kaposi's	1	11	0.09 (0.01–0.71)	0.02
Lymphoma	3	10	0.30 (0.08–1.10)	0.07
[1]Cervical cancer in 1 participant in the Immediate Group.				

Table 2. Non-AIDS Cancers

	Immediate Group	Deferred Group
Cancer, non-AIDS	9	18
Anal cancer	1	2
Lung cancer	2	2
Prostate cancer	2	3
Other non-AIDS cancer[2]	4	11
[2]Other non-AIDS cancers: *Immediate Group:* squamous cell carcinoma, fibrosarcoma, plasma cell myeloma, bladder cancer. *Deferred Group:* breast cancer, thyroid cancer, ureteric cancer, myeloid leukemia, gastric adenocarcinoma, malignant melanoma, testicular cancer (2), leiomyosarcoma, liver cancer, squamous cell carcinoma of head and neck.		

O5. Long-Term Viral Suppression Predicts Lower Cancer Incidence Among HIV-Infected Veterans, but Higher Than Among Uninfected

Lesley Park[1], Janet Tate[2,3], Amy Justice[2,3], Robert Dubrow[3,4]

[1]Stanford University School of Medicine, Stanford, California, USA; [2]Yale School of Medicine, New Haven, Connecticut, USA; [3]Veterans Affairs Connecticut Healthcare System, West Haven, Connecticut, USA; [4]Yale School of Public Health, New Haven, Connecticut, USA

Background: Viral suppression is a primary marker of HIV treatment success associated with partial reversal of HIV-associated immunodeficiency and decreased mortality. It remains unclear whether increased risks for AIDS-defining cancers (ADC) and several non-AIDS-defining cancers (NADC) persist among HIV-infected (HIV+) patients with long-term viral suppression. We hypothesized that HIV+ subjects with long-term suppression would have lower cancer incidence than unsuppressed subjects, but suppressed HIV+ subjects would have higher cancer incidence rates than demographically similar uninfected subjects.

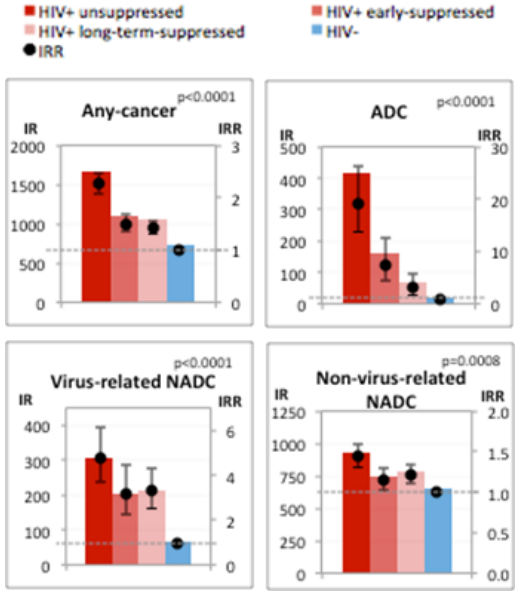


Figure 1. Cancer group standardized incidence rates (bars; IR, per 100,000 person-years) and incidence rate ratios (IRR) with 95% confidence intervals by viral suppression status and p-value for viral suppression trend from 1997-2012.

Methods: The Veterans Aging Cohort Study (VACS) is a prospective, open cohort of HIV+ veterans in medical care in the United States, matched 1:2 by sex, age, race/ethnicity, and clinic site to uninfected veterans. We linked VACS to the VA Central Cancer Registry to identify incident cancer cases. For each subject, we identified the first diagnosis during follow-up of any-cancer (combined), cancer groups (ADC, virus-related NADC, non-virus-related NADC), and specific cancer types. We calculated age-, race-, sex-, and calendar-period-standardized incidence rates (IR) and incidence rate ratios (IRR, HIV+ versus uninfected) by viral suppression status. We classified each day of observation time per subject into the following categories: *unsuppressed* (person-time with HIV RNA ≥ 500 copies/mL), *early-suppressed* (initial 2 years of person-time with HIV RNA < 500 copies/mL), *long-term-suppressed* (person-time after early suppression with HIV RNA <500 copies/mL), and *uninfected in regular VA care* (person-time with at least one inpatient or outpatient visit every 1.5 years for uninfected patients).

Results: For any-cancer, there was a significant decreasing IR trend by viral suppression status (p<0.0001). Compared to uninfected, IRRs were unsuppressed = 2.26 (95% confidence interval [CI]: 2.07-2.46); early-suppressed = 1.50 (95% CI: 1.34-1.68); and long-term-suppressed = 1.43 (95% CI, 1.31-1.56). We found similar trends for ADC (grouped), NADC (grouped), and the following specific cancer types: non-Hodgkin lymphoma, Kaposi sarcoma, Hodgkin lymphoma, multiple myeloma, leukemia, and anal, stomach, larynx, and lung cancers. The unsuppressed had a lower prostate cancer incidence rate than the long-term suppressed. After adjusting for time-updated CD4 count, IRRs were attenuated, but significant viral suppression trends persisted in all cancer groups and several specific cancer types.

Conclusions: Long-term viral suppression was associated with lower cancer incidence among HIV+, suggesting a potential cancer prevention effect of antiretroviral therapy, although cancer risk remained elevated compared with uninfected. Future analyses should examine the effect of lower viral suppression thresholds and further explore the mediating or confounding effects of CD4 count.

O6. Risk of Kaposi Sarcoma in HIV-Positive Adults on ART: A Global Analysis

Julia Bohlius[1], Eliane Rohner[1], Lukas Bütikofer[1], Mhairi Maskew[2], Yi-Ming A. Chen[3], Valeria Fink[4], Chad Achenbach[5], Matthias Egger[1] for International Epidemiologic Databases to Evaluate AIDS (IeDEA) and the Collaboration of Observational HIV Epidemiological Research in Europe (COHERE)

[1]University of Bern, Switzerland; [2]University of Witwatersrand, South Africa; [3]Kaohsiung Medical University, Taiwan; [4]Fundacion Huesped, Argentina; [5]Northwestern University, Chicago, Illinois, USA

Background: Kaposi sarcoma (KS) is a frequent cancer in HIV-positive adults, but no comparisons of KS risk are available between regions with different prevalence of HIV and HHV-8 infection. We did a multi-regional analysis to examine the risk of incident KS among patients on combination antiretroviral therapy (ART) in IeDEA and COHERE.

Methods: We included HIV-positive adults (≥16 years) who started ART after enrollment from 1996 onwards. We compared the risk of incident KS between regions using flexible parametric survival models with region-specific baseline hazards. In multivariable analyses, we adjusted for age, sex, and its interaction with region, time-updated CD4 counts, and year of ART initiation. We excluded the region Asia and Australia from multivariable analyses due to small sample sizes. We present hazard ratios (HR) and 95% confidence intervals (CI) by time since ART start (Figure) and at 2 years after ART initiation.

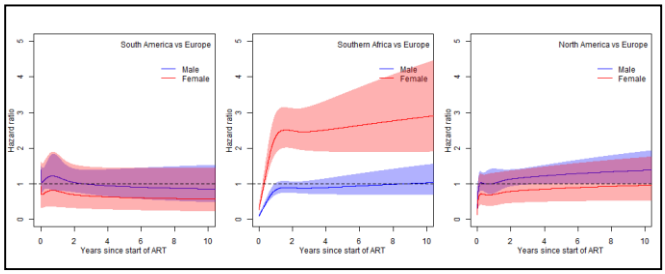


Figure. Hazard ratios and 95% confidence intervals for the risk of developing Kaposi sarcoma in different regions adjusted for time-updated CD4 cell counts, age, sex, and its interaction with region, year of ART initiation.

Results: We included 352,013 patients from 59 countries (Table). Median age at ART initiation was 36.3 years and similar across regions. Median CD4 cell counts at ART initiation were <200 cells/µL in Asia, Southern Africa, and South America, and >200 cells/µL in Australia, Europe, and North America. The proportion of males and the subset who have sex with men (MSM) was highest in Australia, followed by North and South America and Europe.

Table. Patient Characteristics at ART Initiation Stratified by Region

	Asia	Australia	South America	Southern Africa	North America	Europe
n	2,626	343	8,359	165,255	16,538	158,892
Median age	36.3	41.7	35.6	35.1	39.6	37.3
Median CD4	138	285	165	139	234	250
Male	69%	92%	74%	37%	75%	72%
MSM	36%	70%	57%	Not reported	67%	54%

During 1.3 million person-years (pys) 2,935 patients developed KS for an overall incidence rate of 199/100,000 pys (95% CI 192-207). KS incidence was highest in the first months after starting ART and declined thereafter. *Results at two years after starting ART:* Women in Southern Africa had an increased risk of developing KS compared to women in Europe in crude (HR 2.85, 95% CI 2.30-3.55) and adjusted analysis (2.49, 95% CI 1.99-3.12). KS risk was similar in women in South America, North America, and Europe. In crude analysis the risk of developing KS was highest in men in North America compared to Europe (HR 1.54, 95% CI 1.26-1.90); in multivariable analyses this risk declined to HR 1.14 (95% CI 0.93-1.40). The change was mainly explained by adjusting for time-updated CD4 cell counts. The Figure shows that the KS risk was similar in men from South America and Southern Africa compared to Europe.

Conclusions: Women in Southern Africa had an increased risk of developing KS compared to women in Europe which was not explained by HIV-related risk factors. In men the risk of developing KS was similar across regions after adjusting for HIV-related risk factors. This pattern likely reflects different HHV-8 risk profiles: while men were at high risk of HHV-8 infection in most regions (MSM or resident in HHV-8 endemic regions) the main risk factor for HHV-8 infection in women was residence in endemic regions. Migration data were not available for all regions and hence not considered in the analysis.

O7. Determinants of Shedding of Kaposi Sarcoma-Associated Herpesvirus in Saliva Among Ugandan Mothers and Children

Vickie Marshall[1], Robert Newton[2,3,4], Katie Wakeham[5], Romin Roshan[1], Angela Nalwoga[2], Lawrence Muhangi[2], Wendell Miley[1], Elena M. Cornejo Castro[1], Nazzarena Labo[1], Alison Elliott[2,6], Denise Whitby[1]

[1]Viral Oncology Section, AIDS and Cancer Virus Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, Maryland, USA; [2]Medical Research Council/Uganda Virus Research Institute, Uganda Research Unit on AIDS, Entebbe, Uganda; [3]Department of Health Sciences, University of York, York, United Kingdom; [4]International Agency for Research on Cancer, Lyon, France; [5]Institute of Cancer Sciences, University of Glasgow, Glasgow, United Kingdom; [6]London School of Hygiene & Tropical Medicine, London, United Kingdom

Background: After establishing latent infection, KSHV undergoes occasional reactivation and is shed in saliva. Studies of saliva shedding are therefore a fundamental tool to investigate viral replication and transmission.

Methods: The Entebbe Mother and Baby study (EMaBS) is an ongoing longitudinal birth cohort study following over 2000 pregnant women in Uganda. To identify the determinants of shedding of KSHV in saliva, we systematically recruited 563 of the mothers and their 571 six-year-old children. KSHV seroprevalence was determined using ELISAs detecting anti-K8.1 and anti-ORF73 antibodies; saliva viral load was measured using quantitative real time PCR.

Results: We tested 303 seropositive mothers and 115 seropositive children; in addition, 84 seronegative mothers were tested. Virus was detected in the saliva of 70 (18%) mothers and 42 (37%) children. The median viral load was 8 times higher among children than among mothers (P=0.009), but did not differ significantly by sex of the child. Among mothers, detection of KSHV DNA increased with increasing levels of antibodies; this was more marked for anti-K8.1 than for anti-ORF73. Interestingly, KSHV DNA was detected more frequently in the saliva of mothers with anemia, although this was not significant (p=0.078). Among children, KSHV was detected more often from boys than from girls (48% versus 25%; P=0.02).

Conclusions: The observed association between increasing antibody levels and KSHV viral shedding provides important corroboration for the hypothesis that high antibody levels, especially to K8.1, are a marker for KSHV reactivation. Moreover, the high viral load observed in children's saliva and the higher shedding frequency in boys may offer insight on virus transmission and viral pathogenesis.

O8. Kaposi Sarcoma in HIV-Infected Children and Adolescents in Central Malawi: A Novel Clinical Staging Classification Determines Risk Stratification

Nader Kim El-Mallawany[1,2], William Kamiyango[3], Jimmy Villiera[3], Carrie Kovarik[4], Dale Frank[4], Anthony Eason[5], Michael E. Scheurer[2,6], Peter N. Kazembe[3], Dirk P. Dittmer[5], Parth S. Mehta[2,6]

[1]New York Medical College, Valhalla, New York, USA; [2]Baylor College of Medicine, Houston, Texas, USA; [3]Baylor College of Medicine Children's Foundation Malawi, Lilongwe, Malawi; [4]University of Pennsylvania, Philadelphia, Pennsylvania, USA; [5]Lineberger Comprehensive Cancer Center, The University of North Carolina at Chapel Hill, North Carolina, USA; [6]Texas Children's Cancer and Hematology Centers, Houston, Texas, USA

Background: Uldrick et al. first described the Kaposi sarcoma (KS) inflammatory cytokine syndrome (KICS) in HIV-infected adults in 2010. KICS is characterized by high levels of human interleukin-6 (IL-6), viral IL-6, and human herpesvirus-8 (HHV-8) in KS patients in the absence of multicentric Castleman disease (MCD). IL-6 is related to the pathophysiology of HHV-8 in many ways. KICS or extreme IL-6 levels have not been described in KS in children or in Africa. Here we describe our pediatric KICS experience in Lilongwe, Malawi.

Methods: We retrospectively analyzed 6 HIV-infected children diagnosed with KS between August 2010 and June 2013 in Lilongwe that fit the clinical presentation of KICS. Local first-line chemotherapy included bleomycin and vincristine (BV). HAART was prescribed according to national guidelines with nevirapine-based first line regimens. We compared patients that fit the KICS definition to the overall cohort of pediatric KS patients, and because of the overlap in clinical features, we compared them to a sub-group of children with lymphadenopathic KS (LN KS) as well.

Results: There were 3 females and 3 males. All 6 presented with the following constellation of clinical findings: (1) bulging lymphadenopathy, (2) persistent fevers, (3) massive hepatosplenomegaly extending to the umbilicus, and (4) severe cytopenias. Concurrent findings of hyperpigmented KS skin lesions were present in 2 patients, non-hyperpigmented subcutaneous nodules in 3, and facial edema in one. KICS patients demonstrated a lower median age (3.5 years, range 2.2–6.2), than the overall pediatric KS cohort (9.0 years, range 1.7-17.9, p=0.0024) and LN KS sub-group (6.9 years, range 1.7-17.9, p=0.0274). KICS patients presented with a lower median hemoglobin (5.1 g/dL, range 4–6.2 g/dL) than overall KS (9.3 g/dL, range 1.8-13.6 g/dL, p=0.0004) and LN KS (9.5 g/dL, range 1.8-13.6 g/dL, p=0.0008), and a lower median platelet count as well (13 x 10^9/L, range 6–24) compared to overall KS (216 x 10^9/L, range 7-672, p=0.0002) and LN KS (204 x 10^9/L, range 7-672, p=0.0004). Due to the limited availability of platelet transfusions for these patients presenting with severe thrombocytopenia, lymph node biopsy was only obtained in one patient; it revealed KS histology without evidence of MCD (spindle cell infiltrate with immunohistochemical stains positive for HHV-8 LANA and CD31, negative for CD20). Virologic testing was obtained for one patient and was characteristic of KICS, revealing HHV-8 viral load of 1.9 x 10^4 copies/10^6 cells, IL-6 level 450 pg/mL, and interleukin-10 level 320 pg/mL. Failure to respond to BV was apparent, so prednisone was added to treat the systemic inflammatory syndrome. Cytopenias dramatically improved 2 weeks after starting prednisone plus BV, with median platelets reaching 587 (range 148–789) without transfusion. Four patients achieved long-term complete remission with median follow-up for survivors of 44 months (range 31-50). Two patients died early (1 and 3 weeks after presentation) due to complications of other opportunistic infections (severe pneumonia and sepsis).

Conclusions: This case series describes a unique clinical presentation of IL-6 related KS cases in HIV-infected children in an HHV-8 endemic region. It is distinctly different than both pediatric KS in general, as well as the lymphadenopathic subtype of pediatric KS, despite the overlap in clinical features with LN KS. Analogous to adult KICS the clinical features are driven by inflammatory cytokines. Long-term cures in a low resource setting could be achieved by adding prednisone to BV plus HAART.

O9. Comprehensive RNAseq Analysis of KSHV Gene Expression in Kaposi Sarcoma Tumors and Correlation With In Vitro Infection Models

Emilia Gan[1], Warren Phipps[2,3], Serge Barcy[4], Michael Lagunoff[5], Gregory Bruce[6], Matt Fitzgibbon[3], Corey Casper[1,2,3,7], Timothy Rose[4,6]

Departments of [1]Global Health, [2]Medicine, [4]Pediatrics, [5]Microbiology, and [7]Epidemiology, University of Washington; [3]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center,[6]Center for Global Infectious Disease Research, Seattle Children's Research Institute, Seattle, Washington, USA

Background: The development of next-generation RNAseq-based transcriptome analysis has enabled an unbiased approach to compare the global expression patterns of RNA transcripts in culture systems in vitro and tissues in vivo. Targeted studies defined a basic gene expression profile for KSHV latency in infected culture cells and reactivation using protein and chemical induction. Previously, we characterized KSHV gene expression in Kaposi sarcoma (KS) tumors of Ugandan adults using RNAseq analysis. Here, we compare the KS transcript profiles with those obtained from in vitro culture models of KSHV latency and reactivation to further our understanding of the molecular genetics of KSHV infection.

Methods: As shown previously, KS biopsy specimens were obtained from treatment-naïve HIV-infected adults with histologically confirmed KS initiating therapy at the Uganda Cancer Institute, and RNA was isolated. For in vitro infections, gradient-purified KSHV virions were used to infect cell cultures and RNA was extracted 48 hours post-infection. Poly-A selected KSHV mRNA transcripts were quantified using RNA-Seq.

Results: 45 KS biopsies were obtained from 34 HIV-positive individuals. 80-120 million paired-end RNA reads were obtained from each tumor, with 1,000-400,000 reads mapping to the KSHV reference NC_009333. Six KS tumors were analyzed by stranded sequencing to determine directionality of RNA transcripts. Phylogenetic analysis of ORF75 sequences revealed at least 10 different KSHV genetic clusters. RNA reads from the largest cluster (10 individuals) were assembled to obtain the nearly complete genome sequence (98.3%) of a Ugandan KSHV strain, 23B. RNA reads mapped to the NC_009333 and 23B genomes showed close congruence. RNAseq reads were also obtained from typical latent KSHV infections (48 pi) in blood and lymphatic endothelial cells in vitro, with less than 3% of cells expressing the lytic marker ORF59. This level of "spontaneous reactivation" is similar to that generally seen in KS tumors. Quantitation of RNA reads in the cell lines revealed a reproducible transcript profile across the whole genome, consistent with wide-scale lytic cascade of gene expression in the infected cultures in the absence of artificial chemical inducers. A detailed genome-wide transcript map and annotations were developed for novel genes and transcripts, and a system was devised to quantitate RNA reads without errors due to overlapping transcripts. No evidence for a complete lytic gene cascade was observed in the RNA reads from the KS tumors, although specific "lytic" genes, such as ORFs K2 (vIL6), K5 (MIR2), K8 (BZIP), 57, 58, 59, and 65, and T1.1 (PAN) RNA were consistently expressed at elevated though variable levels, as previously shown. Higher levels of RNA reads were detected across the latency region, including ORFs K12 (Kaposin), 71 (vFLIP), 72 (vCYC), 75 (FGARAT), and K15. Minimal RNA reads mapped to ORFs 50 (RTA), K8.1, 73 (LANA), K14 (vOX2), and 74 (vGPCR).

Conclusions: Global transcriptome analysis of KSHV infections in KS tumors and in vitro infection models provided important insights into the genomic architecture and gene expression control of KSHV. Comparison between KS tumor biopsies and in vitro tissue culture models revealed important differences in overall KSHV gene expression profiles. Although RNAseq analysis does not identify the cells responsible for the observed RNA transcripts, it provides an overall assessment of gene expression in the study sample and allows a targeted approach to examine the contributions of specific KSHV genes in the viral life cycle and pathological effects in the KS tumor lesions.

O10. KSHV Induces Revision of B Cell Receptors

Jennifer E. Totonchy, Amy Chadburn, Jessica M. Osborn, Geoffrey Mikita, Ethel Cesarman

Pathology and Laboratory Medicine, Weill Cornell Medical College, New York, New York, USA

Background: KSHV infection of B lymphocytes is almost exclusively restricted to lambda light chain (IgL) expressing cells both in vivo[1] and in vitro[2] and kappa light chain (IgK) cells that are KSHV positive are almost never observed. Interestingly, transgenic mice expressing KSHV-vFLIP in B lymphocytes display increased numbers of IgL positive cells.[3] This specific association of KSHV biology with IgL has been a longstanding conundrum in the field given that IgL and IgK lymphocytes should be physiologically indistinguishable.

Methods: In order to explore early pathogenic events during KSHV infection of lymphocytes, we developed a model using de novo infection of primary Naïve B lymphocytes from human tonsil with cell free BAC16 recombinant KSHV derived from iSLK. Naïve cells are separated from total tonsil lymphocytes by either flow or magnetic sorting techniques. Infected B cells are cultured on irradiated feeder cells expressing FcYR2 (CDW32) in order to preserve their pre-infection phenotype long term in culture. At various times post-infection, we observe phenotypic markers by flow cytometry, genomic markers by PCR, and gene expression by RT-PCR. We correlate in vitro findings with examination of in vivo data from AIDS-lymphadenopathy.

Results: We observe a variety of phenotypic changes in KSHV-infected primary Naïve B lymphocytes, many of which are maturation markers reminiscent of the phenotype of KHSV-infected cells in MCD lymph nodes in vivo. Although at early stages of infection we observe equal infection of B cells bearing immunoglobulin lambda (IgL) and kappa (IgK) light chains, the IgK positive cells are lost over time in the infected cultures. Experiments in which IgK and IgL Naïve cells were sorted and infected separately reveal that KSHV infection induces IgK B cells to become IgL positive via a IgL/IgK double-positive intermediate. Moreover, polyclonal IgL genomic rearrangements are observed in KSHV-infected IgK B cells by PCR. Consistent with these results, we observe that KSHV-infected cells in lymph node biopsies from AIDS patients are uniformly IgL positive, whether these have histological features of MCD or not.

Conclusions: Our in vivo data demonstrate that IgL restriction exists in KSHV-infected B cells in non-MCD AIDS lymphadenopathy, suggesting that IgL bias is a general feature of KSHV replication in B lymphocytes rather than a disease-specific manifestation. Moreover, our in vitro data demonstrate the replacement of IgK light chains with IgL upon KSHV infection, a process called B cell receptor (BCR) revision, providing a new and intriguing explanation for IgL restriction in KSHV infection. The potential implications of these results are far-reaching. BCR revision is associated with the induction of autoimmunity,[4,5] and autoimmunity, in turn, is implicated as a significant driver of lymphomagenesis.[6,7] Thus, our results provide a framework in which KSHV replication in the lymphocyte compartment could create a pro-tumorigenic autoimmune state via BCR revision.

O11. The cryoEM Structure and Structure-Guided Mutageneses of KSHV Capsid

Xinghong Dai[1,2], Danyang Gong[1], Ting-Ting Wu[1], Z. Hong Zhou[2,3], Ren Sun[1,3]

[1]Department of Molecular and Medical Pharmacology, [2]Department of Microbiology, Immunology, and Molecular Genetics, [3]California NanoSystems Institute, University of California, Los Angeles, Los Angeles, California, USA

Background: Kaposi's sarcoma-associated herpesvirus (KSHV) is the etiologic agent of Kaposi's sarcoma and several other malignancies commonly occurring in AIDS patients. Assembling capsid and packaging genome to produce progeny virus is a key step in the cycle of KSHV infection and transmission. The KSHV capsid, or the capsid of any herpesvirus in general, has to endure a pressure exerted by its dsDNA genome as high as tens of atmospheres [Bauer et al. 2013, *J Am Chem Soc* no. 135 (30):11216-21]. Therefore, a small defect introduced into the delicate capsid might produce a lethal effect on the virus. Understanding the capsid structure and identifying key structural elements for capsid stabilization are very likely beneficial to future design of antiviral drugs targeting the capsid or vaccines based on modification of the viral capsid.

Methods and Results: Using the state of the art cryo electron microscopy (cryoEM) techniques, we solved the structure of KSHV capsid to 6 angstrom resolution [Dai et al. 2015, PNAS 112 (7):E649-E656]. Functional analyses, based on the structural information, have demonstrated that the smallest capsid protein (SCP) of KSHV uses a kinked helix to cross-link neighboring major capsid protein (MCP) subunits to reenforce the capsid for genome packaging. Recently we are making progress to improve the resolution to see more details. Based on our recent analyses of the vast interactions among the four capsid proteins guided by the cryoEM structure, we predicted segments of capsid proteins that are potentially crucial for the stability of the entire capsid. Furthermore, we carried out structure-guided mutageneses to verify the critical role of these segments. For example, we predicted that the very end of the N-terminal region of KSHV MCP (ORF25) is involved in binding neighboring hexons together; the N-terminal region of the triplex protein Tri1 (ORF62) adopts a "triskelion" shape to hook the triplexes on the capsid floor formed by MCPs, and seal the holes of the capsid floor. Serial truncations of the MCP or Tri1 in their N-terminals were shown to be lethal mutations by affecting the capsid assembly and/or genome containment, verifying our structure-based predictions. More structure-guided mutageneses will be performed in the future.

Conclusions: With the structural information, we are able to identify the sequences/domains essential for the assembly or stability of the capsid. We believe our cryoEM reconstruction of the KSHV capsid and structure-based mutagenesis studies are helpful to further understanding of herpesvirus capsid assembly and informative to capsid-based design of therapeutics, vaccines, and research tools.

O12. Modeling of the KSHV Episome-Host Chromatin Synapse by Super-Resolution Localization Microscopy

Margaret J. Grant[1], Thomas J. Ellison[1], M. Mitchell Smith[1], Dean H. Kedes[1,2]

[1]Myles H. Thaler Center for AIDS and Human Retrovirus Research, Department of Microbiology, Immunology, and Cancer Biology, University of Virginia, Charlottesville, Virginia, USA; [2]Department of Medicine, Division of Infectious Diseases, University of Virginia, Charlottesville, Virginia USA

Background: Stable infection with herpesviruses such as EBV and KSHV depends on the ability to tether their episome to the host chromatin to prevent dilution over serial cell divisions. For KSHV, this tether comprises a series of homodimers of the virally encoded latency-associated nuclear antigen (LANA). These dimers bind with their C-termini to specific juxtaposed sequences (LBS1, 2 and 3) in the terminal repeat (TR) region of the viral episome and with their N-termini to an acidic patch in host nucleosomes. In standard epifluorescence microscopy, LANA tethers appear as nuclear punctae that correlate tightly with the number of episomal copies; however, light microscopy is unable to resolve the structural details of individual tethers.

Methods: We have applied direct Stochastic Optical Reconstruction Microscopy (dSTORM) to investigate the structure of the LANA tether on latent KSHV episomes at nanoscale resolution in B cell (BCBL-1) as well as epithelial cell (SLKp/Caki-1p) lines stably infected with the virus in its latent form. Specifically, we investigated the nature of the tether during interphase as well as mitosis, a time when efficient partitioning is crucial for viral episome persistence.

Results: Using two different LANA antibodies – one against an N terminal epitope and one against the central repeat region – we measured a longer radius of gyration for the N-terminal antibody, suggesting a model in which the LANA proteins assume a radial arrangement with respect to the underlying TR DNA axis. Furthermore, data from SLKp/Caki-1p suggest that the tether undergoes an ~4-fold condensation during mitosis. Finally, in preliminary data, we have found that a subset of LANA tethers is curvilinear but that the overall volume of interphase tethers within each cell type, regardless of shape, is remarkably similar.

Conclusions: The location of individual LANA proteins within the viral episome-host chromatin tether suggests a possible radial distribution of LANA dimers along the TR DNA axis. In mitotic SLKp/Caki-1p cells, the tethers undergo a marked decrease in volume, suggesting condensation that parallels that of host chromatin. Furthermore, a subset of the tethers assumes a curvature consistent with potential contacts with an underlying 400nm (condensed) metaphase chromosome. Currently, we are determining the spatial relationship between the LANA-bound TR region and the remainder of the episome.

O13. Benefits and Harms of Lung Cancer Screening in HIV-Infected Individuals: A Simulation Study

Chung Yin Kong[1], Keith Sigel[2], R. Scott Braithwaite[3], Juan Wisnivesky[2], Kristina Crothers[4]

[1]Institute for Technology Assessment, Massachusetts General Hospital, Massachusetts, USA; [2]Department of Medicine, Mount Sinai School of Medicine, New York, New York, USA; [3]Department of Population Health, New York University School of Medicine, New York, New York USA; [4]Department of Medicine, University of Washington School of Medicine, Seattle, Washington, USA

Background: Lung cancer is the leading cause of non-AIDS defining cancer (NADC) deaths among HIV-infected (HIV+) individuals. Although lung cancer screening with computed tomography (CT) has been endorsed by both the U.S. Preventive Services Task Force (USPSTF) and the U.S. Centers for Medicare & Medicaid Services (CMS), the benefits and harms of lung cancer screening among HIV+ individuals remain unclear. In this study, we extended and applied a comprehensive simulation model, the Lung Cancer Policy Model (LCPM), to estimate these benefits and harms.

Methods: We created a version of the LCPM tailored to HIV+ individuals using data from literature and the Veterans Aging Cohort Study (VACS) and incorporating long-term relationships between adherence and HIV-mortality from a validated HIV simulation. The LCPM was then used to estimate the lung cancer specific life-years gained and the number of CT exams performed to screen HIV+ individuals with varied smoking histories, CD4 counts, and antiretroviral (ART) adherence. We first compared health outcomes of a no screening scenario to outcomes of a screening scenario using eligibility criteria established by the CMS (55-77 years of age and ≥30 pack-year smoking history, ≤15 years since quitting). We then varied the screening eligibility criteria to identify an optimal screening strategy for HIV+ patients.

Results: Our Results indicated that HIV+ patients benefit from lung cancer screening. For HIV+ current smokers with 100% ART adherence, screening can provide 0.081-0.101 life-years gained using CMS recommendations (Table 1). For HIV+ former smokers, screening provides larger life-years gained (0.112-0.118) because former smokers have longer life expectancy. Our results indicated that lowering the screening age-span from 55-77 to 50-72 can increase life-years gained but would require 13% to 41% more CT screening exams. Additional results also showed that HIV+ patients with CD4 counts of 200-500 will still benefit from screening but will have smaller life-year gains compared to patients with CD4 >500. Among all scenarios examined, the lung cancer specific mortality reduction from screening ranges from 15.6% to 23.2%, which is comparable to the 20.0% mortality reduction observed among HIV- patients in the National Lung Screening Trial [1]. Improving adherence to ART from 50% to 100% increased overall life-expectancy by 2.2-3.5 years, further enhancing the benefit from screening.

Table 1. Health Outcomes of HIV+ Patients Who Underwent Lung Cancer Screening (100% ART Adherence)

	Using CMS recommendations				**Lowering screening age-span to 50-72**			
Smoking status	Former		Current		Former		Current	
CD4 count	200-500	>500	200-500	>500	200-500	>500	200-500	>500
Life expectancy	71.87	72.71	65.82	67.57	71.91	72.74	65.84	67.59
Life-years gained	0.112	0.118	0.081	0.101	0.148	0.155	0.095	0.116
# of screening CT exams per 100K	1.12E+06	1.16E+06	1.04E+06	1.16E+06	1.59E+06	1.63E+06	1.41E+06	1.51E+06

Conclusions: Results from simulation modeling showed that lung cancer screening with CT can provide the HIV+ population with a mortality reduction benefit similar to that of HIV- patients. However, the optimal benefit occurs when screening is both begun and stopped at younger ages than it is in the HIV- population, due to a combination of elevated lung cancer risk and shorter life-expectancy among HIV+ patients.

O14. Lung Cancer Screening and Smoking Behavior in HIV+ Smokers

Keith Sigel[1], Taylor Zuber[1], Matthew Silverstein[1], Kristina Crothers[2], Chung Yin Kong[3], R. Scott Braithwaite[4], Jack E. Burkhalter[5], Juan Wisnivesky[1]

[1]Department of Medicine, Mount Sinai School of Medicine, New York, New York, USA; [2]Department of Medicine, University of Washington School of Medicine, Seattle, Washington, USA; [3]Institute for Technology Assessment, Massachusetts General Hospital, Massachusetts, USA; [4]Department of Population Health, New York University School of Medicine, New York, New York, USA; [5]Memorial Sloan Kettering Cancer Center, New York, New York, USA

Background: HIV-infected (HIV+) persons have a higher risk of lung cancer compared to uninfected persons, partially due to a higher prevalence of smoking in the HIV+ population. Lung cancer screening with computed tomography (CT) has been proposed in high-risk HIV+ persons, but there are no data regarding the impact of screening on smoking behaviors in this group. Studies of lung cancer screening and smoking in uninfected persons have shown annual tobacco quit rates of 7%-23%, higher than usual quit rates of 2%-3%.[1] In this study, we prospectively evaluated smoking behaviors and attitudes in HIV+ persons before and after lung cancer screening with low-dose CT.

Methods: We recruited 60 HIV+ persons for lung cancer screening with low dose CT who met the following criteria: age ≥50 years, current or former (quit <15 years) smoker with ≥25 pack-years smoking history and recent CD4 count >200 cells/mm^3. Radiologists' interpretations of the screening CTs were reviewed to identify scans with nodules that required additional evaluation ("positive scans"). Subjects were surveyed about smoking behaviors prior to screening and then current smokers were surveyed again 3 months after screening. Baseline attitudes towards smoking were assessed using validated instruments including a nicotine dependence scale and a smoking cessation self-efficacy scale. We then compared baseline characteristics between smokers who decreased their smoking quantity and those who did not.

Results: In our screening cohort (n=60), 37 subjects (62%) were current smokers at the time of enrollment. Current smokers had a mean age of 56 years, 51% were Black, and 41% were Latino. Mean pack-year smoking for current smokers was 28 pack-years, and median CD4 count was 636 cells/mm^3. At enrollment, current smokers smoked a mean of 14 cigarettes per day. There was no significant difference in the rate of positive scans by smoking status (11% for current smokers, 4% for former smokers; p=0.4). At 3-month follow-up, 8 (22%; 95% CI: 11-37%) subjects had increased their reported number of cigarettes smoked per day, 9 (24%; 95% CI: 19-47%) had not changed, and 20 (54%; 95% CI: 37-70%) had decreased the number of cigarettes they smoked per day. Two (5%; 95% CI: 1-20%) subjects reported they had quit smoking in the interval. There were no differences in sociodemographics, scan positivity, pack-year smoking exposure, age at smoking initiation, baseline nicotine dependence measures, or domains of smoking cessation self-efficacy measures between persons who decreased their cigarette smoking quantity compared to those who did not (all p-values>0.1). Participants who decreased their smoking were more likely to have had a previous quit attempt compared to those who did not decrease their smoking (72% versus 35%; p-value=0.02).

Conclusions: HIV+ smokers demonstrated smoking reductions after lung cancer screening that were broadly consistent with those observed in studies of uninfected smokers. Presence of a prior quit attempt may identify persons with higher likelihood of smoking behavior change. Our study was limited by self-reported smoking measures. The utility of smoking cessation in the setting of lung cancer screening should be further evaluated in HIV+ smokers.

O15. Characteristics of HIV+ Lung Cancer Patients Undergoing Surgical Resection at an Urban Center With High HIV Prevalence: Implications for Lung Cancer Screening

Vipul Pareek[1], Marina Shcherba[1], H. Dean Hosgood[2], Mindy Ginsberg[2], Linda Fisher[3], Kevin Wilson[3], Julie Chung[3], David B. Hanna[2], Uriel R. Felsen[1], Juan Lin[2], Xiaonan Xue[2], Simon Spivack[1], Steven M. Keller[4], Kathryn Anastos[1], Howard D. Strickler[2], Missak Haigentz, Jr.[1]

[1]Department of Medicine, Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, New York, USA; [2]Department of Epidemiology & Population Health, Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, New York, USA; [3]Health Information Management, Montefiore Medical Center, Bronx, New York, USA; [4]Department of Cardiovascular and Thoracic Surgery, Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, New York, USA

Background: Lung cancer (LCa) is the most common cause of non-AIDS defining cancer death among persons with HIV infection, and screening efforts to detect early stage disease for which curative therapy can be directed may hold great promise for this high-risk population. In the pre-LCa screening era, however, few HIV+ pts underwent curative surgery. To understand the potential impact of surgical resection of LCa among HIV+ pts, we describe the clinical characteristics and outcomes of patients (pts) who underwent complete resection of LCa in the pre-screening era.

Methods: The Cancer Registry (42,967 cancer cases) and the HIV Clinical Core Database (14,936 HIV+s) at Montefiore Medical Center – Einstein (Bronx, New York) were linked to identify HIV+ cancer cases between 2000 and 2012. Chart review was performed for those with LCa.

Results: LCa, diagnosed in 90 out of 935 cancers (10%) in HIV+s, was the most common non-AIDS defining cancer. 14 LCa pts (15% of total LCa; 8M/6F; including all 6 pts with clinical stage I disease and 5 of 9 pts with clinical stage II disease) underwent curative surgical resections. Median age (range) of completely resected pts was 55 (47-72) years; all but one were smokers, with median history of 30 (12-80) pack years. All pts were on antiretrovirals prior to LCa diagnosis, with median CD4+ 266.5 (23-1480) cells/μL; 5 pts had CD4 <200. Histological types were squamous in 6 pts, adenocarcinoma in 5 pts, large cell in 2 pts, and adenosquamous in 1 patient. Pathological staging revealed stage I in 6 pts, stage II in 5 pts, and stage III in 3 pts. Median survival (range) by stage grouping: I, 58.5 (7-126+) mo; II, 63.5 (52-78+) mo; III, 17 (2-70+) mo.

Conclusions: Surgical outcomes for LCa appeared comparable to the general population, with many pts (including those with evidence of severe immunosuppression) experiencing long-term survival. HIV+ LCa pts with early stage disease benefit from standard surgical procedures; however, as we previously demonstrated, most HIV+s diagnosed with LCa do not meet current LCa screening guidelines due to younger age or limited smoking histories at diagnosis [J Clin Oncol 32:5s, 2014 (suppl; abstr 1569)]. Given the clinical benefit from surgical resection, efforts for early detection should be directed to make low dose CT screening more applicable to this population with known LCa care disparities.

O16. Homologous Prime-Boost With an HPV-E6/E7 Immunotherapy Plus PD-1 Checkpoint Inhibition Results in Tumor Regression and Reduction in PD-L1 Expression

Adrian E. Rice[1], Yvette Latchman[1], Joseph Balint, Jr.[1], John Lee[2], Elizabeth Gabitzsch[1], Frank Jones[1]

[1]Etubics Corporation, Seattle, Washington, USA; [2]Sanford Cancer Research Center, Sioux Falls, South Dakota, USA

Background: Patients with human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS) have an elevated risk for the acquisition of certain cancers including Kaposi's sarcoma, non-Hodgkin's lymphoma, and human papillomavirus (HPV)-associated malignancies. We have developed an immunotherapy targeting HPV-associated cancers using a viral gene delivery platform to immunize against HPV 16 genes E6 and E7 (Ad5 [E1-, E2b-]-E6/E7).

Methods and Results: Using a homologous prime-boost treatment regimen in pre-clinical studies, we demonstrated that this targeted immunotherapy induced cell-mediated immune (CMI) responses against both E6 and E7 in a dose-specific manner. Intracellular cytokine staining of CD8+ T lymphocytes revealed that these HPV-specific T cells were multifunctional, having expressed high levels of both interferon-γ and tumor necrosis factor alpha. We further investigated tumor growth and survival in HPV-associated cancer and found that immunotherapy using Ad5 [E1-, E2b-]-E6/E7 alone resulted in the complete clearance of small tumors and an overall survival benefit in larger established tumors. When we treated with Ad5 [E1-, E2b-]-E6/E7 in combination with programmed death-ligand 1 (PD-1) blockade, we observed an increased level of anti-tumor activity against large tumors, including the complete clearance of large tumors in some mice and increased overall survival. Analysis of the tumor microenvironment in drug treated mice revealed elevated CD8+ tumor infiltrating lymphocytes (TILs) compared with untreated controls; however, we also observed the induction of suppressive mechanisms such as expression of programmed death-ligand 1 (PD-L1) on tumor cells and an increase in PD-1+ TILs. When Ad5 [E1-, E2b-]-E6/E7 immunotherapy was combined with anti-PD-1 antibody, we observed the same high level of CD8+ TILs but also a reduction in PD-L1 expression on tumor cells and reduced PD-1+ TILs.

Conclusions: These data provide a mechanism by which combination therapy may improve tumor clearance. The FDA has granted us an orphan drug designation for Ad5 [E1-, E2b-]-E6/E7 and we plan to move forward with clinical trials treating patients with HPV+ head and neck squamous cell and cervical carcinoma by pairing Ad5 [E1-, E2b-]-E6/E7 with checkpoint inhibition.

O17. High-Risk Human Papillomavirus Oncoprotein E6 Induces FoxM1 in Human Keratinocytes Through Transcriptional Regulation by Grainyhead-Like 2 (GRHL2)

Wei Chen[1], Tetsu Shamine[1,5], Saaket Varma[1], Ki-Hyuk Shin[1,2,3], Reuben H. Kim[1,2,3], No-Hee Park[1,2,3,4], Mo K. Kang[1,2,3]

[1]School of Dentistry, [2]Dental Research Institute, [3]Jonsson Comprehensive Cancer Center, [4]David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California, USA; [5]Oral and Maxillofacial Surgery, Shinshu University, Matsumoto, Japan

Background: Squamous cell carcinoma (SCC) is disproportionately prevalent in patients infected with human immunodeficiency virus (HIV) and is linked to co-infection with "high-risk" human papillomavirus (HPV).[1,2] Reducing the morbidity and mortality associated with SCCs in HIV+ patients therefore requires better understanding of pathogenesis and disease progression. Forkhead box M1b (FoxM1b) is an oncogene essential for cell cycle progression in human cancers. Our prior study showed that FoxM1b is regulated by Grainyhead-Like 2 (GHRL2),[3] which is epithelial-specific transcription factor involved in carcinogenesis. The purpose of the current study is to elucidate the pathobiological role of GRHL2 and FoxM1b in HPV-associated carcinogenesis.

Methods: Real time-PCR and Western blot assays were exploited to examine the gene expression and protein levels. Chromatin immunoprecipitation (ChIP) and luciferase reporter assays were used to investigate the enrichment of trans-regulators at the FoxM1b promoter resulting in altered promoter activity. Tumorigenic effects of FoxM1b were assessed by tumor spheroid formation and xenograft transplantation in immunocompromised mice. FoxM1 signaling in human oral keratinocytes (HOKs) was determined by microarray analysis using the cells with and without FoxM1b overexpression. K14-HPV 16E6 knock-in mice were used to establish the relationship between HPV viral gene expression and FoxM1 level in vivo.

Results: Our data showed that both GRHL2 and FoxM1 levels were markedly increased in HOKs immortalized with type 16 HPV (HOK-16B) compared with normal human oral keratinocytes (NHOK). We also confirmed induction of GRHL2 and FoxM1b in NHOK infected with HPV virus capable of expressing E6 and E7. When the E6 and E7 viral oncogenes were independently overexpressed in NHOK, GRHL2 and FoxM1b levels were induced by E6 but not E7. Furthermore, expression of GRHL2 and FoxM1b were elevated in epidermis of HPV-16E6 mice compared with the tissues from the control Fvb mice. ChIP and luciferase reporter assay revealed that GRHL2 directly binds the promoter region of FoxM1 for transregulation. Despite induced FoxM1b level in HOK-16B, the cells were non-tumorigenic; further elevation of FoxM1b level by retroviral vector led to acquisition of tumorigenicity in vivo and enhanced sphere formation in vitro. Microarray-based gene expression profiling revealed downstream targets of FoxM1b to include EGFR and HOX-10A signaling pathways in HOKs harboring HPV genome.

Conclusions: These data indicate that HPV 16E6 induces GRHL2 and FoxM1b expression in human keratinocytes and that further enhancement of FoxM1b level led to tumorigenic conversion of HPV-harboring HOKs. Further studies will elucidate the functional role of FoxM1 in maintenance of cancer phenotype and whether GRHL2 and FoxM1 may be effective therapeutic targets of HPV-associated SCCs in HIV+ patients.

This study was funded by the grants from NIDCR/NIH (R01DE18295; K02DE18959; R56DE024593).

References

1. Gami B, Kubba F, Ziprin P. (2014) Human papilloma virus and squamous cell carcinoma of the anus. Clin Med Insights Oncol 8:113-119.
2. Schim van der Loeff MF, Mooij SH, Richel O, de Vries HJ, Prins JM. (2014) HPV and anal cancer in HIV-infected individuals: a review. Curr HIV/AIDS Rep 11:250-262.
3. Chen W, Liu XZ, Oh JE, Shin KH, Kim RH, Jiang M, Park NH, Kang MK. (2012) Grainyhead-like 2 (GRHL2) inhibits keratinocyte differentiation through epigenetic mechanism. Cell Death Dis 3:e450.

O18. Lenalidomide and Pomalidomide Inhibit KSHV-Induced Downregulation of MHC Class I Expression in Primary Effusion Lymphoma Cells

David A. Davis[1], Holda A. Anagho[1], Suraj K. Mishra[1], Robert F. Carey[1], Yuki Takamatsu[1], Kenji Maeda[1], Jerome B. Zeldis[2], Robert Yarchoan[1]

[1]HIV and AIDS Malignancy Branch, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland, USA; [2]Celgene Corp., Summit, New Jersey, USA

Background: Kaposi's sarcoma-associated herpesvirus (KSHV) is the causative agent of Kaposi's sarcoma and two B-cell lymphomas, multicentric Castleman's disease (MCD) and primary effusion lymphoma (PEL). Generally resistant to chemotherapeutic drugs, PEL has a poor prognosis, with a median survival of 6 months. KSHV has latent and lytic replicative programs. During lytic replication, KSHV E3 ubiquitin ligases K3 and K5 function to down-regulate surface major histocompatibility class I (MHC-I) molecules to prevent cytotoxic T cell recognition. Our group has shown that thalidomide and pomalidomide are clinically active in Kaposi's sarcoma. The target of thalidomide, lenalidomide and pomalidomide, is the cellular protein cereblon, which is part of the cellular U3 ubiquitin ligase complex. With this background, we sought to explore the utility of these drugs in PEL and their effects on MHC-1 expression in PEL cells.

Methods: We analyzed proliferation and MHC-I levels in KSHV-positive PEL cell lines BC-3, BCBL-1, and JSC-1 cells treated with lenalidomide or pomalidomide and/or butyrate (to induce lytic replication) by FACS and western blot. We also looked at levels of viral and cellular proteins in PEL cells by western blot. Finally, we transfected BJAB cells with K3- or K5- expressing plasmids and analyzed the effect of lenalidomide and pomalidomide on MHC I levels by FACS and western blot.

Results: All three compounds suppressed replication of PEL cell lines. In addition, the immunomodulatory drugs, lenalidomide and pomalidomide prevented MHC I downregulation in butyrate-induced PEL cells. In K5-transfected cells, inhibition of MHC-I downregulation was seen at the protein level by western blot but downregulation of surface expression was not observed by FACS analysis when corrected for protein expression levels. However, in K3-transfected cells, MHC-I was downregulated and this was consistently prevented by pomalidomide at 1 and 10 μM when analyzed by western blot and FACS, suggesting specific interference of the K3-induced downregulation. Treatment with butyrate led to an increase in expression of ORF45, a lytic protein, as expected, but this was not substantially affected by pomalidomide. In addition, pomalidomide treatment did not affect K3 or K5 expression in BC-3 cells.

Conclusions: These data show that lenalidomide and pomalidomide inhibit proliferation of PEL cells and also inhibit KSHV-induced downregulation of MHC-1. Furthermore, the data suggest that inhibition of MHC-I by these drugs was not mediated by interference of the expression of K3 or K5. Inhibition of MHC-I downregulation appears to involve at least in part interference with the K3 (and possibly also K5) mediated pathway of MHC-I downregulation. We are currently investigating the mechanisms by which these drugs prevent MHC-I downregulation in PEL cells. By preventing MHC-I downregulation, these drugs may enable infected cells to be recognized and destroyed by the immune system. The two activities may provide an impetus for its use as a potential treatment for PEL or other KSHV-related cancers.

This work was supported by the intramural program of the NIH, National Cancer Institute (NCI), and a CRADA between the NCI and Celgene, Corp.

O19. Radiation-Sparing Treatment of Acquired Immune Deficiency Syndrome (AIDS)-Related Primary Central Nervous System Lymphoma (PCNSL) With Highly Active Antiretroviral Therapy (HAART), Rituximab (R), and High-Dose Methotrexate With Leucovorin Rescue (HD-MTX)

Priscila H. Goncalves[1], Thomas S. Uldrick[1], Karen Aleman[1], Mark N. Polizzotto[1], Kathleen M. Wyvill[1], Seth M. Steinberg[2], Stefania Pittaluga[3], Maryalice Stetler-Stevenson[3], Constance Yuan[3], Richard Little[1,4], Robert Yarchoan[1]

[1]HIV and AIDS Malignancy Branch, [2]Biostatistics and Data Management Section, [3]Laboratory of Pathology, Center for Cancer Research, [4]Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, Maryland, USA

Background: AIDS-PCNSL lymphoma is generally an Epstein-Barr (EBV)-associated tumor that occurs in patients (pts) with advanced human immunodeficiency virus (HIV)-associated immunosuppression. Historically, overall survival (OS) is poor (usually <6 months). African American (AA) race and history of central nervous system (CNS) infections have been associated with inferior outcomes.[1] Treatment often consists of whole brain radiation, which can lead to devastating neurotoxicity. We are prospectively evaluating curative-intent radiation-sparing immunochemotherapy consisting of HAART, the anti-CD20 monoclonal antibody, rituximab, and HD-MTX.

Methods: Patients with AIDS-PCNSL receive HAART and 6 cycles of rituximab 375 mg/m^2 with MTX 6 g/m^2 and leucovorin rescue (R-HD-MTX). Responses are evaluated by modified International Working Group Response Criteria for PCNSL. Pts obtaining a complete response (CR) or complete response unconfirmed (CRu) after R-HD-MTX received 2 additional cycles of HD-MTX. Responses, immune reconstitution, and OS were evaluated using descriptive statistics and Kaplan-Meier methods.

Results: Patient characteristics: 8 men, 3 women; median (med) age 31 years (range: 21-45); 7 AA, 3 Hispanic, 1 white non-Hispanic; med Eastern Cooperative Oncology Group performance status 2 (1-3); med baseline Mini Mental State Examination (MMSE, maximum score = 30) was 22 (5-29). Most pts were not on HAART prior to diagnosis (5) or had been on HAART <4 months (4). Med time from HIV to PCNSL diagnosis was 4 months (0-23 years). Med CD4$^+$ T-cell count at PCNSL diagnosis was 19 cells/μL (0-409). Diagnosis of PCNSL was biopsy confirmed (10) or made by ^{18}fludeoxyglucose positron emission tomography/cerebral spinal fluid (CSF) EBV viral load criteria.[1] One pt had an EBV-negative tumor. Flow cytometry showed leptomeningeal disease in 4 pts. 3 had concurrent CNS infections. 10 were evaluable for response to R-HD-MTX. 1 non-adherent pt completed only 1 cycle and was not evaluable (treatment failure, TF). Responses after R-HD-MTX: CR (2), CRu (3), partial response (PR) (4) and progressive disease (PD) (1). One pt developed a newly described paradoxical PCNSL immune reconstitution inflammatory syndrome (IRIS) successfully treated with steroids.[2] 2 pts received second-line temozolomide due to PR at end of R-HD-MTX and obtained a subsequent CR. 2 pts with PD received second-line therapy; 1 received the Cancer and Leukemia Group B (CALGB) 50202 induction regimen and obtained a subsequent CR; 1 received rituximab and temozolomide and had PD. There were 3 deaths on study: 1 pulmonary embolism, 1 CNS fungal infection in setting of PD, 1 TF. Including pts who received second line therapy, 8 (73%) obtained a durable CR. Median CD4$^+$ T-cell increase during R-HD-MTX was +35 cells/μL (-54 - +369). In surviving pts, med MMSE after R-HD-MTX was 28 (27-30). With med 52.9 months potential follow-up, estimated OS beyond 12 months is 71.6% (95% CI 35-89.9%).

Conclusions: Preliminary evidence suggests treatment of AIDS-PCNSL with HAART, and R-HD-MTX, may be associated with a high response rate, CD4$^+$ immune reconstitution, and very good OS, even in a high-risk pt population. Protocol accrual is ongoing; results of this study will be important to define effective radiation-sparing therapy for AIDS-PCNSL and evaluate long-term neurocognitive outcomes.

O20. AMC-085: A Pilot Trial of AVD and Brentuximab Vedotin in the Upfront Treatment of Stage II-IV HIV-Associated Hodgkin Lymphoma. A Trial of the AIDS Malignancy Consortium

Paul G. Rubinstein[1,2], Page Moore[3], David Henry[4], Lee Ratner[5], Elad Sharon[6], Ariela Noy[7]

[1]Department of Medicine, John H Stroger, Jr. Hospital of Cook County; [2]The Ruth M. Rothstein CORE Center, Chicago, Illinois, USA; [3]Department of Biostatistics, University of Arkansas for Medical Sciences, Little Rock, Arkansas; [4]Abramson Cancer Center, Pennsylvania Hospital, Philadelphia, Pennsylvania, USA; [5]Division of Oncology, Washington University School of Medicine, St. Louis, Missouri, USA; [6]Cancer Therapy Evaluation Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, Maryland, USA; [7]Memorial Sloan Kettering Cancer Center, New York, New York, USA

Introduction: Patients (pts) infected with HIV have a 6-8 fold increased risk of classic Hodgkin lymphoma (cHL). Incidence may have increased with the implementation of combined antiretroviral therapy (cART) in the mid 1990s. Frontline therapy for HIV-associated cHL (HIV-cHL) using doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) in the pre-cART era showed a 2-year overall survival (OS) of 48%, but outcomes are currently similar to the non-HIV population. Pts with advanced disease have a 30% chance of relapse with ABVD. Brentuximab vedotin (BV), an anti-CD30 antibody drug conjugate, selectively induces apoptosis of CD30+ cells with a complete response of 34% in patients with relapsed/refractory cHL. An international trial of BV with doxorubicin, vinblastine, and dacarbazine (AVD) vs. ABVD is ongoing. Here we present the Phase I portion of the first trial using BV with AVD in the upfront treatment of HIV-cHL. The Phase II portion is actively accruing in both the United States and France as part of an AIDS Malignancy Consortium (AMC)/Lymphoma Study Association (LYSA) collaboration.

Methods: The Phase I was a 3+3 dose de-escalation design evaluating 3 dose levels of BV (1.2 mg/kg, 0.9 mg/kg, and 0.6 mg/kg) every 2 weeks combined with standard, fixed doses of doxorubicin 25 mg/m^2, vinblastine 6 mg/m^2, and dacarbazine 375 mg/m^2 (AVD) in a 28-day cycle. Eligibility: HIV+ pts diagnosed with untreated cHL stage II-IV with CD4 counts ≥50 cells/mm^3 were required to take cART regimens for at least 1 week before treatment. Ritonavir, zidovidine, and cobisistat were excluded. Baseline, cycle 2, and post-treatment PET/CT scans were required. Dose limiting toxicities (DLTs) were defined during cycle 1.

Results: Six pts (5 men and 1 woman) were treated in the Phase I portion from 3/2013 to 5/2015. Staging: II (n=1), III (n=1), IV (n=4). Pathology: mixed cellularity (n=2), nodular sclerosis (n=3), and lymphocyte depleted/mixed cellularity (n=1) HIV-cHL. The median CD4 T cell count at lymphoma diagnosis was 499 cells/mm^3 (range 86-784) and the median viral load was 44 copies/ml (range 20-77). No cycle 1 DLTs were identified in the first 6 eligible patients and only 3 grade 3 adverse events in later cycles were noted, pneumonia, n=1, neuropathy, n=2, and neutropenia, n=1. In 2 pts, toxicity required delays in therapy of over 3 weeks (after c5d1 and after c6d1) resulting in subject removal from further protocol therapy. One pt had a decrease in the diffusion lung capacity for carbon monoxide (DLCO) to 65% after cycle 2, and BV was withheld while AVD continued as per protocol. Two pts were later deemed ineligible, and excluded from any analysis, due to the concomitant use of ritonavir-based cART at enrollment. Both developed febrile neutropenia and one developed a grade 3 pancreatitis during cycle 1, emphasizing the importance of not treating patients with BV + AVD with concurrent CYP3A4 inhibitors. Five of the 6 pts achieved cycle 2 PET/CT negativity as defined by a Deauville score 1-3. The PET/CT positive patient ultimately had a negative post-therapy scan. With a median followup of 13 months, the progression free survival (PFS) is 100%. Phase II is enrolling at BV 1.2 mg/kg in combination with AVD.

Conclusions: AVD-BV in stage II-IV HIV-cHL was well-tolerated therapy as no DLT were identified. Five of the 6 patients achieved a negative C2 PET/CT and 5/5 of the patients who completed therapy thus far achieved a CR. With a median followup of 13 months, the PFS is 100%. The recommended Phase II dose is 1.2 mg/kg +AVD every other week. The Phase II portion (51 subjects) is actively accruing in both the USA and France, in an AMC/LYSA collaboration, clinicaltrials.gov NCT01771107.

O21. Non-Myeloablative Haploidentical Allogeneic Bone Marrow Transplantation in HIV-Infected Individuals

D. Xu[1], Adam Capoferri[1,2], H. McHugh[1], A. Cash[1], O. Laeyendecker[1,3], S. Sakoian[4], C. Bullen[1], C. Pohlmeyer[1], P. Pham[1,5], L. Schoch[4], J. Lai[1], J. Gallant[5], C. Flexner[1], R. Jones[4], Y. Kasamon[4], R. Siliciano[1,2], C. Durand[1], R. Ambinder[4]

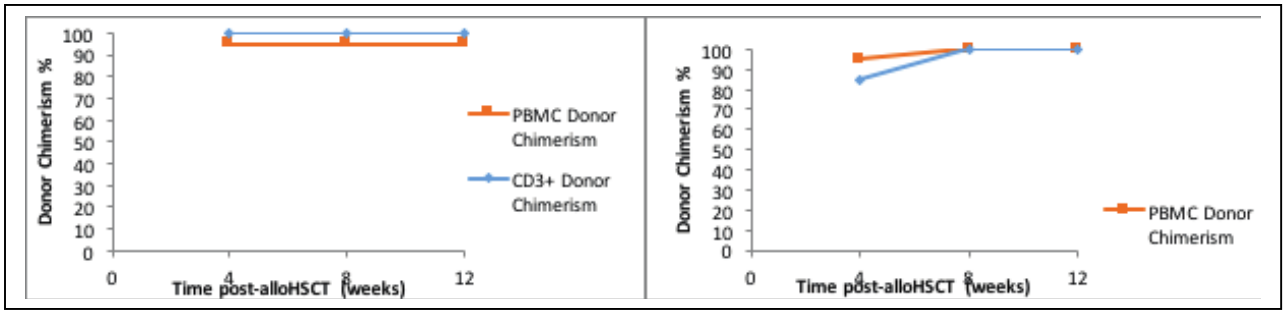
[1]Johns Hopkins University School of Medicine, Department of Medicine, Baltimore, Maryland, USA; [2]Howard Hughes Medical Institute; Chevy Chase, Maryland, USA; [3]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA; [4]Johns Hopkins University, The Sidney Kimmel Cancer Center, Baltimore, Maryland, USA; [5]Johns Hopkins University, Department of Pharmacy, Baltimore, Maryland, USA; [1]Southwest CARE Center, Santa Fe, New Mexico, USA

Background: In HIV-infected (HIV+) individuals, a latent reservoir of infected resting CD4+ resting T cells is thought to pose the major barrier to cure. Destroying the cells comprising this latent reservoir while simultaneously preventing new infection events from occurring in non-infected cells is thought to be a viable strategy for a complete, eradicating cure. Allogeneic bone marrow transplant (alloBMT), coupled with protection of new infection events through uninterrupted antiretroviral (ART) treatment, has been proposed as an implementation of this strategy. In an ongoing trial, our group is investigating this approach in HIV-infected (HIV+) patients requiring bone marrow transplants. Here, we present a report of two patients in our study who received alloBMT from related HLA-haploidentical donors.

Method: Patients received standard non-myeloablative conditioning with fludarabine, cyclophosphamide, and total body irradiation. Graft versus host disease prophylaxis was with post-transplant cyclophosphamide, tacrolimus, and mycophenolate. Patients were monitored for graft-versus-host disease and PCR-based clinical chimerism measurements of patients' PBMC (peripheral blood mononuclear cells) and CD3+ T cell replacement due to graft-versus-host effects were longitudinally taken. The HIV latent reservoir of these patients was quantified using a Viral Outgrowth Assay (VOA) developed by our group which specifically measures replication-competent HIV and excludes defective, mutated HIV variants.

	BMT Transplant	Malignancy	Oncology Outcome	Baseline HIV IUPM	12 Week post-BMT IUPM
Patient 1	1/2/15	Non-Hodgkins	Alive, Cancer Free	0.174356	0.072964
Patient 2	3/11/15	Non-Hodgkins	Alive, Cancer Free	2.318592	0.06027

Complete donor chimerism was achieved quickly in both PBMC and CD3+ T cell compartments.



Results: Relative to pre-transplant baseline, we observe a reduction of the latent reservoir at a 12-week time point in both patients (Table); patients have remained negative for replication-competent virus as measured by VOA for 12 weeks post-alloBMT. The value reported here is the lower limit of detection of the VOA based on the input number of cells. Both patients are alive and well more than 6 months after transplantation.

Conclusions: Non-myeloablative haplo transplant is effective treatment for a variety of hematologic malignancies. The results in these two patients suggest that it may be also effective in substantially reducing the size of the long term reservoir to undetectable levels.

HIGHLIGHTED POSTERS

These posters were selected by the organizers for showcasing on both days of the ICMAOI

1. A More Representative and Less Biased Approach to Estimating Mortality After Diagnosis of Kaposi Sarcoma Among HIV-Infected Adults in Sub-Saharan Africa in the Era of Antiretroviral Therapy

Aggrey Semeere[1,2], Esther Freeman[3], Megan Wenger[2], Naftali Busakhala[4,5], Elyne Rotich[5], Chite F. Asirwa[5,6], Mwebesa Bwana[7], Michael Kanyesigye[7], Elima Jedy-Agba[8], Vivian Kwaghe[9], Kenneth Iregbu[10], Francois Dabis[11], Antoine Jaquet[11], Sam Phiri[12], Julia Bohlius[13], Matthias Egger[13], David Glidden[2], Constantin Yiannoutsos[6], Kara Wools-Kaloustian[6], Jeffrey Martin[2]

[1]Infectious Diseases Institute, Kampala, Uganda; [2]University of California, San Francisco, California, USA; [3]Harvard Medical School, Boston, Massachusetts, USA; [4]Moi University, Eldoret, Kenya; [5]Academic Model Providing Access to Healthcare (AMPATH), Eldoret, Kenya; [6]Indiana University, Indianapolis, Indiana, USA; [7]Mbarara University, Mbarara, Uganda; [8]Institute of Human Virology, Abuja, Nigeria; [9]University of Abuja Teaching Hospital (UATH), Nigeria; [10]National Hospital of Abuja (NHA), Nigeria; [11]INSERM U897 & ISPED, Université Bordeaux, France; [12]Lighthouse Trust Clinic, Lilongwe, Malawi; [13]University of Bern, Switzerland

Background: In sub-Saharan Africa, Kaposi sarcoma (KS) is among the most common malignancies in HIV-infected individuals, and, given the high prevalence of HIV, among the most common cancers in the entire population. Prior to the availability of antiretroviral therapy (ART), survival after KS in Africa was poor (60%-70% 1-year mortality). As ART has now become available in Africa, it is important to understand contemporary survival of KS, but most previous estimates have suffered from either nonrepresentativeness (coming from cancer referral centers or non-comprehensive registries) and/or bias stemming from populations fraught with high rates of losses to follow-up (LTFU).

Methods: We identified all HIV-infected adults who were newly diagnosed with KS between 2009 and 2012 while receiving their primary care at one of five HIV care programs participating in the East, West, and Southern Africa regions of the International Epidemiologic Databases to Evaluate AIDS (IeDEA) Consortium (ISS Clinic, Mbarara, Uganda; AMPATH, Kenya; Lighthouse Clinic, Lilongwe, Malawi; and NHA and UATH in Abuja, Nigeria). Patients specifically referred to these sites for oncologic care were excluded. In East Africa, as a comparator to the patients with KS, we also selected (a) patients with newly diagnosed tuberculosis or cryptococcosis while not yet on ART ("Serious OI group") and (b) patients first meeting eligibility for ART by CD4 count criteria but with no prior WHO Stage III/IV diagnosis ("CD4 group"). Patients' clinic experience was evaluated until death (the primary outcome), transfer to another care facility, or administrative database closure. Among those LTFU (absent from clinic for at least 3 months), all those in the KS group, as well as a random sample of the Serious OI and CD4 groups, were tracked in the community to update vital status. Outcomes among the tracked patients were incorporated via probability weights in subsequent "corrected" analyses.

Results: A total of 1197 patients with KS were included (57% from Kenya, 24% Malawi, 14% Uganda, and 4.8% Nigeria); 63% were men, and the median age was 35 years (interquartile range [IQR]: 30-41) and median CD4 count 169 cells/mm^3 (IQR: 68-320). Of the 447 patients with KS who became LTFU, we updated vital status in 79% via tracking in the community. Prior to incorporating updated vital status among those tracked, "naïve" mortality was 18% (95% CI: 16-21) at 1 year and 26% (95% CI: 22-30) at 3 years. After incorporating the updated vital status of those LTFU, "corrected" mortality was 32% (95% CI: 29-35) at 1 year and 45% (95% CI: 41-49) at 3 years. In the East Africa region, we compared mortality in those with KS to 3819 patients with a "Serious OI" and 10,603 in the "CD4 group." After adjusting for age, sex, BMI, hemoglobin, CD4, and nature of diagnosis (prevalent or incident), being diagnosed with KS was associated with substantially higher mortality than being diagnosed with a Serious OI (hazard ratio 3.2 [95% CI: 2.4-4.3]) or becoming ART eligible by CD4 count alone (hazard ratio 3.4 [95% CI: 2.6-4.7]).

Conclusion: Despite growing ART availability, mortality after diagnosis of KS among HIV-infected African adults is still high in absolute terms and substantially higher than in other HIV-infected patients. The high mortality is especially notable given the representativeness of the primary care-based study population. The findings suggest the need for faster and more complete ART access and more effective interventions above and beyond ART for persons with KS. Methodologically, high rates of LTFU among patients with KS create considerable selection bias in survival estimation, but this can be overcome by tracking lost patients in the community.

2. Anti-Tumor Activity of DLX1008, an Anti-VEGF-A Antibody Fragment With Low Picomolar Affinity, in an In Vivo Model of Kaposi Sarcoma

Anthony Eason[1], Sang-Hoon Sin[1], Douglas Phillips[2], Japar Shamshiev[2], Miriam Steinwand[2], Nicole Dreier[2], Anna Bianca Howald[2], Marco Landi[2], Andrea Marti[2], Camilla Winnewisser[2], Julia Molitor[2], Titus Kretzschmar[2], Dirk Dittmer[1]

[1]Lineberger Comprehensive Cancer Center, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; [2]Delenex Therapeutics AG, Schlieren, Zurich, Switzerland

Background: Kaposi sarcoma (KS) is the most angiogenic cancer in the human population. KS and hemangioma are the only cancers that are of endothelial cell lineage origin. This cancer is caused by KS-associated herpesvirus (KSHV) and is singularly dependent on VEGF. Another property of KS is vascular leakage and edema. KS exists in a limited form where lesions develop predominantly on the skin only, and as a systemic disease is rapidly fatal if untreated. The current standard of care is based on liposomal anthracycline (Doxil™), which is effective in 50%-70% of cases. Recurrence treatment is limited by severe cardiac toxicity and a lifetime limit for Doxil. No targeted or adjuvant therapy exists at this point or has entered Phase III clinical trials. KS is unique among sarcomas, because not only is the cancer dependent on neo-angiogenesis, as all cancers are, but the cancer cells themselves are dependent on VEGF and secrete VEGF as a paracrine factor. Thus, neutralizing VEGF has a dual effect in KS: it starves the tumor by inhibiting neo-angiogenesis and it directly inhibits tumor cell proliferation. DLX1008 is a Delenex PENTRA®Body that shows exceptionally high affinity to human and mouse VEGF-A, and offers advantages in tissue penetration and distribution as compared to IgG antibodies due to its small size, as well as options for local or topical administration.

Methods: We previously developed a xenograft model for KS; using this model, 10^5 KSHV-positive human endothelial cells (ATCC® VR-1802™) were implanted in 200µl matrigel s.c. into NGS or C.B.17-SCID mice (Jackson lab) and treated with 15mg/kg DLX1008 intraperitoneally five times weekly for 21 days, starting at day 5, i.e., after tumors had formed. Tumor growth was followed by caliper measurements and tumor pathology evaluated by histochemistry. We used n=10 mice per group, and either treated with DLX1008 or irrelevant control (DLX1084). This represents a repeated measurement design, treating each animal as random variable. We used Box-Cox as a variance stabilizing transformation.

Results: The difference in tumor growth between groups was significant to $p \leq 0.004$. Immunohistochemistry with H&E stain showed that the tumor died from the inside out in response to DLX1008, with increased enucleated cells when compared to the healthy cells in the center of the control tumors, consistent with lack of oxygen and nutrient supply.

Conclusions: These initial data demonstrate that DLX1008 significantly retards tumor growth vis-à-vis control. This result is biologically even more impressive because of the biology of the implant growth in this model. Cells on the outside of the skin implant will receive oxygen by passive diffusion as well as angiogenesis; the cells most dependent on angiogenesis are on the center of the implant. Because both the tumor cell itself as well as the tumor's non-neoplastic sustaining vasculature are dependent on VEGF secreted by KS, cell culture studies only capture a fraction of the possible effect of any anti-VEGF therapy. Our in vivo model provides the necessary structure to evaluate the efficacy of DLX1008 in an appropriate tumor microenvironment. Therefore we propose that DLX1008 is an excellent antibody candidate to advance into clinical trials in Kaposi sarcoma.

3. Changes in Clinical Context for Kaposi Sarcoma and Non-Hodgkin Lymphoma Among HIV-infected People in the United States

Elizabeth Yanik[1], Chad Achenbach[2], Satish Gopal[3], Anna Coghill[1], Stephen Cole[4], Eric Engels[1], and the Centers for AIDS Research Network of Integrated Clinical Systems

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, Maryland, USA; [2]Department of Medicine, Northwestern University, Chicago, Illinois, USA; [3]Lineberger Comprehensive Cancer Center, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; [4]Department of Epidemiology, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Background: Given earlier antiretroviral (ART) initiation and increasing linkage to HIV care, the fraction of Kaposi sarcoma (KS) and non-Hodgkin lymphoma (NHL) cases occurring among HIV patients on effective ART may be increasing. Understanding these changes in KS and NHL burden is important, as HIV-associated cancers may differ depending on the immunologic and HIV virologic context in which tumors occur.

Methods: KS and NHL diagnoses between 1996 and 2011 were identified among adult HIV-infected patients in the U.S. Centers for AIDS Research Network of Integrated Clinical Systems. KS and NHL cases were categorized as occurring before or after entering routine HIV clinical care (defined as two clinical visits occurring ≥1 month and <6 months apart). Among those in care, cases were categorized by ART exposure, virologic suppression, and CD4 count at cancer diagnosis. The proportion of cases in each category was described across calendar time. Person-time in each category was calculated to determine if changes in proportions were driven by changes in person-time at risk or by changes in incidence rates.

Results: A total of 466 KS and 258 NHL cases were identified. For KS and NHL, diagnoses in recent years occurred more often after entry into routine care (KS: 42% in 1996-2001 vs. 58% in 2007-2011, P-trend<0.01; NHL: 61% in 1996-2001 vs. 72% in 2007-2011, P-trend=0.18). In more recent years, KS cases in care more frequently occurred among people on ART (55% in 1996-2001 vs. 76% in 2007-2011, P-trend=0.02). The proportion of NHL cases on ART was higher but stable over time (83% over all time, P-trend=0.81). Over time, an increasing proportion of both KS and NHL occurred at higher CD4 counts (Figure, P<0.05 for KS and NHL) and with undetectable HIV RNA (P<0.05 for KS and NHL). In more recent years, more person-time was contributed by patients on ART, patients with CD4 counts ≥200 cells/mm^3, and patients with suppressed HIV RNA. When person-time was accounted for, incidence rates were stable or declined over time in almost every category of ART use, CD4 count, and HIV RNA.

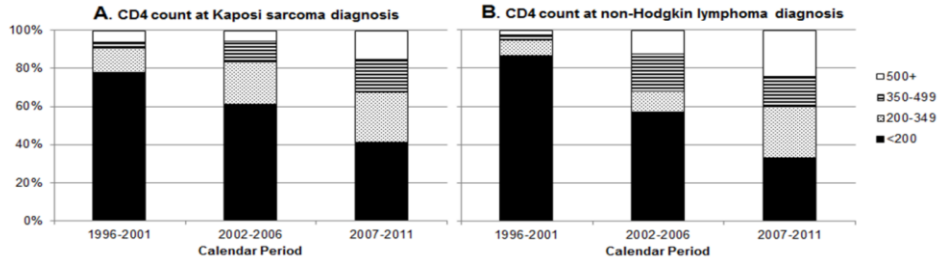


Figure. CD4 count (in cells/mm^3) at diagnosis for KS (A) and NHL (B) patients in the Centers for AIDS Research Network of Integrated Clinical Systems.

Conclusions: In the most recent calendar period, most KS and NHL were diagnosed in people already in routine clinical care. Among these cases, KS and NHL occurred at higher CD4 counts and lower HIV RNA values in more recent years, and KS occurred more frequently in people already on ART. These changes were not driven by changes in incidence, but rather by an increasing proportion of the HIV population being on ART, with higher CD4 counts and suppressed HIV RNA. These trends will likely continue with further improvements in linkage to care and earlier ART initiation. Improved understanding of HIV-associated cancer pathogenesis, management, and outcomes in the current context versus earlier in the ART era is therefore important.

4. Disparities in Cancer Treatment Among HIV-Infected Individuals

Gita Suneja[1], Chun Chieh Lin[2], Edgar P. Simard[3], Xuesong Han[2], Eric A. Engels[4], Ahmedin Jemal*[2]*

[1]University of Utah School of Medicine, Salt Lake City, Utah, USA; [2]American Cancer Society, Atlanta, Georgia, USA; [3]Emory University, Rollins School of Public Health, Atlanta, Georgia, USA; [4]National Cancer Institute, Bethesda, Maryland, USA

Background: Prior studies reported that HIV-infected cancer patients are less likely to receive cancer treatment compared with HIV-uninfected individuals; however, data on comorbidities and insurance status were lacking.

Methods: We used data from the National Cancer Database to study non-elderly adults diagnosed with diffuse large B-cell lymphoma (DLBCL), Hodgkin lymphoma, and head and neck, lung, upper gastrointestinal, colorectal, anal, prostate, breast, and cervical cancers from 2003 to 2011. Cancer treatment was defined as chemotherapy, surgery, radiotherapy, or any combination during the first course of treatment. We used multivariate logistic regression to examine associations between HIV status and lack of cancer treatment for all cancer patients adjusted for clinical and sociodemographic covariates. In a separate analysis, we examined associations for privately insured patients only. We identified predictors of lack of treatment among HIV-infected patients.

Results: A total of 10,390 HIV-infected cases and 2,228,642 HIV-uninfected cases were included in the study. In multivariate analysis, HIV-infected cancer patients were more likely to not have received treatment for DLBCL (adjusted odds ratio [aOR] 1.82, 95%CI 1.65-2.00), Hodgkin lymphoma (1.92, 1.66-2.22), and head and neck (1.48, 1.09-2.01), lung (2.46, 2.19-2.76), upper gastrointestinal (2.62, 2.04-3.37), colorectal (1.70, 1.17-2.48), prostate (2.16, 1.69-2.76), breast (2.14, 1.16-3.98), and cervical (2.81, 1.77-4.45) cancers. Among privately insured patients, HIV infection was associated with lack of cancer treatment for all cancers, except head and neck and anal cancer; DLBCL (adjusted odds ratio [aOR] 1.57, 95%CI 1.34-1.84), Hodgkin lymphoma (1.87, 1.50-2.33), and lung (2.47, 1.89-3.22), upper gastrointestinal (3.61, 2.17-6.03), colorectal (2.24, 1.05-4.77), prostate (2.25, 1.48-3.42), breast (4.24, 1.36-13.17), and cervical (2.73, 0.80-9.29) cancers. Predictors of lack of cancer treatment among HIV-infected patients varied by type of malignancy (solid tumor vs. hematologic), with black race and insurance status being predictors in both groups. Stage IV disease was associated with lack of treatment for solid malignancies, whereas advanced stage was predictive of treatment receipt for hematologic malignancies.

Conclusions: In the United States, HIV-infected cancer patients are less likely to receive cancer treatment relative to HIV-uninfected individuals regardless of their insurance status and comorbidities. Further investigation is required to identify factors contributing to the observed disparity with the goal of improving survival and quality of life for HIV-infected cancer patients.

5. Does Inflammation at Antiretroviral Therapy Initiation Increase Risk of Early Death and Disease Progression Among HIV-Infected Adults With Kaposi Sarcoma?

Stephen Asimwe[1,2], Miriam Laker-Oketta[2,3], Helen Byakwaga[2], Adrienne Mocello[2], Yong Huang[2], Toby Maurer[2], David V. Glidden[2], Russell Tracy[4], Tricia Burdo[5], Michael Lederman[6], Albert Davalos[7], Edward Mbidde[3,8], Peter Hunt[2], Jeffrey Martin[2]

[1]Mbarara Regional Referral Hospital, Mbarara, Uganda;[2]University of California, San Francisco, San Francisco, California, USA; [3]Infectious Diseases Institute, Kampala, Uganda; [4]University of Vermont, Burlington, Vermont, USA; [5]Boston College, Chestnut Hill, Massachusetts, USA; [6]Case Western Reserve University, Cleveland, Ohio, USA; [7]Buck Institute for Research on Aging, Novato, California, USA; [8]Uganda Virus Research Institute, Entebbe, Uganda

Background: HIV-infected patients with Kaposi sarcoma (KS) in sub-Saharan Africa have a substantially higher risk of death following initiation of antiretroviral therapy (ART) than comparable HIV-infected individuals without KS. The biological mechanisms underlying this inferior clinical response to ART are not well understood, but we hypothesized that inflammation may play a role.

Methods: We studied HIV-infected adults who were participants in the AntiRetrovirals for KS ("ARKS") clinical trial in Uganda. None had an urgent indication for chemotherapy, and each was initially treated with ART alone. Cryopreserved plasma samples were tested for 12 biomarkers of inflammation, monocyte activation, microbial translocation, and coagulation using individual assays. We assessed whether levels of these biomarkers at ART initiation were causally related to either death by 48 weeks or the composite outcome of death or development of an indication for chemotherapy. Each biomarker was assessed in isolation for its relationship with each of the outcomes after adjustment for a directed acyclic graph-based set of confounders (age, sex, history of tuberculosis, body mass index, plasma HIV RNA level, and CD4+ T cell count) using proportional hazards regression.

		Death		Death or Chemo. Indication	
Biomarker*	**Quartile**	**AHR† (95% CI)**	**P**	**AHR (95% CI)**	**P**
Soluble CD14 (sCD14)	1 (lowest)	Ref.	-	Ref.	-
	4 (highest)	11.1 (2.5-49.4)	0.002	3.3 (1.7-6.6)	0.001
C-Reactive protein (CRP)	1	Ref.	-	Ref.	-
	4	9.0 (2.6-30.7)	<0.001	4.7 (2.2-10.0)	<0.001
Kynurenine/ tryptophan ratio (K:T ratio)	1	Ref.	-	Ref.	-
	4	8.6 (1.9-38.5)	0.005	3.5 (1.5-8.2)	0.003
Interleukin-6 (IL-6)	1	Ref.	-	Ref.	-
	4	5.4 (1.8-16.8)	0.003	3.6 (1.8-7.5)	<0.001
D-Dimer	1	Ref.	-	Ref.	-
	4	5.1 (1.8-14.0)	0.002	4.6 (2.3-9.2)	<0.001
Soluble tumor necrosis factor - receptor I (sTNF-RI)	1	Ref.	-	Ref.	-
	4	2.6 (1.0-6.7)	0.05	3.5 (1.7-7.1)	0.001
sTNF-RII	1	Ref.	-	Ref.	-
	4	4.3 (1.4-13.1)	0.01	3.4 (1.6-7.2)	0.001
Soluble CD27 (sCD27)	1	Ref.	-	Ref.	-
	4	2.7 (1.0-7.3)	0.04	2.0 (0.95-4.0)	0.07

*Intestinal fatty acid binding protein, high mobility group box 1 protein, soluble CD163, and interferon-gamma inducible protein 10 were not associated with either outcome

†Adjusted hazard ratio comparing, for each outcome, the hazard of outcome among subjects in the 4th quartile (highest levels) of biomarker concentration at ART initiation to the hazard of outcome among subjects in the 1st quartile (lowest levels).

Results: We evaluated 224 subjects (56% men). Median values at ART initiation were age = 34 years (IQR 28-40); CD4+ T cell count = 119 cells/mm^3 (IQR 24-265); and HIV RNA level = 5.4 $\log_{10}$ copies/ml (IQR 5.0-5.6). By 48 weeks, 19% of subjects died; 35% either died or developed a chemotherapy indication. sCD14, CRP, K:T ratio, IL-6, D-Dimer, sTNF-RI, sTNF-RII, and sCD27 were each associated with at least one of the outcomes, either in dose-response or threshold relationships (Table).

Conclusions: Among HIV-infected adults with KS treated with ART in East Africa, increased levels of several biomarkers of inflammation, as well as abnormal coagulation, were temporally associated with either clinical progression of KS and/or death. The findings suggest that many of the same biological mechanisms responsible for KS occurrence may also influence clinical outcomes and that adjunctive interventions aimed at reducing inflammation might improve clinical course.

6. Expression of HIV-1 Matrix Protein and Correlation With B Cell Lymphoma in HIV-1 Transgenic Mice

Virginia Carroll[1], Mark K. Lafferty[1], Joseph L. Bryant[1], Robert C. Gallo[1], Alfredo Garzino-Demo[1,2]

[1]Institute of Human Virology, University of Maryland School of Medicine, Baltimore, Maryland, USA; [2]Department of Molecular Medicine, University of Padova, Italy

Background: HIV infection is associated with an increased risk for Non-Hodgkin's Lymphoma (NHL). How HIV infection promotes the development of lymphoma is unclear, but may involve chronic B cell activation, inflammation, and/or impaired immunity, possibly leading to loss of control of oncogenic viruses and reduced tumor immunosurveillance. The HIV-1 transgenic mouse, Tg26, has a pNL4-3 proviral transgene lacking most of the gag/pol region and exhibits a variety of pathologies, including B cell lymphoma in 15% of animals. Defects in the viral genome and non-permissivity of the host render the virus non-infectious, but viral genes are expressed via the LTR in tissues such as skin, lymph node, spleen, and kidney. Given that Tg26 mice are immunocompetent and lack viral replication, we hypothesize that HIV proteins themselves may promote lymphomagenesis. Our group has previously characterized the lymphoma in Tg26 mice and shown elevated expression of HIV-1 matrix protein p17, gp120, and Nef in spleens of affected animals. p17 is a structural protein that is also secreted by infected cells and can persist in the lymph nodes of HIV-infected patients in the absence of viral replication. p17 is known to mediate many biological effects, including enhancement of lymphocyte proliferation and cytokine production, chemotaxis, and angiogenesis/lymphangiogenesis.

Methods: In order to more carefully define a role for p17 in lymphomagenesis in this model, we evaluated p17 expression in lymph nodes and bone marrow of Tg26 mice at different disease stages by Western blot and immunohistochemistry (IHC). In addition, we investigated whether asymptomatic Tg26 mice displayed alterations in B cell development or survival that may predispose to the development of lymphoma via flow cytometry.

Results: p17 protein signal was detected by Western blot in lymph nodes of Tg26 mice with early and late stage lymphoma. We observed gp120 and Nef expression in lymph nodes only during the late stage of lymphoma. Interestingly, a high level of p17 was detected by IHC in bone marrow of asymptomatic Tg26 animals, suggesting p17 may influence B cells early in development. Normal mouse B cells bound fluorochrome-labeled p17 by flow cytometry. A comparison of bone marrow cells from asymptomatic Tg26 mice vs. WT controls revealed a near 2-fold increase in frequency of $IgD^{+}IgM^{lo}$ mature B cells as well as increased expression of CD21/complement receptor 2, a marker of mature B cells and the receptor for Epstein-Barr virus. Survival of splenic B cells from asymptomatic Tg26 mice was enhanced as measured by dye exclusion after 1 day of culture with or without IL-4 and anti-CD40 mAb stimulation.

Conclusions: Tg26 mice express the HIV-1 matrix protein, p17, in bone marrow prior to development of lymphoma, suggesting the protein may contribute to lymphomagenesis in this model. Preliminary studies indicate the HIV-1 transgene leads to changes in B cell homeostasis in the bone marrow and B cell survival in the periphery. Further study of HIV transgenic mouse models will allow a greater understanding of the role of HIV proteins in lymphomagenesis.

7. HO-1 Promotes KSHV Infection of Endothelial Cells Through Inhibition of TLR4 Signaling

Sara Botto, Ashlee V. Moses

Vaccine & Gene Therapy Institute, Oregon Health & Science University, Beaverton, Oregon, USA

Background: KSHV infection of endothelial cells (EC) in vitro is associated with robust induction of the host gene *heme oxygenase-1* (*HO-1*), a stress-inducible cellular enzyme responsible for heme catabolism. HO-1 is also strongly expressed in spindle cells in KS lesions, which are notable for extravasation of heme-rich erythrocytes (1). Heme degradation releases iron and produces carbon monoxide (CO) and biliverdin, which is converted into bilirubin (BR) via biliverdin reductase. KSHV-infected EC proliferate in response to free heme in a HO-1-dependent manner, implicating virus-enhanced HO-1 activity in KS tumorigenesis (1). We have recently shown that KSHV induces HO-1 in a biphasic manner, characterized by an early transient induction (4-6 hours PI) and a more sustained upregulation co-incident with the establishment of latency (3 days PI and later) (2). Using a BAC16-derived recombinant KSHV lacking one of the KSHV miRNAs, miR-K12-11, we showed a key role for the viral miRNA in KSHV-induction of HO-1 during latency, through the downregulation of the HO-1 transcriptional repressor BACH1 (2). Interestingly, neither KSHV de novo gene expression nor miR-K12-11 are required for the early peak of HO-1 induction, suggesting that other virion and/or host components regulate this initial phase of HO-1 expression.

Methods: Virus infections were performed in vitro in lymphatic EC (LEC) using recombinant KSHV-BAC16 in the presence of an inhibitor of TLR4 signaling (CLI-095), a CO releasing molecule (CORM-2) and a selective HO-1 inhibitor (OB-24). HO-1 mRNA and protein levels were determined by qPCR, immunoblotting, and immunofluorescence microscopy.

Results: Using HO-1 knockdown LEC, we showed that HO-1 was important for efficient de novo KSHV infection, promoting initial infection and replication as well as virus spread. Moreover, we were able to recapitulate this phenotype by treating LEC with OB-24, an azole-based inhibitor of HO-1 activity (3). Accordingly, we investigated the mechanism(s) whereby HO-1 promotes KSHV infection. It has been previously shown that TLR4 mediates antiviral immunity against KSHV and that the virus employs countermeasures: KSHV binding to endothelial cells activates TLR4 signaling, which is then inhibited by KSHV proteins and miRNAs (4). Additionally, TLR4-deficient cells showed a higher susceptibility to KSHV infection (4). Interestingly, the heme metabolite CO is able to block TLR4 activation, by inhibiting the binding of TLR4 to its signaling partners (5,6); consequently, we explored a potential role for CO in blocking KSHV-activation of TLR4 to facilitate viral infection. Our data show that virions devoid of the KSHV genome (B-type capsids) induce early HO-1 and trigger TLR4 in LEC, suggesting that viral structural components mediate both phenotypes. Additionally, we show that LEC treated with the TLR4 inhibitor CLI-095 are more permissive for KSHV infection, and that CORM-2 both blocks TLR4 signaling in LEC and enhances the efficiency of KSHV infection. We are currently examining the mechanism of TLR4 inhibition in HO-1 knockout LEC using CRISPR technology.

Conclusions: Our data suggest that in the context of KSHV infection, HO-1 functions as a novel inhibitor of the antiviral immune response in LEC and facilitates the earliest stages of de novo infection. Therefore, we propose HO-1 as anti-viral as well as anti-tumor target for KS therapy.

8. Immunodeficiency and the Risk of Cervical Intra-epithelial Neoplasia 2/3 and Cervical Cancer: A Nested Case-Control Study in the Swiss HIV Cohort Study

Gary M. Clifford[1], Silvia Franceschi[1], Olivia Keiser[2], Franziska Schöni-Affolter[3], Matthias Egger[2], Swiss HIV Cohort Study

[1]International Agency for Research on Cancer, Lyon, France; [2]Institute of Social and Preventive Medicine (ISPM), Bern, Switzerland; [3]Coordination and Data Center, Swiss HIV Cohort Study, Lausanne, Switzerland

Background: HIV-infected women are at increased risk of cervical intra-epithelial neoplasia (CIN) and invasive cervical cancer (ICC), but it has been difficult to disentangle the influences of heavy exposure to HPV infection, inadequate screening, and immunodeficiency.

Methods: A case-control study including 364 CIN2/3 and 20 ICC cases matched to 1,147 controls was nested in the Swiss HIV Cohort Study (1985-2013).

Results: CIN2/3 risk was significantly associated with low CD4+ cell counts, whether measured as nadir (odds ratio (OR) per 100-cell/µL decrease=1.15, 95% CI: 1.08, 1.22), or at CIN2/3 diagnosis (1.10, 95% CI: 1.04, 1.16). An association was evident even for nadir CD4+ 200-349 versus ≥350 cells/µL (OR=1.57, 95% CI: 1.09, 2.25). After adjustment for nadir CD4+, a protective effect of >2-year cART use was seen against CIN2/3 (OR versus never cART use=0.64, 95% CI: 0.42, 0.98). Despite low study power, similar associations were seen for ICC, notably with nadir CD4+ (OR for 50 versus >350 cells/µL= 11.10, 95% CI: 1.24, 100). HPV16-L1 antibodies were significantly associated with CIN2/3, but HPV16-E6 antibodies were nearly exclusively detected in ICC.

Conclusions: Worsening immunodeficiency, even at only moderately decreased CD4+ cell counts (200-349 CD4+ cells/µL), is a significant risk factor for CIN2/3 and cervical cancer.

9. HPV16 Infection and Oncogenesis on the Epigenome of Human Tonsil Epithelium

Sa Do Kang[1], Sreejata Chatterjee[1], Mohd Israr[2], Anna Salzberg[1], Arthur Berg[1], Willard Freeman[3], Craig Meyers[1]

[1]Penn State College of Medicine, Hershey, Pennsylvania, USA; [2]The Feinstein Institute for Medical Research, Manhasset, New York, USA; [3]Oklahoma University Health Sciences Center, Oklahoma City, Oklahoma, USA

Background: In recent decades, the rate of HNSCCs caused by HPV16 has steadily increased, becoming a major cause of oropharyngeal cancers that are located predominantly in the lingual and palatine tonsillar areas. In particular, patients infected with human immunodeficiency virus (HIV) have higher rates of HPV16 infection and are at greater risk for developing HPV16-related cancers including HNSCC. Environmental factors, including viruses, can affect the epigenetics of the host genome and play a role in the oncogenic process. Moreover, studies have shown that epigenetic changes also occur in the viral genome and are associated with oncogenesis. In this study, we screen for genes that are epigenetically altered with HPV16 infection and identify genes that could help elucidate the oncogenic process and provide targets for therapy. By using our in vitro three-dimensional organotypic tissue culture system, we can examine changes in the epigenetic profile of both the host and viral genome at different stages of the HPV life cycle. With the same tissue culture system, we will also measure the impact of ART therapy on the epigenetic modifications induced by HPV16, and examine whether there is any tissue-specific response by comparing the epigenetic profiles of HPV16-infected tonsil and cervical epithelium.

Methods: HPV16 infected keratinocytes are grown in a raft culture model. In order to measure the epigenetic changes that occur at different stages of the HPV life cycle, both host and viral methylomes will be analyzed at 4, 10, and 20 days of growth in raft culture. Additionally, we will set up raft cultures of HPV16-infected keratinocytes at every 5 passages to examine histological progression into cancer and measure changes in the expression of proteins associated with proliferation and differentiation. These experiments will be repeated at identical conditions, but with ART treatment (Kaletra and Ampenavir). For host methylome analysis, we will apply a capture enrichment next generation sequencing approach, and for HPV16 methylome analysis we have developed an approach that hybridizes traditional bisulphite conversion followed by PCR with “personal” next generation sequencing. Methylome analysis will be compared with previous microarray analyses.

Results: When comparing changes in gene expression of HPV16 infected tissues with uninfected tissues, cervix, foreskin, and tonsil tissues differed in the number of genes affected. Microarray analyses showed that the cervix had 1418, foreskin had 1737, and tonsil had 178 total changes in gene expression following HPV16 infection. All three tissues only had 30 changes in gene expression in common following HPV16 infection, whereas the cervix and foreskin tissues had 345 changes in gene expression and cervix and tonsil tissues or foreskin and tonsil tissues had only 54 or 68 gene expression changes, respectively, following HPV16 infection.

Conclusions: Different sets of genes are affected by HPV16 infection in human tonsil, cervix, and foreskin tissues. Overall, tonsil tissue had much fewer genes affected by HPV16 infection as compared to the cervix and foreskin. We hope to soon have methylome data to compare with the gene expression data.

10. Phase I Trial of Cabozantinib (XL184) for Advanced Solid Tumors in Persons With HIV Infection: AIDS Malignancy Consortium (AMC) Trial 087 – Trial in Progress

Elizabeth Y. Chiao[1], Michelle A. Rudek[2], Page C. Moore[3], David M. Aboulafia[4], Richard F. Ambinder[5], Robert Baiocchi[6], Kelly A. Shimabukuro[7], Virginia C. Broudy[8], Jennifer W. Chuy[9], Jeannette Y. Lee[3], Richard F. Little[10], Ronald T. Mitsuyasu[11], Missak Haigentz, Jr.[8]

[1]Baylor College of Medicine, Houston Health Services Research and Development Center of Excellence, Michael E. DeBakey Veterans Affairs Medical Center, Houston, Texas, USA; [2]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, Maryland, USA; [3]University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA; [4] Virginia Mason Medical Center, Seattle, Washington, USA; [5]Johns Hopkins University, Baltimore, Maryland, USA; [6]Comprehensive Cancer Center, Ohio State Medical Center, Columbus, Ohio, [7]Center for Personalized Cancer Therapy, and Division of Hematology and Oncology, UCSD Moores Cancer Center, La Jolla, California, USA; [8]University of Washington School of Medicine, Seattle, Washington, USA; [9]Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, New York, USA; [10]Clinical Investigations Branch, National Cancer Institute, Bethesda, Maryland, USA; [11]University of California, Los Angeles, UCLA Clinical AIDS Research and Education Center, Los Angeles, California, USA

Background: With the aging of the HIV-infected population, cancer has now become the leading cause of death among this population, and solid tumors have become increasingly prevalent. Treatment of solid tumors with novel, targeted treatments, including tyrosine kinase inhibitors, in HIV-infected individuals may be complicated by drug-drug interactions with these agents and antiretroviral therapy (ART). To study these interactions, the AMC has launched clinical studies with novel and standard therapies to address this emerging epidemic with the goal of formulating treatment recommendations to treat solid tumors in HIV+ patients. To this end, the AMC is conducting a Phase 1 trial of Cabozantinib (XL184), a multiple receptor tyrosine kinase implicated in tumor growth, metastasis, and angiogenesis. The primary target is c-MET and VEGFR2; additional targets include RET, AXL, KIT, and TIE-2.

Methods: The primary objective of AMC087 is to determine the safety and tolerability of cabozantinib in HIV+ cancer patients. Secondary objectives include effects of ART on cabozantinib pharmacokinetics, effects of cabozantinib on CD4+ count and HIV viral load, and to evaluate preliminary efficacy signals of cabozantinib in commonly represented cancers. Subjects are stratified based on their ART regimen: (A) ritonavir or cobicistat-containing ART (potent CYP3A4 inhibitors), (B) efavirenz or etravirine-containing ART (CYP3A4 inducers), and (C) other ART regimens (including no ART). A standard Phase 1 dose escalation design (3+3) is used to determine the MTD of cabozantinib (in stratum-based cohorts at 20, 40, 60, 80, and 100 mg PO daily in 28 day cycles) in each group. PK analysis is performed on C1D1 and D22-23 (steady state). The trial was initiated in 2013 and is open at AMC sites; up to 42 pts will be enrolled.

Results: As of 8/2015, 20 pts have been enrolled, 18 men and 2 women. Thirteen participants were white, 6 black, 2 Hispanic, and 1 multiple races. The cancer diagnoses of the enrollees are 12 Kaposi sarcoma (KS), 1 anal cancer, 1 head and neck cancer, and 6 other types of cancer. The maximally tolerated dose (MTD) of cabozantinib for Stratum C was determined to be 60mg/day, without observed dose limiting toxicity (DLT). One patient has experienced a DLT at the 60 mg/day level of Stratum A. Among the KS patients, 2 have had a partial response, and a patient with a non-KS sarcoma had near partial response by RECIST 1.1.. Three participants remain on study therapy for >1 year. Updated information on the safety and initial pharmacokinetic analyses will be presented.

Conclusion: Preliminary evidence suggests that Cabozantinib may be safely administered in HIV-infected inividuals. NCT01822522.

11. Regulation of p53-Dependent DNA Damage Responses by Hepatitis C Virus Infection

Jonathan Mitchell, Stanley Lemon, David McGivern

Lineberger Comprehensive Cancer Center and Department of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Background: Globally, approximately one-quarter of HIV-infected persons are co-infected with HCV. Among HIV-infected patients undergoing antiretroviral therapy, HCV-related liver disease is an increasingly important cause of mortality. In part, this is due to the fact that chronic HCV infection promotes the development of hepatocellular carcinoma (HCC). The mechanisms underlying the progression of HCV infection to HCC are poorly defined due to the lack of good model systems to study tumorigenesis. Several lines of evidence support a direct role for HCV infection in carcinogenesis by disruption of tumor suppressor pathways. The p53 protein is a canonical tumor suppressor that is stabilized and activated by a variety of cellular stress pathways, including the DNA damage response (DDR). Overexpression studies have demonstrated that HCV core, NS3, and NS5A can interact with p53 and modulate its function as a transcriptional activator. However, the impact of HCV infection on p53 activity remains undefined, primarily because cell-lines that are most permissive for HCV (e.g., Huh-7 cells and their derivatives) lack functional p53.

Methods: HepG2 cells express wild-type p53 and support HCV replication upon ectopic expression of the HCV co-factor microRNA-122 (miR-122), but only a minority of cells become infected. To monitor p53-dependent DDR signaling in HCV-infected cells vs. uninfected bystander cells, a multicolor staining approach was combined with flow cytometry detection.

Results: Accumulation of p53 was blocked in HCV-infected HepG2/miR-122 cells following treatment with the DNA damaging agent etoposide. Consistent with this finding, expression of p21 (a p53-dependent gene) was also abrogated in HCV-infected cells. Analyses of mRNA levels demonstrated that p53 transcript levels were not different in infected vs uninfected cells. HCV infection also blocked p53 accumulation following treatment with proteasome inhibitors (MG115 or epoxomicin) or with nutlin3, a specific inhibitor of MDM2, the E3 ubiquitin ligase that normally mediates p53 degradation.

Conclusions: HCV infection can block p53-dependent DDR signaling. Inhibition of p53 accumulation following DNA damage is post-transcriptional and proteasome- and MDM2-independent. These findings provide the first evidence for p53 inhibition during HCV infection and thus reveal a direct mechanism whereby HCV may contribute to hepatocarcinogenesis.

12. Role of Histone H3.3 in the Establishment and Maintenance of KSHV Latency

Hong Seok Choi[1], Viacheslav Morozov[2], Vaibhav Jain[1], Kevin Brulois[4], Peter Turner[1], Jianhong Hu[3], Alexander Ishov[2] Rolf Renne[1]

[1]Molecular Genetics & Microbiology, University of Florida, Gainesville, Florida, USA; [2]Anatomy and Cell Biology, University of Florida, Gainesville, Florida, USA; [3]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, USA; [4]Stanford University, Palo Alto, California, USA

Background: Kaposi's sarcoma (KS) is the most common oral cancer in human immunodeficiency virus (HIV)-infected patients. AIDS-associated KS is highly frequent in the oral cavity, which is also the major site for KSHV shedding via saliva. KSHV infected tumor cells are predominately latently infected. The viral encoded latency-associated nuclear antigen (LANA) is a multifunctional protein required for latency and also interacts with multiple transcriptional regulators and chromatin modifying factors. Our preliminary ChIP-seq data demonstrate that histone variant H3.3, which is frequently mutated in human malignancies and plays important roles in transcriptional regulation, occupies viral episomes at specific regions that correlate with LANA binding. Furthermore, LANA associates with Daxx and SSRP1/HIRA, chaperone proteins involved in the deposition of H3.3. We hypothesize that H3.3 deposition onto viral episomes is crucial for viral gene expression during latency, and furthermore that this process is in part mediated by LANA's interactions with H3.3 chaperones.

Methods: To address this question, we have used CRISPR-Cas9 to engineer cells that lack Daxx or HIRA. Using the elegant Red-Green-Blue (RGB) BAC16 KSHV system, we have studied the establishment and maintenance of latency, and reactivation. Furthermore, we will compare H3.3 deposition and histone modification by ChIPseq and gene expression in wt and knock-out cells under latent and lytic conditions.

Results: Characterization of RGB-BAC16 infected Hep2 cells revealed that latency is controlled less tightly in both HIRA and Daxx knock-out cells as indicated by an increase in green cells. Gene expression analysis by qRT-PCR revealed higher expression of the immediate early genes RTA, ORF45, and ORF57. Interestingly, in HIRA knock-out but not in Daxx knock-out cells w3e also saw increased LANA promotor activity. ChIP-PCR on selected promoters for H.3.3., H3K4me3, H3K27me3, and LANA occupancy as well as genome-wide ChIPseq analysis is currently ongoing.

Conclusion: Our initial data indicate that genetically disrupting the H3.3 chaperone pathways HIRA and Daxx by CRISPR-Cas9 leads to marked changes in latency control. The long-term goal of this project is to create proof of concept data that modulating histone variant deposition can potentially be harnessed as a novel KSHV-specific therapeutic strategy to tip the balance between latent and lytic replication.

13. Systematic Analysis of KSHV Specific T Cell Responses in Healthy Seropositive Donors Measured by IFN-γ ELISPOT Using Proteome-wide Overlapping Peptides

Romin Roshan, Nazzarena Labo, Wendell Miley, Vickie Marshall, Hannah Perez, Ray Sowder, Matt Trivett, Victor Ayala, Claes Ohlen, David Ott, Denise Whitby

Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, Maryland, USA.

Background: The adaptive cellular immune response to KSHV is less well explored and understood compared to what is known for other human herpesviruses. To date, studies on a limited number of subjects have identified only a few T-cell epitopes from a handful of KSHV antigens, displaying no commonly shared immunodominance. We have recently shown that the antibody responses to KSHV can be highly variable in breadth and depth, suggesting that an unbiased analysis may also facilitate the understanding of the cellular immune response to KSHV.

Methods: We analyzed systematically the IFN-γ response of healthy research donors by ELISPOT. Using over 7,500 overlapping 15-aa peptides, we made 82 pools, each spanning one KSHV ORF. First, PBMC from 12 KSHV seropositive and 4 seronegative donors were tested to detect responses to individual ORFs, and then matrices of partial pools were utilized to identify individual peptides that served as specific T-cell epitopes.

Results: None of the seronegative donors responded to any peptide pool. Two of the 10 KSHV seropositive donors also showed no responses. The remaining 8 had responses ranging widely in depth and breath. The median number of recognized ORFs was 3 (IQR 3-6), and although most were lytic genes, there was very little overlap between subjects. The mean net response intensity for recognized antigens also varied greatly. In five of the responding donors, individual peptides/epitopes were identified for the predominant response: four donors responded to only one peptide per ORF, while one recognized five.

Conclusion: Healthy KSHV infected individuals can display IFN-γ responses to diverse KSHV antigens, consistent with a lack of shared immunodominance; the intensity of responses also varied considerably. Ongoing studies will analyze the cell mediated correlates of immune control, or lack thereof, in healthy individuals and in patients with KSHV associated diseases.

14. Targeting an Immune Kinase to Purge KSHV Persistent Infection

Junjie Zhang[1], Hao Feng[2], Emily R. Feldman[1], Xiangshu Wen[1], Si-Yi Chen[1], Weiming Yuan[1], Scott Tibbetts[3], Pinghui Feng[1]

[1]Department of Molecular Microbiology and Immunology, Norris Comprehensive Cancer Center, University of Southern California, California, USA; [2]Key Laboratory of Protein Chemistry and Developmental Biology of Education Ministry of China, College of Life Sciences, Hunan Normal University, Changsha, Hunan, China; [3]Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida, Gainesville, Florida, USA

The inhibitor of κB kinase epsilon (IKKε) is important for interferon-mediated innate immune defense.[1] We have previously reported that KSHV infection activates IKKε to enable its latent infection via promoting NF-κB activation.[2] Furthermore, IKKε-dependent NF-κB activation also contributes to inflammatory response underpinning the development of KSHV-associated malignancies, such as Kaposi's sarcoma.[3] Activation of nuclear factor of activated T cells (NFAT) is crucial for immune responses and chiefly achieved via dephosphorylation. Countable kinases have been identified to phosphorylate NFAT and negatively regulate NFAT activation. The function of IKKε remains obscure in T cells, despite its abundant expression.

To study roles of IKKε in NFAT activation induced by KSHV vGPCR, we discovered that IKKε potently phosphorylated NFAT and inhibited NFAT activation. Mass spectrometry identified key phosphorylation sites within the regulatory domain of NFAT and a phosphorylation-resistant mutant was more active than wild-type NFAT in activating NFAT and T cell immune response. A recently identified pharmacological inhibitor inactivated IKKε, elevated NFAT activation, and boosted T cell immune response. With murine gamma herpesvirus 68, a model virus for human KSHV and EBV, we found that loss of IKKε elevated CD8 T cell immune response by 2-3-fold and conversely reduced viral latent infection by >50-fold. When CD8 T cells were depleted, viral latent infection was restored to indistinguishable levels in wild-type and IKKε-deficient mice. Adopting the "humanized" BLT (bone marrow-liver-thymus) NSG mouse model for KSHV infection that was recently reported by Dr. Charles Wood's group, we detected robust KSHV persistent infection and CD8 T cell immune response against lytic antigens of KSHV. Given the multiple roles of IKKε in KSHV infection and pathogenesis, small molecules inhibiting IKKε are expected to purge KSHV persistent infection and alleviate KSHV-associated diseases. We are developing small molecules that target IKKε to boost T cell immunity against KSHV infection, and progress will be updated in the meeting.

DAY ONE POSTERS

These highlighted abstracts will be presented on both days. Their abstracts are on pages 47 to 60.

1. A More Representative and Less Biased Approach to Estimating Mortality After Diagnosis of Kaposi Sarcoma Among HIV-Infected Adults in Sub-Saharan Africa in the Era of Antiretroviral Therapy

2. Anti-Tumor Activity of DLX1008, an Anti-VEGF-A Antibody Fragment With Low Picomolar Affinity, in an In Vivo Model of Kaposi Sarcoma

3. Changes in Clinical Context for Kaposi Sarcoma and Non-Hodgkin Lymphoma Among HIV-infected People in the United States

4. Disparities in Cancer Treatment Among HIV-Infected Individuals

5. Does Inflammation at Antiretroviral Therapy Initiation Increase Risk of Early Death and Disease Progression Among HIV-Infected Adults With Kaposi Sarcoma?

6. Expression of HIV-1 Matrix Protein and Correlation With B Cell Lymphoma in HIV-1 Transgenic Mice

7. HO-1 Promotes KSHV Infection of Endothelial Cells Through Inhibition of TLR4 Signaling

8. Immunodeficiency and the Risk of Cervical Intra-epithelial Neoplasia 2/3 and Cervical Cancer: A Nested Case-Control Study in the Swiss HIV Cohort Study

9. HPV16 Infection and Oncogenesis on the Epigenome of Human Tonsil Epithelium

10. Phase I Trial of Cabozantinib (XL184) for Advanced Solid Tumors in Persons With HIV Infection: AIDS Malignancy Consortium (AMC) Trial 087 – Trial in Progress

11. Regulation of p53-Dependent DNA Damage Responses by Hepatitis C Virus Infection

12. Role of Histone H3.3 in the Establishment and Maintenance of KSHV Latency

13. Systematic Analysis of KSHV Specific T Cell Responses in Healthy Seropositive Donors Measured by IFN-γ ELISPOT Using Proteome-wide Overlapping Peptides

14. Targeting an Immune Kinase to Purge KSHV Persistent Infection

15. Breast Cancer in Patients With Human Immunodeficiency Virus (HIV) Infection: The Bronx Experience

Hina Khan, Oleg Gligich, Ana Acuna, Joseph Sparano, Jesus Anampa

Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, New York, USA

Background: Advances in therapeutic options for patients with HIV infection have increased their life expectancy, which also translates in more time exposed to develop malignancies. Breast cancer is the most common cause of cancer and cancer death worldwide among women. There are limited data on the clinico-pathological features, treatment, and outcomes of patients with HIV and breast cancer. Currently, they are being treated, staged, and screened following the same guidelines as for the non-HIV population. More research is needed to understand the impact of HIV infection in patients with breast cancer, and evaluate the need for different screening or treatment guidelines for the management of these patients

Methods: The purpose of this study is to describe the baseline clinico-pathological features of breast cancer in women with HIV infection and to describe their treatment outcomes. Using the clinical looking glass (institutional clinical data-search software), we retrospectively identified patients with HIV infection by ICD9 codes, and then selected the cases also diagnosed with breast cancer between December 1999 and December 2014 at Montefiore Medical Center, a major referral center for breast cancer patients in the Bronx.

Results: A total of 49 patients were included, all of which were females, and 31 patients were African-American. The median age at breast cancer diagnosis was 50 years [range: 22-70 years] and 55.5% were premenopausal, with a median BMI of 24. Evaluation of HIV disease at breast cancer diagnosis revealed a median CD4 count of 565 cells/µL, with CD4 values <200 only in 8.3 % cases [n=4]. Breast cancer characteristics revealed that most patients presented with stage I-II (77%, n=34), while only 7%(n=18) and 5%(n=2) presented with stage III and IV respectively. About 57% tumors were poorly differentiated, 65.1% [n=28] were found to have hormone receptor positive tumors, HER2/neu overexpression was present in 36% [n=15] cases, and 23% tumors [n=10] were triple negative. Comorbidities were measured as Charlson index (we did not include breast cancer in the calculation, normal range 0-40); the median index was 7 [range: 0-14]. Overall disease recurrence rate was 10% [n =5]. The median time from HIV infection to breast cancer diagnosis was 78 months (1-288 months). An unusually high death rate of 35% [n=17 deaths] was seen; 12 of these patients met the criteria for AIDS. The median PFS until last date of follow-up to date was 40 months. Among the 30 cases treated with chemotherapy, adverse events were documented in 46.6% [n=14]. Almost 60% received granulocyte-colony stimulating factor support [n=18]. Twenty percent [n=6] of the patients treated with chemotherapy required some form of dose reductions, delay, or interruption of treatment due to adverse events. Targeted therapy with Herceptin was well tolerated.

Conclusions: Our study showed that breast cancer in HIV patients presented at a younger age than the national average. While HER2 targeted therapy and hormonal therapy are well tolerated, standard dose systemic chemotherapy can cause significant side effects in HIV infected patients being treated for breast cancer. Our study has several limitations due to its retrospective design; therefore, future research is required to confirm our results. Caution needs to be exercised in weighing the benefits of chemotherapy against the risks of complications from treatment.

16. A Community-Based, Respiratory Biomarker Feedback Intervention to Increase Smoking Cessation Resources Uptake Among Low Income Persons Living With HIV/AIDS: Results of a Randomized Controlled Feasibility Trial

Jack E. Burkhalter[1], Erica I. Lubetkin[2], John Guidry[3], Ingmar Gorman[4], Julie Kumar[1], James Sullivan[1], Marc Feinstein[1], Mohammed Mujawar[1], Kieth Siegel[5], Yuelin Li[1]

[1]Memorial Sloan Kettering Cancer Center, New York, New York, USA; [2]The City College of New York, New York, New York, USA; [3]GMHC, New York, New York, USA; [4]The New School, New York, New York, USA; [5]Mount Sinai School of Medicine, New York, New York, USA

Background: U.S. prevalence of smoking among persons living with HIV/AIDS (PLWHA) is nearly double that in the general population (37.6% vs. 20.6%), and quit rates are lower among PLWHA.[1] Rates of tobacco-related cancers, i.e., lung and oral cancers, among PLWHA are rising, and respiratory and cardiovascular diseases in an aging HIV/AIDS population are a health care challenge. To address this tobacco-related health disparity, innovative treatments are needed in diverse settings to broaden the reach of evidence-based tobacco cessation resources. Such settings include not only medical clinics but community-based AIDS agencies (ASOs), which provide financial, housing, food, transportation, and psychosocial services to PLWHA and constitute a unique tobacco intervention venue.

Methods: A pilot randomized controlled trial examined the feasibility of a community-based intervention to increase uptake of free/low cost public health smoking cessation resources in low income PLWHA smoking ≥20 cigarettes/week. N=52 PLWHA recruited at 3 community-based ASOs were randomized to either Treatment as Usual (TAU, n=27: brief counseling, print materials, offer to refer to state quitline) or AIR (n=25: TAU + biomarker feedback using CO level, respiratory symptoms, and spirometry lung age, delivered within a motivational framework). One in-person session was provided at participants' home ASO by trained ASO staff (TAU) or tobacco treatment clinicians (AIR). New York State provides free quitline support and nicotine patches, and Medicaid covers quit medications. Primary outcome was feasibility, defined as ≥67% treated and retained at 1-month follow-up. Secondary outcomes included intervention impact, motivation changes, quitline utilization, and treatment satisfaction.

Results: Trial participants' mean age was 53; 52% were women, 71% were African American, and 52% had high school education or less. Most (71%) smoked daily a mean 11 cigarettes/day, and 65% smoked within 5 minutes of waking. Primary outcomes demonstrated feasibility: (1) reached accrual goals in 3 months; (2) delivered treatment to 85%; (3) exceeded (81%) the threshold feasibility indicator of 67% treated and retained at 1-month follow-up. Secondary analyses showed that verified uptake of quitline services was 40% for TAU and AIR. Motivation to quit smoking from baseline to immediately post-intervention was higher in the AIR arm ($p<.05$), but at 1 month the two study arms did not differ. AIR participants whose assessed lung age was older compared with their birth age were more likely to take up quitline services ($p<.05$). Satisfaction with AIR was higher than for TAU ($p=.01$). Qualitative interviews verified satisfaction ratings and the impact of spirometric testing and indicated desire for more ongoing counseling contact.

Conclusion: These findings support treatment acceptability, feasibility, and the motivational role of respiratory biomarker feedback. This trial is unique in targeting low income PLWHA who smoke and non-medical community ASOs and their staff as providers, facilitating future dissemination. Future work will examine intervention modifications based on qualitative feedback and entail conducting a larger efficacy trial.

17. Anal Cancer Screening in Men Who Have Sex With Men in the Multicenter AIDS Cohort Study

Gypsyamber D'Souza[1], Alicia Wentz[1], Dorothy Wiley[2], Nisha Shah[1], F. Barrington[1], Teresa Darragh[3], N. Joste[4], Michael Plankey[5], Sushell Reddy[6], Elizabeth Breen[7], Stephen Young[8], Ross Cranston[9]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA; [2] University of California, Los Angeles, California, USA; [3]University of California, San Francisco, San Francisco, California, USA; [4]University of New Mexico Health Sciences Center, Albuquerque, New Mexico, USA; [5]Georgetown University Medical Center, Washington, D.C., USA; [6]Northwestern University, Chicago, Illinois, USA; [7]University of California, Los Angeles, Los Angeles, California, USA; [8]Tricore Reference Laboratories. Albuquerque, New Mexico, USA; [9]University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Background: To evaluate the prevalence of anal cytology (ACyt) abnormalities among HIV-infected and HIV-uninfected men who have sex with men (MSM)

Method: Multicenter cohort study of 723 HIV-infected and 788 HIV-uninfected MSM with ACyt, with a second ACyt collected 2 years later. Referral for high-resolution anoscopy (HRA) was suggested for abnormal ACyt. ACyt samples were collected using a polyester swab and liquid cytology media and read in a central laboratory.

Results: Prevalence of any abnormal ACyt was 25% in HIV-uninfected MSM, and increased to 38%, 41%, and 47% among HIV-infected MSM with current CD4+ T-cell counts ≥500, 350-499, and <350 cells/mm^3 ($p<0.001$), respectively. Anal HPV16 DNA was also more common in HIV-infected than HIV uninfected MSM (26% vs 16%, $p<0.001$). Abnormal baseline ACyt together with prevalent HPV16 DNA detection was present in only 6% of HIV-uninfected MSM compared to 18% of HIV-infected MSM with current CD4<350, $p<0.001$.

Among HIV-infected men, half the men with LSIL and 78% of men with ASC-H/HSIL had lower grade ACyt findings 18-30 months later ("regressed"). However, 22% of untreated HIV-infected men with ASC-H/HSIL cytology maintained that same grade of cytology at their second test approximately 2 years later, and 4% with LSIL "progressed" to ASC-H/HSIL. Abnormal ACyt had high sensitivity (96%) but low specificity (17%) for $_b$HSIL.

Conclusions: Prevalence of abnormal ACyt remains elevated in HIV-infected men during the current ART era.

18. Assessing the Effects of Storage Time and Cancer Type on the Quality of Formalin Fixed Paraffin Embedded Tissues: A Fit-for-Purpose Study

Anna Yakovleva, Jordan Plieskatt, Sarah Jensen, Nidia Kitice, Razan Humeida, Jonathan Lang, Sylvia Silver, Jeffrey Bethony

George Washington University, Washington, D.C., USA

Background: Fit-for-Purpose (FFP) studies on the integrity of cellular products extracted from biobanked specimens such as Formalin Fixed Paraffin Embedded (FFPE) tissue are limited. The available studies compare the performance of extraction kits or sample processing methods. Limited consideration has been given to factors such as the duration of storage time and the cancer type on the "fitness" of these cellular products for purposes such as proteomics, or Next Generation Sequencing (NGS). Even less consideration has been given to sampling and statistical modeling of these effects with previous studies composed of small sample sizes and utilizing descriptive statistics (means, standard deviations, etc.). In the current study, we attempt to measure the effects of "storage time" and "cancer" on the integrity and the quality of proteins and nucleic acids from FFPE using a stratified random sampling method and mixed effects modeling.

Methods: One hundred and twenty (n = 120) FFPE cases were selected by first stratifying the FFPE inventoried at the George Washington University AIDS and Cancer Specimen Resource Regional Biospecimen Repository (GWU-ACSR RBR) by storage time (10-year intervals) and cancer type (adenocarcinoma, papillary, and squamous carcinoma) and then randomly sampling within these strata to achieve a balanced design. Half of the sampled cases were stored for >11 years (1996-2001) and the other half stored for <11 years (2002-2013). Initial tissue sections were discarded from the FFPE block followed by 10 micron sections taken for each purification workflow. DNA, RNA, and micro-RNA (miRNA) were purified using the Qiagen QIAamp DNA FFPE, RNeasy FFPE, and miRNAasy FFPE kits, respectively, using the 10 micron tissue sections. Extracted nucleic acids were evaluated with both spectroscopy (Molecular Devices, SpectraDrop Micro-Volume Microplate) and BioAnalyzer 2100 (Agilent Technologies) or 2200 TapeStation (genomic DNA only). Proteins were isolated from the FFPE using Qiagen Qproteome Kit.

Results: This is a work in progress. To date, we have evaluated sample absorbance at their respective wavelength(s), absorbance ratio(s) where applicable, concentration, and integrity of sample where applicable (e.g., RNA Integrity Number or RIN or DNA Integrity Number or DIN). Yield, product purity, and product quality were scored as "quality attributes" and assembled into a database to be assessed with cumulative logit models in the near future.

Conclusions: Results will be presented that show the utility of our statistical approach in combination with established extraction protocols to assess the quality of FFPE samples. Special emphasis was placed on using extraction routines that, though of "moderate-throughput," were consistent, had a quality output for sample analysis, and could be easily implemented in other laboratory or institutional settings. To date, we have assessed product yield in combination with the integrity score on RNA, DNA, miRNA, and protein to identify the effects of storage time and carcinoma type on the quality of cellular products from FFPE. We now plan to assemble a mixed effects model to determine the magnitude of the effects associated with duration of storage and/or carcinoma type on sample quality. We also plan to plot the quality scores of all the samples, encompassing the full time period of consideration, to facilitate identification of possible degradation trends in the quality of the products.

19. Association Between HIV and Persistent HPV Infections Among Nigerian Women

Sally Adebamowo[1,2,3], Ayotunde Famooto[3], Eileen Dareng[3,4], Toyosi Olawande[3], Olayinka Olaniyan[5], Richard Offiong[6], Clement Adebamowo[2,3,7]

[1]Center for Research on Genomics and Global Health, National Genome Research Institute, Bethesda, Maryland, USA; [2]Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA; [3]Institute of Human Virology Nigeria, Abuja, Nigeria; [4]University of Cambridge, Cambridge, United Kingdom; [5]National Hospital Abuja, Abuja, Nigeria; [6]University of Abuja Teaching Hospital, Abuja, Nigeria; [7]University of Maryland, Baltimore, Maryland, USA

Background: The incidence of cervical cancer has remained stable in HIV positive women but the prevalence, persistence, and multiplicity of high-risk HPV infection appears different comparing HIV positive to HIV negative women. We examined the association between HIV and prevalent and persistent HPV infections among women in a prospective cohort in Nigeria.

Methods: We enrolled women presenting at cervical cancer screening programs in Abuja, Nigeria, between 2012 and 2014 and collected information on demographic characteristics, risk factors of HPV infection, and cervical exfoliated cells samples at baseline and 6 and 12 month follow-up visits. DNA enzyme immunoassay (DEIA) and Roche Linear Array HPV Genotyping Test® were used to characterize HPV. Persistent HPV infection was defined as positive results on 2 consecutive DEIA tests. We used logistic regression models to estimate the association between HIV and the risk of HPV infections.

Results: Among the 1020 women enrolled, the mean age (±SD) was 37(8) and 44% and 56% were HIV positive and HIV negative, respectively. The prevalence of any HPV infection was 53% (58% among HIV positive; 42% among HIV negative, p-value <0.001); the prevalence of persistent HPV infection was 17% (78% among HIV positive; 22% among HIV negative, p-value <0.001); The multivariate odds ratio (OR) and 95% confidence interval (95 % CI) was 3.22 (95% CI 2.40 – 4.32, p-value <0.001) for any HPV infection and 5.52 (95% CI 3.61 - 8.44, p-value <0.001) for persistent HPV infections, comparing HIV positive to HIV negative women, adjusted for variables that reached statistical significance in univariate analyses: age, age at sexual initiation, total number of lifetime sexual partners, marital status, and level of education.

Conclusions: HIV infection is associated with increased risk of any HPV and persistent HPV infections. Previously, we reported that HPV35 (8.7%) and HPV56 (7.4%) were the most prevalent hrHPV among HIV positive women, while HPV52 and HPV68 (2.8%, each) were the most prevalent hrHPV types among HIV negative women, from a subset of this population. As data accrue, we will present the results of the specific HPV types at the forthcoming conference.

20. The Burden of Human Papilloma Virus Associated Cancers in Nigeria, 2012-2014

Elima Jedy-Agba[1,2], Eileen Dareng[1,3], Emmanuel Oga[1,4], Michael Odutola[1], Sally Adebamowo[1,5], Festus Igbinoba[6], Theresa Otu[7], Emmanuel Ezeome[8], Ima-Obong Ekanem[9], Ramatu Hassan[10], Clement Adebamowo[1,4]

[1]Institute of Human Virology, Abuja, Nigeria; [2]Department of Non-communicable Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, United Kingdom; [3]Center for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, United Kingdom; [4]Department of Epidemiology and Public Health, University of Maryland, Baltimore, USA; [5]Center for Research on Genomics and Global Health, National Human Genome Research Institute, Bethesda, Maryland, USA; [6]National Hospital Abuja, Nigeria; [7]University of Abuja Teaching Hospital Gwagwalada, Nigeria; [8]University of Nigeria Teaching Hospital Enugu, Nigeria; [9]University of Calabar Teaching Hospital, Calabar, Nigeria; [10]Federal Ministry of Health, Abuja, Nigeria

Background: The Human Papilloma Virus (HPV) is a necessary cause of cervical cancer and is associated with other cancers including vulval, vaginal, anal, penile, and oropharyngeal cancers. In this study, we evaluate the burden of HPV associated cancers using data from population based cancer registries (PBCR) in Nigeria.

Methods: We obtained data on cancers that are considered to be associated with HPV based on the IARC monograph 100b including cancers of the Cervix (C.53), Vulva (C.51), Vagina (C.52), Anus (C.21), Penis (C.60), and Oropharynx (C.01,C.09,C.10) from PBCR in Abuja (Central Nigeria), Enugu (Eastern Nigeria), and Calabar (South Eastern Nigeria). Previous literature using prevalence data and relative risks suggest that the Population Attributable Fractions (PAFs) for HPV associated cancers in developing countries were Cervical (100%), Vulval and Vaginal (40%), Anal (90%), Oropharynx (12%) in women, and Penile (40%), Anal (90%), and Oropharynx (12%) in men.

Results: Among women, the 3 PBCR reported a total of 2,986 cases of cancer between 2012 and 2014 with 493 HPV associated cancers contributing 16.5% of the total cancers. Of the 493 HPV associated cancers, 430 were cervical cancers, 27 vulval cancers, 20 anal cancers, 8 vaginal cancers, and 8 oropharyngeal cancers. Of these, 463 (94%) were attributable to HPV infection. The PBCR reported 1,875 cancers in men between 2012 and 2014. Of these, 40 were HPV associated cancers including 22 anal cancers, 16 oropharyngeal cancers, and 2 penile cancers constituting 2% of all cancers in men. Some 23 (57.5%) of the 40 HPV associated cancers were attributable to HPV infection.

Conclusion: Cervical and vulva cancers were the most common HPV associated cancers among Nigerian women and anal cancer was the commonest HPV associated cancer in Nigerian men. Our findings suggest that approximately 57.5% of all HPV associated cancers in men and over 90% of all HPV associated cancers in women can be prevented if HPV infection is eliminated.

21. Cancer Mortality Among HIV-Infected Individuals in Botswana

Scott Dryden-Peterson[1,2,3], Gita Suneja[4], Heluf Medhin[5], Memory Bvochora-Nsingo[6], Mukendi Kayembe[5,7], Neo Tapela[1,2,5], Shahin Lockman[1,2,3]

[1]Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana; [2]Brigham and Women's Hospital, Boston, Massachusetts, USA; [3]Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA; [4]University of Utah, Salt Lake City, Utah, USA; [5]Botswana Ministry of Health, Gaborone, Botswana; [6]Department of Oncology, Gaborone Private Hospital, Gaborone, Botswana; [7]National Health Laboratory, Gaborone, Botswana

Background: With declining mortality due to infections, cancer has become the leading cause of death among HIV-infected individuals in high-income countries. However, in sub-Saharan Africa, where more than two-thirds of HIV infections occur, the relative contribution of infection and malignancy to HIV-associated mortality is unknown. We sought to estimate cancer mortality since the availability of antiretroviral treatment (ART) in Botswana, and compare with mortality due to tuberculosis (TB), the leading infectious cause of death in sub-Saharan Africa.

Methods: Incidence by cancer type was estimated from 8479 incident cases from the Botswana National Cancer Registry during the period of ART expansion, 2003-2008. We utilized Poisson regression in an inverse probability weighted population with known HIV status and projected cancer incidence through 2013. Cancer mortality was estimated using parametric Weibull models from observed survival in a separate prospective cancer cohort in Botswana (2010-2015). Survival probabilities for each cancer type were assumed to be constant during the study period and all deaths were attributed to cancer. We utilized estimates from the WHO|Global TB Program (derived from Botswana government data) to estimate TB-HIV deaths.

Results: A total of 808 patients with HIV and cancer followed for median of 12.2 months (IQR 6.1 to 24.3 months) contributed to survival estimates (1.2% loss-to-followup). Estimated 5-year survival was low: cervix 3.9%, head and neck 4.4%, breast 19.3%, non-Hodgkin lymphoma 39.7%, Kaposi sarcoma 52.1%, and combined other sites 15.1%. Mortality due to Kaposi sarcoma declined over the study period (4.2%, 95% CI 5.0 to 3.3%), but cervical cancer mortality increased (13.3%, 95% CI 11.7 to 14.9). Overall cancer mortality increased (1.2%, 95% CI 0.7 to 3.1%) while TB mortality declined substantially between 2003 and 2013. In 2013, projected cancer mortality (293 per 100,000, 95%CI 264-331) approximated projected TB mortality (324 per 100,000, estimate range 241-419).

Conclusions: Despite ART coverage exceeding 90%, mortality due to cancer in HIV-infected individuals has increased in Botswana and now likely exceeds mortality due to TB. Cervical cancer mortality is rising sharply. Interventions to reduce cancer risk, establish screening programs, and improve access to treatment are urgently needed for HIV-infected individuals.

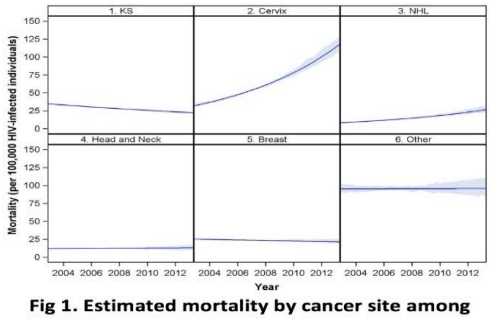


Fig 1. Estimated mortality by cancer site among HIV-infected individuals in Botswana.

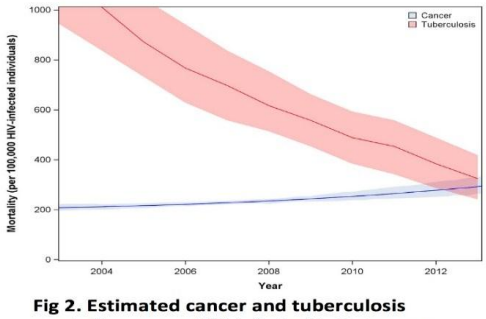


Fig 2. Estimated cancer and tuberculosis mortality among HIV-infected in Botswana.

22. Cancer Presentation and Outcomes of Patients With HIV vs. AIDS

Surbhi Grover[1], Yuezhou Jing[2], James Goedert[3], Michael J. Silverberg[4], Kate Salters[5], Michael A. Horberg[6], Chad Achenbach[7], Angel Mayor[8], Keri Althoff[2], for the North American AIDS Cohort Collaboration on Research and Design

[1]Abramson Cancer Center, University of Pennsylvania, Philadelphia, Pennsylvania, USA; [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA; [3]National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA; [4]Kaiser Permanente Northern California, Oakland, California, USA; [5]BC Centre for Excellence in HIV/AIDS, Simon Fraser University, Vancouver, British Columbia, Canada; [6]Kaiser Permanente Mid-Atlantic States, Rockville, Maryland, USA; [7]Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA; [8]Universidad Central del Caribe, Bayamón, Puerto Rico, USA

Background: HIV-associated immune suppression has been linked to an increased risk of certain cancers; this risk may be higher in patients with a history of AIDS-defining illnesses (ADIs), which indicates at least one prior episode of advanced HIV disease progression. Cancer stage at presentation and the risk of death after cancer diagnosis in HIV-infected adults with vs. without ADI prior to cancer diagnosis has not been fully described in the modern treatment era. We aim to describe cancer stage and risk of death by ADI status at type-specific cancer diagnosis in HIV-infected adults who have successfully linked into HIV care in North America.

Methods: HIV-infected adults (≥18 years of age) diagnosed with anal, oropharynx (OP), cervical, lung cancer, or Hodgkin lymphoma from January 2000 to December 2010 in the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) were included. Patient characteristics, stage of cancer at diagnosis, and mortality rates and adjusted rate ratios (using Poisson regression models) were estimated.

Results: Of the 81,865 participants reviewed for cancer outcomes, 814 had a cancer diagnosis (162 with anal cancer, 5 with cervical cancer, 444 with lung cancer, 114 with OP cancer, and 89 with Hodgkin's lymphoma); 642 (79%) of whom had a history of ADI at cancer diagnosis. Regardless of cancer type, the majority of patients were taking ART at the time of cancer diagnosis time since ART initiation ranged from median 1.2 [interquartile range 0.3, 3.7] years for cervical cancer to 5.3 [3.3, 7.9] for anal cancer). All 5 cervical cancer cases had a prior ADI, unlike the other cancers. For all the type-specific cancers, cancer stage at diagnosis was not different between those with and without a prior ADI. Mortality rates after cancer diagnosis were higher in those with an ADI prior to cancer for all type-specific cancers (Figure 1), and rates increased with increasing cancer stage at diagnosis. After adjustment for age, sex, race, ART use, CD4 count, and cancer stage at diagnosis, the mortality rate ratios comparing those with and without a history of ADI at cancer diagnosis were as follows: anal: 1.5 (0.8, 2.6); lung: 1.6 (1.3, 2.0); OP: 1.9 (1.0, 3.7); and Hodgkin's lymphoma: 1.2 (0.5, 2.8).

Conclusion: There was no difference in cancer stage at diagnosis among HIV-infected adults with or without a prior ADI; however, those with a history of ADI had higher mortality rates compared with those who did not, particularly for lung and OP cancers that remained statistically significant after accounting for confounders. This suggests more aggressive biology of disease in patients with ADIs possibly due to greater degree of immunosuppression or worse performance status.

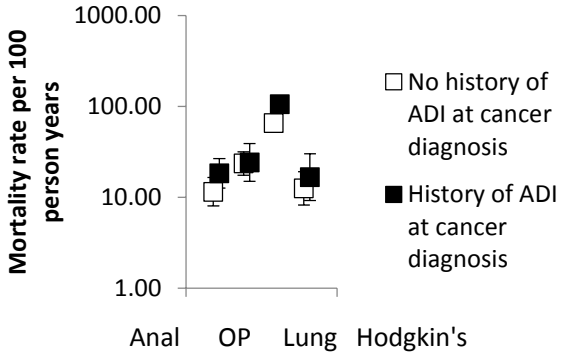


Figure 1: Mortality rates (and 95% confidence intervals) after anal, OP, lung, and Hodgkin's lymphoma cancer diagnoses, by a history of AIDS-defining illness at cancer diagnosis.

Cervical cancer is not included on this figure as there were no deaths among the 5 patients with cervical cancer diagnoses. The confidence intervals around the estimates for lung cancer are so small that they cannot be seen outside of the mark for the point estimate.

23. Cancer Treatment in HIV-Infected and Uninfected Individuals With Colorectal Cancer

Gita Suneja[1], Kathleen McGinnis[2], Roger Bedimo[3], Kristina Crothers[4], Cynthia Gibert[5], Lesley Park[6], David Rimland[7], Meredith Shiels[8], Eric Engels[8], Keith Sigel[9]

[1]University of Utah, Salt Lake City, Utah, USA; [2]VA Connecticut Healthcare System, West Haven, Connecticut, USA; [3]VA North Texas Healthcare System, Dallas, Texas, USA; [4]University of Washington School of Medicine, Seattle, Washington, USA; [5]Veterans Medical Center, Washington, D.C., USA; [6]Stanford University School of Medicine, Stanford, California, USA; [7]Atlanta VA Medical Center, Atlanta, Georgia, USA; [8]National Cancer Institute, Bethesda, Maryland, USA; [9]Mount Sinai Hospital, New York, New York, USA

Background: Prior work has demonstrated that HIV-infected (HIV+) individuals are less likely to receive cancer treatment compared with uninfected individuals; however, the impact of insurance coverage is not known. The aim of this study was to examine patterns of cancer care in U.S. veterans with colorectal cancer with and without HIV infection in the setting of universal access to health care.

Methods: We used data from the Veterans Aging Cohort Study (VACS) linked to the Veterans Affairs Central Cancer Registry, a national registry of cancer cases diagnosed or treated in the Veterans Health System, to identify HIV+ and uninfected (HIV-) adults with incident colon and/or rectal cancer from October 1999 to December 2012. HIV+ cases were matched on demographics to HIV- cases. Cancer treatment was defined as chemotherapy, radiotherapy, surgery, or a combination of the three treatments, and was identified using procedure codes and Pharmacy Benefit Management (PBM) datasets. We examined demographic and clinical variables, including age at cancer diagnosis, sex, race/ethnicity, history of tobacco use, cancer stage, co-morbidities, and for HIV-infected patients, viral load, CD4 count, and antiretroviral therapy use (ARV). Demographic and clinical characteristics were compared by HIV status using chi-square tests and t-tests. Logistic regression models were developed to assess differences in treatment by HIV status. Odds ratios with 95% confidence intervals for univariate models were generated.

Results: We identified 344 HIV- and 137 HIV+ colorectal cancer cases. Demographic characteristics were similar by HIV status; overall median age was 60 years, 99% were male, 39% were white, 53% African-American, and 9% Hispanic/other. 53% were current smokers. Prevalence of diabetes and hypertension were higher in the HIV- group (diabetes 47.7% vs. 32.1%, p=0.002; hypertension 70.6% vs. 53.3%, p<0.001). Local stage cancer was more common among uninfected patients (42.4% vs. 29.2%), and regional or distant stage was more common among HIV+ patients (46.7% and 21.2% vs. 38.7% and 16.3%, respectively); however, differences were not significant (p=0.06). Among those with HIV infection, 80% had CD4 count ≥200, 68% had suppressed viral load (<500), and 72% were on ARV. In unadjusted models, HIV infection was significantly associated with chemotherapy use (52% HIV+ vs. 41% HIV- received chemotherapy; OR 1.57, 95%CI 1.05-2.33), but not with radiotherapy (18% HIV+ vs. 12% HIV-; OR 1.53, 95%CI 0.88-2.63), surgery (80% HIV+ and HIV-; OR 0.97, 95%CI 0.59-1.60), or any cancer treatment (93% HIV+ vs. 88% HIV-; OR 1.67, 95%CI 0.81-3.44).

Conclusions: In contrast to prior population-based studies, we did not observe differences in colorectal cancer treatment rates by HIV status. Treatment rates may be similar due to universal access to health care within the VA system.

24. Cervical Cancer Survival in a Resource-Limited Setting—North Central Nigeria

Jonah Musa[1], Joseph Nankat[1], Chad J. Achenbach[2], Iornum H. Shambe[1], Babafemi O. Taiwo[2], Barnabas Mandong[3], Atiene S. Sagay[1], Robert L. Murphy[2]

[1]Department of Obstetrics and Gynecology, University of Jos/Jos University Teaching Hospital, Jos, Plateau State, Nigeria; [2]Department of Medicine, Division of Infectious Diseases, Center for Global Health, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA; [3]Department of Pathology, University of Jos/Jos University Teaching Hospital, Jos, Plateau State, Nigeria

Background: Organized cervical cancer screening services are presently lacking and Invasive cervical cancer (ICC) poses management challenges in most gynecologic units in Nigeria. We evaluated outcomes of ICC treatment at Jos University Teaching Hospital (JUTH) to better understand factors associated with survival in similar resource limited settings.

Methods: We performed a retrospective cohort study with prospective followup data to estimate time from diagnosis to mortality among women diagnosed with ICC at JUTH. Women who were diagnosed with ICC between January 2011 and May 2013 were followed up after initial management at JUTH and referral for chemo-radiation elsewhere. The main outcome measured was all-cause mortality rate and overall survival (OS). We conducted Cox proportional hazard regression to assess factors associated with death.

Results: During the study period, 65 histologically confirmed ICCs were followed up. The median age of the cohort was 50 years with a median parity of 7. The HIV prevalence in the cohort was 8.2% and the majority (72.3%) were diagnosed at advanced stages (AD) of ICC. Simple total abdominal hysterectomy (TAH) was performed in 38.9% of patients who were diagnosed at early stage disease (ED). After a cumulative followup of 526.17 months, 35 deaths occurred with an overall death rate of 79.8 per 100 women-years. Death rate was significantly higher among women with AD (rate ratio=4.7) and those with baseline anemia (rate ratio= 4.8). We also found a significantly higher hazard of death in women with AD (HR=3.3) and baseline anemia (HR=3.0). In the subgroup of women who were diagnosed with ED, the OS was significantly higher for those who had TAH compared to those who did not (26.5 versus 11.6 months, respectively).

Conclusion: The HIV prevalence among ICC patients was higher than the Nigerian national average. The HIV status did not significantly affect survival of the cohort. AD and baseline anemia were independently associated with higher death rate. TAH performed in patients diagnosed at ED stages may improve survival in settings lacking standard chemo-radiation treatment facilities.

25. Clinicians' Perceptions and Practices Following a Training Program for the Early Detection of Kaposi Sarcoma in Uganda

Miriam Laker-Oketta[1], Lisa Butler[2], Philippa Makanga[1], Merridy Grant[3], Toby Maurer[4], Edward Mbidde[5], Jeffrey Martin[4]

[1]Infectious Diseases Institute, Kampala, Uganda; [2]Harvard Medical School, Boston, Massachusetts, USA; [3]Centre for Rural Health, Durban, South Africa; [4]University of California, San Francisco, San Francisco, California, USA; [5]Uganda Virus Research Institute, Entebbe, Uganda

Background: We developed the Early Detection of Kaposi Sarcoma (EDKS) Training Program to enhance understanding about KS amongst front-line clinicians in government-owned health facilities in Uganda. In a one-day session, training focused on promoting early diagnosis of KS through inquiring with patients about skin changes ("Ask"), skin and oral exam ("Look"), and skin punch biopsy ("Test"). While clinicians demonstrated learning on tests administered at the end of the session, it is unknown how well such training affects clinicians' subsequent practices and how clinicians perceived the training.

Methods: Clinicians who had participated in the EDKS training were invited to undergo an in-depth qualitative interview if they were still practicing in any facility in the respective districts, and had performed at least one of the following: skin punch biopsy, referral for skin punch biopsy, or contacted the EDKS team about a patient with suspected KS. Interviews focused on clinicians' experiences with early KS diagnosis practices, and their perception of the EDKS training. Audio recordings of the interviews were transcribed and subsequently analyzed using an inductive approach with descriptive thematic coding (NVivo qualitative analysis software version 10).

Results: We interviewed 20 clinicians who had taken part in the EDKS training: 15 (75%) Clinical Officers (equivalent to U.S. nurse practitioners), 3 (15%) nurses, and 2 (10%) nursing assistants. Eleven (55%) were men, and their median age was 35 years (IQR: 28 to 48). In the realm of "Ask," all the clinicians were able to incorporate specific questioning of their patients about their skin into their daily practice.

"In the past, we used to ask, 'Do you have any problems?' Now I ask, 'Have you noticed anything?' because we Africans think that a problem is what may hinder you from doing your daily work." (Clinical Officer #10758)

Yet, sometimes they were unable to ask about the skin issues because of large patient load. *"Most of the times we get very many patients and the queues are so long. So asking questions regarding KS may not be applicable to all clients …." (Clinical Officer #10745)*

Regarding "Look," 8 (40%) of the clinicians reported that complete skin exams were challenging. *"Well, it could probably be my personal weakness but exposing a patient's body, when he feels he is okay, is culturally difficult." (Clinical Officer #10494)*

Concerning "Test," many clinicians expressed fear of uncontrollable bleeding when performing biopsies. "*What I had feared in the beginning was, 'What if it bleeds too much.' But, when I followed the procedure —insert the Gelfoam — there wasn't any serious problem." (Clinical Officer #10494)*

Delays in getting skin biopsy results were a common complaint. *"Getting back the results has been a challenge because out of the 8 samples we sent, we got only one back. We got that result through constant reminders." (Nurse Midwife #10585)*

In general, most clinicians found the training useful but several felt the 1-day session was insufficient. *"Before the training, I had no knowledge concerning KS and I had not been doing biopsies. It is because of the training; otherwise, I would not have known which patient has KS." (Clinical Officer #10075) "The length was very short ….I think it needs more time than the one it was given." (Clinical Officer #10075)*

Conclusion: Amongst primary care practitioners in Uganda, a 1-day training program — featuring an "Ask, Look, Test" approach — was favorably perceived and enhanced screening practices for the early detection of KS. More research is needed in determining how to alter external influences (e.g., cultural views on undressing and pathology services) and establishing whether the training can reduce KS morbidity and mortality at the population level.

26. Cohort Profile: African Collaborative Center for Microbiome and Genomics Research (ACCME) Study

Sally N. Adebamowo[1,2,3], Eileen O. Dareng[3,4], Ayotunde Famooto[3], Michael Odutola[3], Olayinka Olaniyan[5], Rasheed Bakare[6], Clement A. Adebamowo[2,3,7], and the H3A Consortium.

[1]Center for Research on Genomics and Global Health, National Genome Research Institute, Bethesda, Maryland, USA; [2]Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA; [3]Institute of Human Virology Nigeria, Abuja, Nigeria; [4]University of Cambridge, Cambridge, United Kingdom; [5]National Hospital Abuja, Abuja, Nigeria; [6]University of Ibadan, Oyo, Nigeria; [7]University of Maryland, Baltimore, Maryland, USA

Background: Cervical cancer is the second commonest cancer in Africa. Much remains unknown about the prevalence and pathogenicity of human papilloma virus (HPV) types and mechanism of disease, and there is a need for new biomarkers for screening programs.

Methods: ACCME is a multicenter prospective cohort study of host germline, somatic and HPV genomics and epigenomics, and vaginal microenvironment, and their association with cervical cancer in 10,000 HIV negative women in Nigeria. Data on demography, lifestyle, medical history, serum, germline DNA, HPV genotype, and vaginal pH are collected at baseline and during follow-up visits every 6 months. Samples of exfoliated cervical cells are analyzed for high-risk HPV with Roche LINEAR ARRAY® and vaginal bacterial composition and abundance are characterized by deep sequencing of barcoded 16S rRNA gene fragments (V4) on Illumina MiSeq platform. Colposcopy and biopsy are conducted on participants with clinical lesions and those with persistent high-risk HPV infections.

Results: By July 2015, ~8500 participants had been enrolled unto the cohort. The mean (SD) age of the study participants at baseline was 40 (10) years. Most of the participants were married (76%), attended university (44%), and had professional jobs (37%). All the study participants have had vaginal sex, 17% have had oral sex, and only 2% have ever had anal sex. We found 30% of the study participants were HPV positive and 70% were HPV negative. The mean (SD) vaginal pH in the study population was 5.2 (0.5). Baseline enrollment will be completed by October 2015. Further analyses to characterize high-risk HPV types and determine persistence will be conducted at each follow-up visit. Also, characterization of cervical cytokines and vaginal microbiome will be conducted after the follow-up visits for all participants have been conducted.

Conclusions: ACCME is a paradigm for translational research in biomarker discovery that addresses high-impact public health challenges affecting women's health in Africa and the rest of the world.

27. Comparison of Gleason Scores in HIV Infected vs. Uninfected Patients With Prostate Adenocarcinoma in the VACS-9 Utilizing the Voogo Text Extraction Tool

Roxanne Wadia[1,2], Lesley S. Park[3], Cynthia Brandt[1,2], Michal Rose[1,2], Cynthia Gibert[4,5], David Rimland[6,7], Maria Rodriguez-Barradas[8,9], Amy Justice[1,2] for the VACS Program Team

[1]Yale School of Medicine, New Haven, Connecticut, USA; [2]Veterans Affairs Healthcare System, West Haven, Connecticut, USA; [3]Stanford University School of Medicine, Stanford, California, USA; [4]Washington, D.C. Veterans Affairs Medical Center, Washington, D.C., USA; [5]George Washington University School of Medicine and Health Sciences, Washington, D.C., USA; [6]Atlanta Veterans Affairs Medical Center, Atlanta, Georgia, USA; [7]Emory University School of Medicine, Atlanta, Georgia, USA; [8]Michael E. DeBakey Veterans Affairs Medical Center, Houston, Texas, USA; [9]Baylor College of Medicine, Houston, Texas, USA

Background: Prostate adenocarcinoma grade, as defined by Gleason score, is a well-defined prognostic indicator; patients with higher Gleason score have more aggressive disease with a higher propensity to metastasize. The purpose of our study is to compare the Gleason score of prostate adenocarcinoma in HIV–infected (HIV+) vs. uninfected patients in the modern combination antiretroviral therapy (ART) era.

Methods: We linked the VA Central Cancer Registry (VACCR) to the Veterans Aging Cohort Study-9 (VACS-9) to identify patients diagnosed with prostate cancer. VACS-9 is a multi-institutional cohort of HIV+ and uninfected patients matched 1:1 on age, race, sex, and clinic site. Using the Voogo text searching tool, all medical progress notes were searched for the term "Gleason" and permutations of this word. Snippets containing the search terms were analyzed manually and Gleason scores were extracted for each patient. Gleason scores were grouped by clinical risk stratification: Gleason 3-4 (dysplastic tissue but not adenocarcinoma), 5-6 (low risk adenocarcinoma), 7 (intermediate risk), and >8 (high risk). Utilizing SAS, a univariate analysis was performed comparing Gleason scores in HIV+ vs. uninfected patients.

Results: In the VACS-9 cohort (N=3,728 HIV+; 3,787 uninfected) there were 117 HIV+ and 169 uninfected subjects with incident prostate cancer diagnoses identified by VACCR. Average age and distribution of race/ethnicity were similar between the HIV+ and uninfected groups. Of the 286 prostate cancer cases, 267 had notes that contained the term "Gleason" or a permutation of the term. High risk Gleason scores were found more frequently among HIV+ patients (15% vs 10%, Table 1). Intermediate risk scores were the most frequent among HIV+ patients (53, 45%), while low risk scores were the most frequent in the uninfected cohort (74, 44%). 27 patients (12 HIV+, 15 uninfected) had no mention of the term "Gleason" in their notes or the term Gleason was not associated with a numerical value.

Table 1. Distribution of Gleason Scores by HIV Status

	HIV+ (N=117)		**HIV- (N=169)**		
Gleason grade	**N**	**(%)**	**N**	**(%)**	**p-value**
Dysplastic (Gleason 3-4)	1	(1)	2	(1)	0.1461
Low risk (Gleason 5-6)	34	(29)	74	(44)	
Intermediate risk (Gleason 7)	53	(45)	61	(36)	
High risk (Gleason 8-10)	17	(15)	17	(10)	
Gleason unknown	12	(10)	15	(9)	

Conclusions: Based on our results, HIV+ patients who undergo prostate biopsies have more biologically aggressive prostate adenocarcinoma, although this difference is not statistically significant. Further analysis and larger scale studies need to be done to further investigate this potential difference and to investigate the role of possible confounders, such as screening, delayed diagnosis, and the role of immunosuppression. We plan to expand this study to the entire VACS virtual cohort (approximately 130,000 patients total) utilizing Voogo as a text searching tool and Voogo.REDEx as a text search and automated extraction tool to identify Gleason scores. In the past, large scale studies of Gleason score have been limited, as it has required the manual analysis of text notes, but with the expanded use of automated text search and extraction tools, it will be feasible to do analyses on large cohorts examining the relationship of Gleason score to stage at diagnosis, CD4 counts, prostate specific antigen (PSA) levels, response to therapy, and outcomes.

28. Effect of HIV Infection on Human Papillomavirus Type Distribution in Invasive Cervical Cancer in Africa

Gary Clifford, Hugo de Vuyst, Stephen Tully, Vanessa Tenet, Silvia Franceschi

International Agency for Research on Cancer, Lyon, France

Background: HIV infection is known to worsen the outcome of cervical human papillomavirus (HPV) infection, and may do so differentially according to HPV type.

Methods: Eighteen eligible studies were included in a meta-analysis of HPV type-distribution in invasive cervical cancer (ICC) among women infected with HIV in Africa. Type-specific HPV DNA prevalence was compared with African data from a published meta-analysis of HIV-negative ICC.

Results: Prevalence of HPV type was similar in 721 HIV-positive (91.6%) and 2,264 HIV-negative (90.2%) ICC, but HIV-positive ICC harbored significantly more multiple HPV type infections (PR= 2.01, 95% CI 1.70-2.38). HPV16 was the most frequently detected type in HIV-positive ICC (41.1%), followed by HPV18 (22.2%), HPV45 (14.5%) and HPV35 (8.0%). Nevertheless, HIV-positive ICC were significantly less frequently infected with HPV16 than HIV-negative ICC (PR=0.86, 95%CI 0.78-0.94), whereas all other HR types were significantly more prevalent in HIV-positive ICC. However, only for HPV18 and 45 was there evidence for higher prevalence of both single and multiple infections in HIV-positive ICC. For other HR types, increases in prevalence were primarily accounted for by multiple infections. Overall prevalence of HPV16/18 in ICC was not significantly different by HIV status (64.3% versus 63.6%; PR=1.01, 95% CI 0.92-1.11).

Conclusions: HIV infection appears to alter the relative carcinogenicity of HR HPV types, so that a lower fraction of ICC is caused by HPV16. However, the HPV18 fraction is concomitantly higher, so that current HPV16/18 prophylactic vaccines may theoretically prevent a similar proportion of ICC, irrespective of HIV status.

29. Development of Kaposi Sarcoma in a Liver-Kidney Transplant Recipient: The Case for Selective Screening

Sheila C. Dollard[1], Anitha D. Yadav[2], Sridhar V. Basavaraju[1], Bashar Aqel[2], David Douglas[2], Matthew Kuehnert[1]

[1]Centers for Disease Control and Prevention, Atlanta, Georgia, USA; [2]Mayo Clinic Transplant Center, Phoenix, Arizona, USA

Background: Post-transplant KS most often occurs in recipients who were HHV-8 seropositive prior to surgery and less commonly as a donor-derived disease, although the latter results in more aggressive disease. In March 2014, CDC was notified of a liver/kidney recipient who developed mesenteric KS after transplant. We investigated to determine the etiology and the extent of transmission.

Methods: Donor and recipients' medical records were reviewed. Pre- and post-transplant serum specimens from the donor and two organ recipients were evaluated by HHV-8 serology.

Results: The organ donor was a HBV-positive man who had sex with men (MSM) and had a history of frequent drug use. Two recipients received 3 solid organs, liver/kidney and kidney. Ten months after transplantation the liver/kidney recipient presented with visceral KS. Testing of the pre-transplant serum specimen revealed the recipient was HHV-8 seronegative and the donor was HHV-8 seropositive. Post-transplant testing confirmed the recipient with KS had seroconverted for HHV-8. Immunosuppression was changed from tacrolimus to sirolimus and 6 months later computed tomography showed near resolution of the mesenteric mass. Recipient of the patient who received a kidney only was KS-free and HHV-8 seronegative pre- and post-transplant.

Conclusions: Prevention of post-transplant KS should be considered through the practice of HHV-8 serologic screening of donors and recipients at elevated risk for HIV and HHV-8 (MSM, IDU). Donor testing may result in improved informed consent of recipients and guide recipient follow-up and clinical management, including immunosuppression selection. With recent U.S. passage of the HOPE Act allowing the use of organs from HIV+ donors for HIV+ recipients, transplant centers in non-HHV-8 endemic areas should consider the possibility of increasing numbers of cases of KS.

30. Factors Associated With Attrition in a Prospective Cohort Study in Nigeria

Olayinka Olaniyan[1,3], Eileen Dareng[2,3], Sally Adebamowo[3,4], Richard Offiong[5], Patrick Dakum[2], Clement Adebamowo[3,6,7]

[1]Department of Obstetrics and Gynecology, National Hospital, Abuja, Nigeria; [2]Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom; [3] Institute of Human Virology, Nigeria; [4]Center for Research on Genomics and Global Health, National Human Genome Research Institute, Bethesda, Maryland, USA; [5] Department of Obstetrics and Gynecology, University of Abuja Teaching Hospital, Abuja, Nigeria; [6]Department of Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, Maryland, USA; [7]Marlene and Stewart Greenbaum Cancer Center, University of Maryland School of Medicine, Baltimore, Maryland, USA

Background: Prospective cohort studies with a high proportion of attrition can suffer from selection bias, with limited generalizability of results. It is important to assess the factors associated with attrition and reasons for study dropout, as results may help in study design and the implementation of effective strategies to reduce attrition.

Methods: We designed a prospective cohort study to evaluate epidemiological, host, and HPV-viral factors associated with cervical pre-cancer in a screening program in Nigeria. We recruited 1020 women in Abuja, Nigeria, and followed them over a median period of 14.4 months with scheduled clinic visits at 6 months and 12 months. Women with at least 2 visits during the study period were considered to be responders in this study. We conducted exit phone interviews for non-responders. We compared demographic, lifestyle, reproductive, and sexual characteristics of the responders and the non-responders by logistic regression models, and explored the reasons for attrition among non-responders.

Results: Of the 1020 women enrolled in the cohort, 717 (70%) returned for at least one followup clinic visit. Of the sociodemographic characteristics evaluated (age, marital status, length of time at residence, educational level, religion, and socioeconomic status), only age was statistically significantly associated with attrition, with older women being less likely to drop out than younger women (OR: 0.96, CI: 0.94-0.98, p <0.001). Of the lifestyle risk factors evaluated (smoking, alcohol consumption, exercise frequency, presence of other chronic ailments, personal perception of health status, and HIV status), HIV infection was statistically significantly associated with attrition, with HIV positive women being less likely to drop out than HIV negative women (OR: 0.46, CI: 0.34, 0.62, p <0.001). The main reasons for study dropout were inability to reach participants (39%, 118/303), no show at appointments (34%, 103/303), ineligibility during study period (16%, 47/303), and voluntary withdrawal (11%, 33/303).

Conclusion: Inability to reach participants was the commonest reason for study dropout. Future prospective cohort study designs in a developing country like Nigeria need to account for this in sample size considerations and plan to reduce this by collecting adequate tracking information at recruitment.

31. Frequency of Malignancies in Persons Living With HIV/AIDS 2010-2015 in North Carolina: A Center Cohort

Carly J. Sherrod[1,5], Margaret Foreshag[2], Davina Chen[2], Oksana Zakharova[2], Kristen Sweet[1], Joseph Eron[2], Kristy Richards[3,4,5], Dirk P. Dittmer[1,5]

[1]Department of Microbiology and Immunology, [2]Division of Infectious Diseases, [3]Division of Hematology/Oncology, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; [4]Department of Biomedical Sciences, Cornell University, Ithaca, New York, USA; [5]Lineberger Comprehensive Cancer Center at the School of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Background: Human Immunodeficiency Virus (HIV) causes Acquired Immunodeficiency Syndrome (AIDS). Initially the malignancies most strongly associated with the development of AIDS included non-Hodgkin's lymphoma (NHL), cervical cancer, and Kaposi's sarcoma (KS). Even today, these cancers are the most common among AIDS patients. As life expectancy for persons living with HIV/AIDS (PLWHA) increases due to combination antiretroviral therapy (cART), so does the risk of developing a broader spectrum of cancers that show strong age dependent incidence rates in the general population such as lung cancer, colon cancer, and liver cancer. In addition, human papillomavirus-associated anal cancer is increasing in PLWHA.

Methods: We recorded the frequencies of cancers over time that develop in PLWHA seen at NC Cancer hospital in Chapel Hill in an effort to study the etiology and pathology of these malignancies. HIV-positive patients at UNC diagnosed or suspected of having cancer were considered for the study. One blood draw and any excess blood and/or body fluids and/or tissue remaining after diagnostic procedures were procured for this study. The blood was separated within 48 hrs following collection and viable peripheral blood mononuclear cells (PBMCs) stored. 1 mL of plasma was used to isolate total DNA and evaluated for the presence of cytomegalovirus, Epstein-Barr virus, and Kaposi sarcoma-associated herpesvirus.

Results: The study is ongoing. Current analyses reflect patients enrolled between 2010 and 2015, i.e., many years after the rollout of cART in the United States. The mean age was 49 years, though the range was large. Many more men than women were enrolled. 43.8% of the patients were black and 53.9% were white. 70.8% had a CD4 count >200, with a median of 300. Between 2010 and 2015 the prototypical AIDS defining cancers, NHL and KS, were the most frequent. This was followed by non-melanoma skin cancer, which encompasses a variety of cancers. The majority of cases had low CD4 counts, though variation was large; 50% of cancers, regardless of type, developed in patients with suppressed or very low HIV viral load.

A more detailed analysis will be presented. When we focused on cancers that develop in patients with CD4 >200 counts the ranking of cancer by individual frequency was head and neck squamous cell carcinoma (HNSCC), KS, NHL, lung, breast, prostate, melanoma, non-melanoma, liver, CML, multicentric Castleman's disease (MCD), and lymphoproliferative disorder.

Conclusion: There are limitations to our study, as it is hospital based and reflects the unique patient population of mostly rural North Carolina. Despite the presumed availability of antiretroviral therapy (ART) for almost two decades, the original AIDS-defining malignancies are disproportionally frequent in this population and do not seem to diminish with time. We expect them to persist at this frequency for the foreseeable future, and we expect additional cancer types to increase in frequency following an overall increase in the mean age of PLWHA.

32. Human Herpesvirus Type-8-Associated Large B Cell Lymphoma (HHV-8-LBL). A Non-serous Extra-cavitary Variant of Primary Effusion Lymphoma in an HIV-Infected Man: A Case Report and Review of the Literature

William Foster[1], David M Aboulafia[2,3]

[1]New York Medical College, Valhalla, New York, USA; [2]Virginia Mason Medical Center, Seattle, Washington, USA; [3]University of Washington, Seattle, Washington, USA

Background: Primary effusion lymphoma (PEL) is a rare non-Hodgkin lymphoma subtype primarily seen in HIV-infected individuals with low CD4+ cell counts and elevated HIV viral loads. It is always associated with human herpesvirus type-8 (HHV-8) and in 80% of cases is also associated with Epstein Barr virus (EBV). Less commonly, PEL is associated with advanced age and other conditions associated with altered immunity, including malignancy, liver cirrhosis, and immunosuppressive medications. It is a tumor of B-cell lineage; however, it shows a "null" phenotype, rarely expressing pan-B cell surface antigens. It does usually express CD45, CD30, CD38, CD138, and MUM1 and is characterized by lymphomatous effusions in body cavities but not lymphadenopathy. It is an aggressive lymphoma; average survival time is less than a year. HHV-8-associated large B-cell Lymphoma (HHV-8-LBL) is a second variant of PEL that is both solid and extra-cavitary. It has immunoblastic and/or anaplastic morphological features and a distinct immuno-histochemical staining pattern, and may have a different clinical presentation than classic PEL.

Methods: Herein, we describe the case of a 57-year-old HIV-infected man who presented with a slowly growing and asymptomatic abdominal mass. An excisional biopsy showed malignant large cells with prominent cytoplasm that were positive for pan-B cell antigen CD20, HHV-8, and EBV, and negative for CD138, CD10, BCL-6, CD3, and CD30. Ki-67 labeling index was 95%. He was diagnosed with stage III HHV-8-LBL, and he was treated with six cycles of R-EPOCH (rituximab, etoposide, vincristine, doxorubicin, cyclophosphamide, and prednisone) infusional chemotherapy. He remains in remission 12 months post-treatment. We also performed a Medline and Embase search to better understand the clinical findings of this patient and the unique attributes of HHV-8-LBL. Focusing our search on English language articles we identified 83 cases of HHV-8-LBL without an effusion component. We compared this to 118 reported cases of classic PEL.

Results: The median age of HHV-8-LBL patients was 41 years (range, 24-77) and 95% were HIV-associated vs. 41 years (range, 26-86) and 96% HIV–association for patients with classic PEL. 47% (30/64) of HHV-8-LBL patients had a pre-existing AIDS diagnosis, and 80% (52/65) were co-infected with EBV. In contrast, 72% (69/96) of classic PEL patients had a pre-existing AIDS diagnosis and 82% (40/49) were co-infected with EBV. The mean CD4+ count of HHV-8-LBL patients was 256 cell/µL (range, 18-1126) compared to 139 cell/µL (range, 2-557) for classic PEL patients. Median survival time for both groups was similar: 5.5 months for patients with HHV-8-LBL (range, 25 days-25+ months) and 4 months (range, 2 days-113+ months) for those with classic PEL. However, more patients with HHV-8-LBL were alive at time of the follow-up (56% vs 18%). For patients who achieved a complete remission and for whom long-term data were available, 100% (7/7) of KSHV-LBL were in CR vs. 38% (8/21) of classic PEL patients.

Conclusions: Our patient's high CD4+ cell count, lack of pre-existing AIDS diagnosis, and excellent response to chemotherapy highlights that HHV-8-LBL may have a distinct clinical picture and possibly a better response to chemotherapy than classic PEL. HHV-8-LBL should be included in the differential diagnosis of HIV patients with solid lesions. It is essential that patients' CDC HIV clinical status and HIV viral load at time of diagnosis of PEL and HHV-8-LBL be reported and that clinical results include longer term follow-up, so that a more complete clinical picture of this little appreciated or understood PEL variant exists.

33. The Impact of Recreational Drug Use on Oral HPV Clearance in HIV-Infected and HIV-Uninfected Individuals

Jennifer Lam[1], C. Coles[1], E. Sugar[1], K. Weber[2], R.D. Cranston[3], J. Margolick[1], L. Jacobson[1], S. Reddy[4], H. Minkoff[5], H. Strickler[6], D. Wiley[7], M. Gillison[8], G. D'Souza[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA; [2]CORE Center at John H. Stroger, Jr. Hospital of Cook County, Chicago, Illinois, USA; [3]University of Pittsburgh, Pittsburgh, Pennsylvania, USA; [4]Northwestern University, Chicago, Illinois, USA; [5]Maimonides Medical Center, Brooklyn, New York, USA; [6]Albert Einstein College of Medicine, Bronx, New York, USA; [7]University of California, Los Angeles, Los Angeles, California, USA; [8]Ohio State University Comprehensive Cancer Center, Columbus, Ohio, USA

Background: Persistent oral human papillomavirus (HPV) infection is a risk factor for oropharyngeal squamous cell carcinoma (OPSCC). HIV-infected individuals have a higher prevalence of oral HPV16 infection and HPV-related OPSCC. Risk factors for oral HPV persistence are not well understood, but initial studies suggest HIV may be associated with reduced clearance. Recreational drug use can have immunomodulatory effects and is common in people with or at risk for HIV, but its role on oral HPV clearance has not been previously explored.

Methods: From 2010 to 2014, Scope® oral rinse and gargle samples were collected from 1,666 participants semi-annually. Samples were tested for 37 types of oral HPV DNA using PCR with PGMY 09/11 primers followed by reverse line blot hybridization. At each visit, data were collected on recent (past 6 months) drug use (alcohol, cigarette, marijuana, tranquilizers, crack, cocaine, heroin, and amphetamines). The relationship between drug use and oral HPV clearance was evaluated with Wei-Lin-Weissfeld regression models, accounting for within-subject clustering of HPV infections. The final multivariate model adjusted for known oral HPV risk factors, including HIV status, CD4 T cell count (>500, 351-500, ≤350 cells/μL), gender, HPV infection type (prevalent vs. incident), oral hygiene (saliva amount: normal/too little/too much), and age (<45, 45-54, ≥55).

Results: A total of 1,358 type specific oral HPV infections in 594 participants were detected during 21,749 person-months of follow-up. At time of first oral HPV detection, participants with HPV DNA detected reported recent use of alcohol (65%), cigarettes (47%), marijuana (27%), tranquilizers (21%), crack (10%), cocaine (6%), heroin (2%), and amphetamines (2%). Median time to clearance was 6.7 months (IQR=6.0-17.4). Among HIV-uninfected participants, recent drug use (of any type) was not associated with oral HPV clearance. Among HIV-infected participants only, recent cocaine (HR=0.55; 95% CI 0.34-0.87) and recent tranquilizer (HR=0.79; 95%CI 0.67-0.95) use were each associated with decreased oral HPV clearance. Results were similar when restricted to only HIV-infected participants within any one of the CD4 T cell strata (>500, 351-500, ≤350 cells/μl). In multivariate analysis, recent cocaine use remained strongly associated with reduced oral HPV clearance among HIV-infected participants (aHR=0.53; 95%CI=0.33-0.85) but not HIV-uninfected (aHR=1.10; 95%CI=0.57-2.15) participants (p-interaction=0.10). Recent tranquilizer use also appeared to be associated with oral HPV clearance among HIV-infected participants (aHR=0.88; 95%CI=0.73-1.06), but was no longer statistically significant.

Conclusions: This study suggests that cocaine use and tranquilizer use may be associated with reduced clearance of oral HPV infection among HIV-infected participants. Other types of recreational drug use did not influence oral HPV clearance.

34. The Impact of ART on the Incidence of AIDS-Defining Cancers (ADC) and Non-ADC

Ian Jen[1], Pei-Hung Chuang[2], Gerald Sharp[3], Julia Bohlius[4], Yen-Ling Liu[5], Yi-Ming Arthur Chen[5,6]

[1]School of Medicine, National Yang-Ming University, Taipei, Taiwan; [2]Department and Institute of Public Health, National Yang-Ming University, Taipei, Taiwan; [3]Epidemiology Branch, Basic Science Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA; [4]Institute of Social and Preventive Medicine (ISPM), University of Bern, Bern, Switzerland; [5]Center for Infectious Disease and Cancer Research, Kaohsiung Medical University, Kaohsiung, Taiwan; [6]Department of Microbiology and Institute of Medical Research, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

Background: Although combination antiretroviral therapy (ART) has substantially improved survival from HIV/AIDS related infectious complications, large-scale studies are needed to evaluate the impact of ART on malignancies and mortality. The objective of this study was to investigate the effect of HIV therapy and other factors on the incidence of AIDS defining cancer (ADC) and non ADC (NADC).

Methods: A retrospective nationwide cohort study was conducted using the treatment service database from Taiwan's Bureau of National Health Insurance and the death registration database from the Ministry of Health and Welfare from January 2000 to December 2010. We examined the associations of socioeconomic status, urban vs. rural residence and ART treatment with ADC, NADC, and mortality, comparing 5,799 HIV/AIDS patients who had never received ART and 9387 such patients who received ART, adjusting for confounders. ART users were further divided into two groups based on prescription timing: Group 1: patients who were symptomatic and had started ART on their first or second visit to an AIDS clinic (n= 4,373); Group 2: patients who were asymptomatic for whom ART treatment was delayed until after their second visit to an AIDS clinic (n= 5,014).

Results: In total, 15,186 adults with HIV/AIDS were identified. Among them, there were 142 cases with ADC, 174 cases with NADC, and 1,299 deaths. Compared to the untreated group, patients in both Groups 1 and 2 had a significantly higher incidence of ADC (group 1 vs. untreated: adjusted HR: 2.43; 95% CI, 1.48-3.98; p <.001; group 2 vs. untreated: aHR: 2.13; 95% CI, 1.30-3.48; p =.003). In contrast, the incidence of NADC was significantly lower for both groups of patients receiving ART (group 1 vs. untreated: aHR: 0.56; 95% CI, 0.38-0.83; p =.004; group 2 vs. untreated: aHR: 0.572; 95% CI, 0.40-0.83; p =.003). Multivariate Cox regression analysis also disclosed other characteristics associated with significantly lower risks of death: (Group 2 patients vs. untreated [aHR, 0.84; 95% CI, 0.74-0.98; p=0.02], higher vs. lower income [aHR, 0.53; 95% CI, 0.6-0.62; p <.001], urban vs. rural residence [aHR, 0.76; 95% CI, 0.66-0.88; p <.001]). Current analysis is sorting out if the ADC/ART finding is due to ADCs bringing HIV patients to diagnosis or some other factors.

Conclusions: For HIV/AIDS patients, ART is significantly associated with lower risks of NADC. Besides ART, higher income and living in urban areas are associated with lower risks of death.

35. The Impact of Highly Active Antiretroviral Therapy on Cancer Survival in the Multicenter AIDS Cohort Study and Women's Interagency HIV Study

Shahar Shmuel[1], Keri Althoff[2], Lisa Flowers[3], Elizabeth Breen[4], Mardge Cohen[5], Ken Ho[6], Kathy Anastos[7], Sunny Wang[8], Lisa Jacobson[2], Eric Seaberg[2]

[1]The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; [2]Johns Hopkins University, Baltimore Maryland, USA; [3]Emory University, Atlanta, Georgia, USA; [4]University of California, Los Angeles, Los Angeles, California, USA; [5]Stroger Hospital and Rush University, Chicago, Illinois, USA; [6]University of Pittsburgh, Pittsburgh, Pennsylvania, USA; [7]Montifiore Medical Center, Bronx, New York, USA; [8]University of California, San Francisco, San Francisco, California, USA

Background: Introduced in the mid-1990s, Highly Active Antiretroviral Therapy (HAART) has altered the natural history of HIV by suppressing HIV replication and maintaining a competent immune system, thus extending the lifespan of seropositive individuals [1]. Coupled with the improved survival expectancies achieved during the HAART era have been dramatic declines in the incidence of the AIDS-defining malignancies (ADMs) Kaposi's sarcoma (KS) and non-Hodgkin's lymphoma (NHL), and a rise in certain non-ADMs (NADMs) among HIV-infected individuals [1–3]. The direct impact of HAART on mortality following cancer diagnosis is less well characterized [1,4,5]. The objective of this study was to determine the effect of HAART on survival following cancer diagnosis using data from the Multicenter AIDS Cohort Study (MACS) and the Women's Interagency HIV Study (WIHS).

Methods: All WIHS and MACS participants diagnosed with cancer during the HAART era (1995-2013) were eligible for this study. Only initial primary cancers that were confirmed in medical records, by cancer registry matches, or via death certificate reviews were included. We evaluated 5-year survival beginning on the date of diagnosis using Kaplan-Meier curves and Cox proportional hazards methods.

Results: Among the 439 eligible study participants, 229 died during follow-up. The overall estimated 1-yr and 5-yr survival probabilities were 70.4% and 40.1%, respectively. Survival did not differ significantly between HIV-infected and –uninfected individuals (adjusted hazard ratio (aHR): 0.70, 95% CI: 0.35 – 1.38**),** and among HIV-infected patients, survival was significantly poorer among participants from WIHS as compared to those from MACS (aHR: 1.78, 95% CI 1.19-2.67). Among HIV-infected individuals, survival did not differ significantly between those diagnosed with AIDS-defining malignancies (ADMs) and those diagnosed with non-AIDS-defining malignancies (NADMs) (aHR: 1.36, 95% CI: 0.90 – 2.05). However, HIV-infected individuals taking HAART at the visit prior to cancer diagnosis had a significantly lower hazard of death than did those not taking HAART (aHR: 0.68, 95% CI: 0.48 – 0.96). Furthermore, HAART was associated with better survival for individuals diagnosed with infection-related cancers (aHR: 0.36, 95% CI: 0.19 – 0.69), but not among those diagnosed with non-infection-related cancers (aHR: 1.07, 95% CI: 0.67 – 1.72), and these effects differed significantly (p=0.002 for interaction).

Conclusions: Among HIV-infected individuals, taking HAART prior to the diagnosis of an infection-related cancer is associated with improved survival, but this was not the case for non-infection-related cancers. Future research is required to examine mechanisms that might explain why HAART is associated with better survival only for infection-related cancers.

36. KSHV and MHV68 LANA Act Reciprocally on Virus Terminal Repeat DNA to Mediate Episome Maintenance

Aline Habison[1], Chantal Beauchemin[1], Min Tan[1], Bruno Correia[2], Marta Pires de Miranda[3], Rajesh Ponnusamy[2], Edward Usherwood[4], Colin McVey[2], J. Pedro Simas[3], Kenneth Kaye[1]

[1]Departments of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA; [2]Instituto de Tecnologia Quimica e Biologica, Universidade Nova de Lisboa, Oeiras, Portugal; [3]Instituto de Microbiologia e Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal; [4]Department of Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire, USA

Requested not to post abstract in online publication.

37. Lung Cancer Among HIV-Infected Individuals at a Single Urban Institution in the Recent Antiretroviral Era

Kristen Hysell, Lydia Aoun-Barakat, Michael Virata, Brinda Emu

Yale-New Haven Hospital, New Haven, Connecticut, USA

Background
Lung cancer is the most common non-AIDS related malignancy among patients with HIV infection. Large cohort studies have found that HIV is an independent risk factor for lung cancer incidence, but detailed characterization of these patients has not been well described. This study sought to characterize demographics, HIV disease status, and treatment, substance abuse, hepatitis coinfection status, and lung cancer outcomes among HIV-infected individuals diagnosed at a single urban institution in the recent antiretroviral era.

Methods: All patients with HIV who were diagnosed with lung cancer between 2001 and 2014 at Yale-New Haven Hospital (New Haven, Connecticut) were analyzed through electronic medical records for demographical information and characteristics of HIV infection, HIV treatment, lung cancer stage and pathology, cancer treatment history, and cancer treatment response.

Results: A total of 27 patients were identified from 2001 to 2014 out of a total clinic population of approximately 2,700 patients during this time span. 15 patients were Black (55.5%), 10 were Caucasian (37%), 1 Hispanic, and 1 unknown. The majority (74%) was male. Median age at time of cancer diagnosis was 52 (range, 40 to 65). The median interval of time from HIV diagnosis to lung cancer diagnosis was 16 years. Mode of HIV transmission included IVDU (48%), MSM (15%), heterosexual sex (15%), or unknown (22%). Only 6 patients (22%) had a diagnosis of AIDS by CDC stage at time of lung cancer diagnosis. The median CD4 count at time of lung cancer diagnosis was 498 cells/mm^3 (range, 26-1240). 81% of patients were on anti-retroviral treatment at time of cancer diagnosis and 63% of patients were on a regimen which involved a protease inhibitor. All patients had a past or present smoking history with median of 30 pack years (range, 7-100). 67% of patients had documented past or present alcohol use and 81% had a documented history of past or present drug use with cocaine and/or heroin. Of note, 66% were co-infected with hepatitis C. 93% of patients had non-small cell lung carcinoma (NSCLC) while 2 patients had small cell lung cancer (7%). Of those with NSCLC, 13 were identified with adenocarcinoma, 8 with squamous cell, and 2 with unspecified NSCLC. 48% of all patients had stage IV lung cancer at time of diagnosis. The one year mortality for all patients was 37%.

Conclusion: This study provides detailed characterization of patients with HIV infection and lung cancer in an urban population. At the time of cancer diagnosis, patients presented with relatively high CD4 count, with the majority on anti-retroviral therapy and virally suppressed. As previously described, presentation occurred at a younger age with high rates of smoking, as well as high rates of illicit drug use. The rate of co-infection with hepatitis C among patients with HIV and NSCLC is higher than the general clinic population, warranting further study about the impact of co-infections on NSCLC incidence.

38. Persistent Human Papillomavirus Infection in a Cohort of Nigerian Women

Eileen O. Dareng[1,2], Toyosi Olawande[2], Ayo O. Famooto[2], Sally N. Akarolo-Anthony[2,3], Richard A.Offiong[4], Olayinka B. Olaniyan[5], Clement A. Adebamowo[6,7]

[1]Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom; [2]Department of Research, Institute of Human Virology, Nigeria; [3] Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA; [4] Department of Obstetrics and Gynaecology, University of Abuja Teaching Hospital, Abuja, Nigeria; [5]Department of Obstetrics and Gynaecology, National Hospital, Abuja, Nigeria; [6]Department of Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, Maryland, USA; [7] Institute of Human Virology and Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, Maryland, USA

Background: Persistent infection with high risk HPV is associated with increased risk of cervical cancer. Therefore, understanding the predictors of persistence may provide some insights in characterizing infections that may have clinical significance.

Methods: From August 2012 to December 2013, we recruited women at our cervical cancer screening clinics in Abuja, Nigeria. Nurses collected ecto-cervical samples for HPV determination which was performed using SPF_{10} DEIA, $LiPA_{25}$ version 1. Relative risks were estimated using Poisson regression models with robust error variance.

Results: Of the 1020 women enrolled, (aged 18-61 years), 727 (71.1%) returned for follow up after mean (SD) 8.6 (4.0) months. Some 42.4% (432/1020) of the participants were HIV positive. Baseline prevalence of any HPV infection was 38.3% (380/992). Of these 248 returned for follow-up and 62.5% (155/248) remained persistently positive for any HPV. Of these participants with persistent any HPV infection, 65.3% (81/124) had persistent high risk HPV infection.

The RR (95%CI, p-value) for an association with prevalent any HPV were 0.99 (0.98 – 0.99, 0.02) for age, 1.23 (1.12 – 1.35, <0.001) for HIV infection, 1.26 (0.97 – 1.63, 0.08) for presence of other STIs, and 1.59 (1.28 – 1.99, <0.001) for abnormal VIA results. The RR (95%CI, p-value) for persistent infection with high risk HPV were 3.68 (2.20 – 6.15, <0.001) for HIV infection and 3.04 (1.80 – 5.12, <0.001) for abnormal baseline VIA.

Conclusions: The preliminary data suggest a high level of persistence of any HPV infection among women with prevalent any HPV infection. Updated analysis, by HPV genotype, will be available in October.

39. Influence of Spirituality and Modesty on Acceptance of Self-Sampling for Cervical Cancer Screening

Eileen O. Dareng[1,2], Elima Jedy-Agba[2,3], Patience Bamisaye[2,4], Fatima Isa Modibbo[5], Lawal O. Oyeneyin[6], Ayodele S. Adewole[6], Olayinka B. Olaniyan[2,7], Patrick S. Dakum[2], Paul D. Pharoah[1], Clement Adebamowo[8,9]

[1]Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom; [2]Department of Research, Institute of Human Virology, Nigeria; [3]Department of Non-communicable Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, United Kingdom; [4]Department of Nursing Services, National Hospital, Abuja, Nigeria; [5]Department of Medical Microbiology and Parasitology, National Hospital, Abuja, Nigeria; [6]Mother and Child Hospital Ondo, Nigeria; [7]Department of Obstetrics and Gynecology, National Hospital, Abuja, Nigeri; [8]Department of Epidemiology and Public Health, School of Medicine, University of Maryland, Baltimore, Maryland, USA; [9]Institute of Human Virology and Greenebaum Cancer Center, School of Medicine, University of Maryland, Baltimore, Maryland, USA

Background: Whereas systematic screening programs have reduced the incidence of cervical cancer in developed countries, the incidence remains high in developing countries. Among several barriers to uptake of cervical cancer screening, the roles of religious and cultural factors such as modesty have been poorly studied. Knowledge about these factors is important because of the potential to overcome them using strategies such as self-collection of cervico-vaginal samples. In this study we evaluate the influence of spirituality and modesty on the acceptance of self-sampling for cervical cancer screening.

Methods: We enrolled 600 participants in Nigeria between August and October 2014 and collected information on spirituality and modesty using two scales. We used principal component analysis to extract scores for spirituality and modesty and logistic regression models to evaluate the association between spirituality, modesty, and preference for self-sampling. All analyses were performed using STATA 12 (Stata Corporation, College Station, Texas, USA).

Results: Some 581 (97%) women had complete data for analysis. Most (69%) were married, 50% were Christian, and 44% were from the southwestern part of Nigeria. Overall, 19% (110/581) of the women preferred self-sampling to being sampled by a health care provider. Adjusting for age and socioeconomic status, spirituality, religious affiliation, and geographic location were significantly associated with preference for self-sampling, while modesty was not significantly associated. The multivariable OR (95% CI, p-value) for association with self-sampling were 0.88 (0.78 – 0.99, 0.03) for spirituality, 1.69 (1.09 – 2.64, 0.02) for religious affiliation, and 0.96 (0.86 – 1.08, 0.51) for modesty.

Conclusion: Our results show the importance of taking cultural and religious beliefs and practices into consideration in planning health interventions like cervical cancer screening. To succeed, public health interventions and the education to promote it must be related to the target population and its preferences.

40. Kaposi Sarcoma in HIV-Infected Children and Adolescents in Central Malawi: A Novel Clinical Staging Classification Determines Risk Stratification

Nader Kim El-Mallawany[1,2], William Kamiyango[3], Jeremy S. Slone[2,4], Jimmy Villiera[3], Carrie Kovarik[5], Gordon E. Schutze[2], Michael E. Scheurer[2,4], Peter N. Kazembe[3], Parth S. Mehta[2,4]

[1]New York Medical College, Valhalla, New York, USA; [2]Baylor College of Medicine, Houston, Texas, USA; [3]Baylor College of Medicine Children's Foundation Malawi, Lilongwe, Malawi; [4]Texas Children's Cancer and Hematology Centers, Houston, Texas, USA; [5]University of Pennsylvania, Philadelphia, Pennsylvania, USA

Background: Kaposi sarcoma (KS) is the most common HIV-associated malignancy in children and adolescents in Africa. Pediatric KS is distinct from adult disease. We evaluated the clinical characteristics associated with favorable and unfavorable outcomes and devised a pediatric KS staging classification to help determine risk stratification.

Methods: We retrospectively analyzed charts of 70 HIV-infected children and adolescents with KS less than 18 years of age diagnosed between August 2010 and June 2013 in Lilongwe, Malawi. Diagnosis was pathology-confirmed in 20%. Local first-line chemotherapy included bleomycin and vincristine (BV). Salvage regimens included doxorubicin plus BV and paclitaxel. Highly active anti-retroviral therapy (HAART) was based on local protocol with nevirapine-based first line regimens.

Results: Median age of the cohort was 8.6 years (range 1.7-17.9); there were 35 females and 35 males. Common sites of presentation were: lymph node (74%), skin (59%), subcutaneous nodules (33%), oral (27%), woody edema (24%), non-woody facial edema (16%), pulmonary (11%), and abdominal visceral (4%). Eighteen (26%) patients presented with lymphadenopathy only, and no classic KS skin, oral, or woody edema findings. Severe CD4 suppression occurred in 28%. Nearly half (49%) of the cohort was naïve to HAART, while 49% were already on HAART at the time of KS diagnosis. Moderate-severe cytopenias were prominent at the time of KS diagnosis: 28% presented with a platelet count <100 x 10^9/L and 21% with platelets <50 x 10^9/L. Anemia was found in 37% with hemoglobin <8 g/dL and 19% with hemoglobin <6 g/dL. Median follow-up for survivors was 29 months (range 15-50), and the 2-year event-free (EFS) and overall survival (OS) were 46% and 58%, respectively. Five patients abandoned treatment; three died and two returned to care. Univariate analysis of variables associated with the highest risk for death included: visceral disease (odds ratio [OR] 19, 95% confidence interval [CI] 2.3-159.2, p = <0.01) and presenting with more than 20 skin/oral lesions (OR 9.5, 95% CI 1.1-83.6, p = 0.01). T_1 vs T_0 TIS staging criteria, presence of cytopenias, and severe immune suppression were not associated with increased risk for death. However, S_1 TIS staging had an OR of 4.22 (95% CI 1.5-11.9, p = <0.01). The *Lilongwe Pediatric KS Staging Classification* was devised as follows: *Stage 1*: limited to skin, flat oral mucosa lesions, and/or subcutaneous nodules, total <10 lesions. *Stage 2*: Having the presence of lymph node involvement, nodular oral lesions, or facial edema, or having >10 but <20 skin/oral lesions. *Stage 3*: woody edema +/- any of the above. *Stage 4*: clinical pulmonary or abdominal visceral involvement, or having >20 skin/oral lesions in widespread distribution, +/- any of the above. There were zero Stage 1 patients; 54% were Stage 2, 21% Stage 3, and 25% Stage 4. Stage 2 patients were younger, mostly represented by lymphadenopathic KS and with high rates of cytopenias; more than half were already on HAART at time of KS diagnosis. Stage 3 patients were older (median age 12.6 years vs. 6.8 years for stage 2 and 9.2 years for stage 4, p= <0.01), while stage 4 patients presented with a lower mean absolute CD4 count of 234 (p=0.02) and poor outcomes when treated with BV. Two-year survival outcomes differed dramatically by stage: stage 2 EFS 73%/OS 75%, stage 3 EFS 29%/OS 79%, and stage 4 EFS 0%/OS 12% (p = < 0.01).

Conclusions: The proposed Lilongwe Pediatric KS Staging Classification differentiates patterns of disease with dramatically contrasting outcomes. Long-term EFS is achievable in stage 2 patients with the combination of BV and HAART. Stage 3 patients have indolent disease with low mortality but low cure rates regardless of the chemotherapy regimen. Identifying stage 4 patients is critical to determine the need for intensified therapy in order to improve overall outcomes.

41. Long-Term Outcomes in Kaposi Sarcoma Herpesvirus-Associated Multicentric Castleman Disease (KSHV-MCD) Patients Treated With Rituximab and Liposomal Doxorubicin

Thomas S Uldrick[1], Mark N. Polizzotto[1], Priscila Goncalves[1], Karen Aleman[1], Kathleen M. Wyvill[1], Seth M. Steinberg[2], Vickie Marshall[3], Denise Whitby[3], Richard F. Little[1], Robert Yarchoan[1]

[1]HIV & AIDS Malignancy Branch and [2]Biostatistics and Data Management Section Center for Cancer Research, National Cancer Institute (NCI), Bethesda, Maryland, USA; [3]AIDS and Cancer Virus Program, Viral Oncology Section, Leidos Biomedical Research, Inc., Frederick, Maryland, USA

Introduction: Rituximab, an anti-CD20 monoclonal antibody, is effective in KSHV-MCD. However, concurrent Kaposi sarcoma (KS) is common and can worsen with rituximab. Liposomal doxorubicin targets CD20-negative KSHV-infected cells and initial clinical results indicated it is useful in treating patients (pts) with KSHV-MCD and concurrent KS or severe KSHV-MCD. However, long-term outcomes and prognostic factors in pts with KSHV-MCD with and without concurrent KS are unknown.

Methods: Pts with symptomatic KSHV-MCD were treated in a prospective pilot study of rituximab 375mg/m^2 and liposomal doxorubicin 20mg/m^2 every 3 weeks (R-Dox) until clinical improvement, followed by antiviral therapy or additional KS therapy if indicated. KSHV-MCD responses were evaluated by NCI criteria. Effect of R-Dox on change in clinical biomarkers was evaluated by Wilcoxon signed rank test, with 2-sided P-value <0.05 considered significant. Overall survival (OS) and progression-free survival (PFS) through 8/2015 was evaluated by Kaplan-Meier and log-rank methods. Baseline clinical factors were evaluated as predictors of overall survival (OS). Continuous parameters were dichotomized near the median for analyses. Longitudinal evaluation of biomarkers will be presented.

Results: 22 HIV-infected pts enrolled, including 17 previously published. Median (med) age 43 (27-55). Prior KSHV-MCD therapy 16 (73%). Ten (45%) had clinical KS, 4 additional had KS in lymph node only, 8 (36%) T1 KS (T1 KS: high-risk tumor features (lymphedema, extensive oral cavity involvement, or visceral disease). Baseline laboratories (Table). Med number of cycles 3 (2-9). At the end R-Dox, clinical benefit responses: complete 77%, partial 9%, stable disease 5% progressive disease 9%. During R-Dox, 8 had improvement in cutaneous KS; only 1 pt had mild transient worsening. Hemoglobin, albumin, c-reactive protein (CRP), KSHV viral load (VL), and serum free light chains all improved with therapy (p<0.0001). With med potential 6.2 year (1.6-9.4) follow up, estimates for 3 years and beyond: PFS 65% (95% CI: 40- 82%), OS 80% (54- 92%). Baseline T1 KS was associated with inferior OS (3-year OS: no T1 KS 91% vs. T1 KS 63%; p=0.03 overall). In contrast, baseline CD4+ <100 cells/μL (p=0.39), hemoglobin (p=0.26), platelets (p=0.31), KSHV VL (p=1), CRP (p=0.98) and serum free light chains (K, p=0.21, L, p=0.33) were not associated with OS.

Conclusions: R-Dox is effective in KSHV-MCD, including many pts with concomitant KS. Baseline measures of KSHV-MCD activity or CD4+ count were not associated with OS. Inferior survival was noted in the few pts with baseline T1 KS. Additional approaches are needed for this population.

Baseline Characteristics

Characteristic	Results	
CD4$^+$ T-cells (cells/uL), med (range)	255	(21-858)
HIV viral load (VL) <200 copies/mL, n(%)	17	(77.3%)
CRP (mg/L), med (range)	81.5	(<4-210)
Hemoglobin (g/dL), med (range)	9.8	(6.8-13.2)
Platelets (K/uL), med (range)	118.5	(11-567)
Albumin (mg/dL), med (range)	2.9	(1.2-4.0)
KSHV VL (copies/10^{6}PMBC), med (range)	18,622	(0-8,780,488)
Serum Free Kappa (mg/dL), med (range)	7.4	(1.9-22.6)
Serum Free Lambda (mg/dL), med (range)	5.7	(1.8-19.8)

42. Metabolic Risk Factors Associated With HCC Development in a HIV/HCV Co-infected Cohort

N.T. Oliver[1], C.M. Hartman[2], J.R. Kramer[1,2], E.Y. Chiao[1,2]

[1]Department of Medicine, Section of Infectious Diseases and Health Services Research, Baylor College of Medicine, Houston, Texas; [2]Center for Innovations in Quality, Effectiveness and Safety, Michael E. DeBakey VA Medical Center, Houston, Texas

Background: Hepatitis C virus (HCV) and HIV co-infection are associated with liver disease progression including hepatocellular carcinoma (HCC) [1]. Persons with HIV/HCV co-infection now live longer due to combined antiretroviral therapy (cART), and they may acquire other chronic diseases that may hasten progression of HCC [2-3]. Statin drug use to mitigate cardiovascular disease risk may impact risk of developing cancer [4]. We attempt to elucidate the association of statin use on development of HCC in an HIV/HCV co-infected VA cohort.

Methods: The HIV Clinical Care Registry (CCR) is a comprehensive VA database that contains clinical information on its HIV/HCV co-infected population nationwide. Variables including diabetes (DM), hypertension (HTN), low-HDL, obesity, and statin use were based upon ICD-9 codes, laboratory tests, and presence of drug prescription. Primary outcome was risk of HCC diagnosis. Cox proportional hazard regression analysis was performed using SAS version 9.1 (SAS Institute Inc., Cary, NC).

Multivariate Analysis, Risk for HCC		
Variable	HR (CI)	*p*-value
Age at HIV diagnosis		
>50	4.36 (2.7-6.9)	<.0001
40-50	2.5 (1.7-3.6)	<.0001
Race		
White/other/unknown	1 (reference)	
Black	0.86 (0.61-1.2)	0.39
Hispanic	1.34 (0.86-2)	0.19
CD4 Count		
<200	1.3 (0.9-1.8)	0.13
200-349	1.5 (1.1-2.1)	0.007
>350	1 (reference)	
Deyo Score		
>2	1.5 (0.97-2.3)	0.06
1	0.8 (0.54-1.2)	0.33
0	1 (reference)	
Cirrhosis	5.1 (3.5-7.4)	<.0001
Low-HDL	1.05 (0.75-1.47)	0.738
Statin Use	0.61 (0.36-1.03)	0.068

Results: This group contained over 8,500 patients with HIV/HCV co-infection, of which 244 had HCC from 1999 to 2010. Median age at HIV diagnosis was 46 years (41-51). Among racial groups, Black race represented 59%, Whites 24.6%, and Hispanics 19.7%.Cirrhosis was found in 86.5% of cases. Patients who were diagnosed with HIV at an older age, had cirrhosis, and had more comorbidities were more likely to develop HCC. Those with CD4 count between 200 and 349 were more likely to have HCC than those with CD4 counts >350. Patients with low-HDL were at a slightly higher risk for HCC, but not statistically significant. Statin use was associated with less HCC, but this effect was marginally significant.

Conclusion: Patients with HIV/HCV co-infection who were diagnosed with HIV later in life, had CD4 counts between 200 and 349 and more comorbidities have greater association with HCC development. Statin drug use to curb the metabolic effects of lipid abnormalities may be protective in this group.

References

1. Graham CS, et al. Influence of human immunodeficiency virus infection on the course of hepatitis C virus infection: a meta-analysis. *Clin Infect Dis*, 2001. 33(4): 562-9.
2. Siegel AB and Zhu AX. Metabolic syndrome and hepatocellular carcinoma: two growing epidemics with a potential link. *Cancer,* 2009. 115(24): 5651-61.
3. Larsson SC and Wolk A. Overweight, obesity and risk of liver cancer: a meta-analysis of cohort studies. *Br J Cancer,* 2007. 97(7): 1005-08.
4. El-Serag HB, et al. Statins are associated with a reduced risk of hepatocellular carcinoma in a large cohort of patients with diabetes. *Gastroenterology,* 2009. 136(5): 1601-08.

43. Outcomes for Aggressive Non-Hodgkin Lymphoma in Malawi With and Without HIV in the Current Antiretroviral Therapy Era

Satish Gopal[1,2,3], Yuri Fedoriw[2], Bongani Kaimila[1], Nathan Montgomery[2], Edwards Kasonkanji[1], Agnes Moses[1,3], Richard Nyasosela[4], Suzgo Mzumara[3,4], Carlos Varela[3,4], Maria Chikasema[1], Victor Makwakwa[1], Salama Itimu[1], Tamiwe Tomoka[3], Steve Kamiza[3], Bal Dhungel[4], Fred Chimzimu[1], Coxcilly Kampani[1], Robert Krysiak[1], Kristy Richards[5], Thomas Shea[2], N. George Liomba

[1]UNC Project, Lilongwe, Malawi; [2]Lineberger Comprehensive Cancer Center, Chapel Hill, North Carolina, USA; [3]University of Malawi College of Medicine, Blantyre, Malawi; [4]Kamuzu Central Hospital, Lilongwe, Malawi; [5]Weill Cornell Medical College, New York, New York, USA

Background: Prospective data are scarce describing outcomes for aggressive non-Hodgkin lymphoma (NHL) patients in sub-Saharan Africa who are HIV- or HIV+ in the current antiretroviral therapy (ART) era.

Methods: We describe a prospective cohort of adults with aggressive NHL receiving CHOP at a national teaching hospital in Malawi between June 2013 and May 2015. Chemotherapy and supportive care are standardized, and HIV+ patients receive concurrent ART.

Results: Thirty-seven of 58 patients with aggressive NHL (64%) during the study period were HIV+. Median age was 47 years [interquartile range (IQR) 38-55] and 34 (59%) were male. Thirty-six patients (62%) had stage III/IV disease which was typically bulky (median largest lymph node mass 10 cm, IQR 7-13). B symptoms were present in 44 (76%) and 29 (50%) had performance status ≥2. Clinical characteristics were similar overall for patients with and without HIV. Thirty-one (84%) HIV+ patients were on ART at lymphoma diagnosis for a median 9.9 months (IQR 1.1-31.7). Median CD4 count at lymphoma diagnosis was 121 cells/μL (IQR 61-244), and 43% had suppressed HIV RNA <400 copies/mL. As of May 31, 2015, vital status was known for all 58 enrolled patients after a median 10.2 months among patients still alive (IQR 4.6-20.7). Kaplan-Meier overall survival (OS) was 49% [95% confidence interval (CI) 34-62%] for the cohort overall 12 months after enrollment. Fifty of 58 patients (86%) survived to cytotoxic treatment initiation, and their estimated OS 12 months after chemotherapy initiation was 57% (95% CI 40-71%). T-cell NHL, hemoglobin, albumin, and international prognostic index (IPI) were associated with mortality (Table). When individual IPI elements were examined, performance status and lactate dehydrogenase were most strongly associated. In adjusted analyses, only IPI was associated with mortality. HIV was not associated with mortality. Of 29 deaths, 20 were attributed to lymphoma (10 HIV+, 10 HIV-), and nine to CHOP complications (7 HIV+, 2 HIV-).

Conclusions: CHOP can be safe and effective for aggressive NHL patients with and without HIV in Malawi. For HIV+ patients, ART use, CD4 count, and HIV RNA at lymphoma diagnosis are comparable to contemporary HIV+ NHL cohorts in the United States, as are outcomes for patients treated with CHOP.

Table. Risk Factors for Mortality Among Aggressive Non-Hodgkin Lymphoma Patients In Malawi

	Unadjusted hazard ratio	95% confidence interval	P value	Adjusted hazard ratio	95% confidence interval	P value
HIV infection	0.76	0.36-1.60	0.47	0.87	0.35-2.16	0.76
T-cell non-Hodgkin lymphoma	3.79	1.25-11.54	0.019	3.22	0.76-13.6	0.11
Female sex	0.69	0.31-1.52	0.36	0.46	0.18-1.16	0.099
Hemoglobin, per g/dL	0.80	0.68-0.95	0.010	0.90	0.71-1.15	0.40
Albumin, per g/dL	0.54	0.33-0.88	0.013	1.03	0.49-2.17	0.95
Body mass index, per kg/m^2	0.95	0.86-1.06	0.36	1.01	0.90-1.13	0.85
International prognostic index, per unit	2.07	1.45-2.95	<0.001	1.74	1.13-2.68	0.012
Age, per year	0.99	0.96-1.02	0.58			
Performance status, per unit	2.11	1.55-2.89	<0.001			
Stage III/IV	2.22	0.98-5.03	0.055			
Lactate dehydrogenase, per 100 IU/L	1.05	1.02-1.09	<0.001			
Extranodal involvement, per site	1.75	1.20-2.55	0.003			

44. Pace of Antiretroviral Therapy Prescription Among HIV-Infected Adults Diagnosed With Kaposi Sarcoma in East Africa

Esther Freeman[1], Aggrey Semeere[2,3], Megan Wenger[3], Naftali Busakhala[4,5], Elyne Rotich[5], Chite F. Asirwa[5,6], Mwebesa Bwana[7], Michael Kanyesigye[7], Toby Maurer[3], Constantin Yiannoutsos[6], Kara Wools-Kaloustian[6], Jeffrey Martin[3]

[1]Harvard Medical School, Boston, Massachusetts, USA; [2]Infectious Diseases Institute, Makerere University College of Health Sciences, Kampala, Uganda; [3]University of California, San Francisco, San Francisco, California, USA; [4]Moi University, Eldoret, Kenya; [5]Academic Model Providing Access to Healthcare (AMPATH), Eldoret Kenya; [6]Indiana University, Indianapolis, Indiana; [7]Mbarara University of Science and Technology, Mbarara, Uganda

Background: In sub-Saharan Africa, Kaposi sarcoma (KS) is amongst the most common malignancies among HIV-infected individuals and carries a substantial mortality in the first year after diagnosis. In all published HIV treatment guidelines, KS is an indication for combination antiretroviral therapy (ART), and, in resource-rich settings, mortality after KS diagnosis has fallen since the availability of ART giving credence to its therapeutic effectiveness for KS. In resource-poor settings such as sub-Saharan Africa, ART is often the only readily available therapy for KS. Despite the relevance of KS, the need to act quickly to prevent mortality, and the growing availability of ART, it is not understood to what extent patients with KS in Africa are being prioritized for ART and how fast ART is being prescribed.

Methods: We identified all ART-naive HIV-infected adults newly diagnosed with KS at any time from January 2009 to July 2012 while receiving their primary care at either of two HIV care programs participating in the East Africa International Epidemiologic Databases to Evaluate AIDS (IeDEA) Consortium: ISS Clinic, Mbarara, Uganda and AMPATH in Kenya. We analyzed data from these patients until they were prescribed ART, died, or transferred care, whichever occurred first. All patients who were lost to follow-up without known ART prescription were attempted to be tracked in the community to update ART use and vital status; those who were not found were represented by those who were found using probability weights. We used the Aalen-Johansen estimator to determine the cumulative incidence of ART initiation from time of KS diagnosis accounting for death as a competing event.

Results: We evaluated 585 patients newly diagnosed with KS during the course of their primary care; 63% were men, and at time of KS diagnosis, median age was 34 years (inter quartile range [IQR]: 29-40) and median CD4+ T cell 183 cells/mm^3 (IQR: 60-335). A total of 56 patients became lost to follow-up without known ART prescription and were tracked in the community, of whom 35 (63%) had their ART information updated either by finding the patient him/herself or an informant. Overall, about 50% of patients were prescribed ART immediately after KS diagnosis, and the cumulative incidence of ART prescription was 71% at 1 month, 83% at 3 months, and 85% by 6 months. Pace of prescription was slightly faster at the Kenyan program (Figure), but there was no evidence for a difference in pace of prescription in those diagnosed with KS at their initial clinic visit versus those diagnosed at follow-up visits.

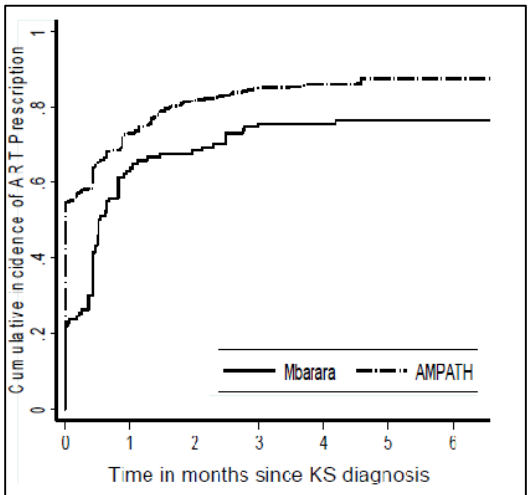


Figure. Cumulative incidence of ART prescription after diagnosis of KS at two HIV primary care programs in East Africa.

Conclusion: Among HIV-infected adults newly diagnosed with KS while in representative HIV primary care settings in East Africa, a sizeable fraction were prescribed ART immediately but an important fraction were not — even by 3 months following KS diagnosis. Given that ART prescription is not always tantamount to dispensing or ingestion, the estimates may be overly optimistic of actual ART use. The findings suggest the need for better understanding decisions to delay ART among patients with KS and/or development of interventions to streamline ART administration.

45. PET-CT in Staging Bone Marrow in HIV-Positive Patients With Lymphoma

Hina Khan, Michael Grant, Nina Kim, Murali Janakiram

Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, New York, USA

Background: In non-HIV-related non-Hodgkin lymphoma (NHL), PET-CT is now part of initial staging for FDG-avid lymphomas, Hodgkin's lymphoma (HL), diffuse large B-cell lymphomas (DLBCL), and other aggressive B and T cell lymphomas specifically. With a high sensitivity of PET-CT, patients with HL and DLBCL can now be spared a staging bone marrow biopsy (BMB) [1]. However, the role of FDG PET-CT in HIV-positive patients with lymphomas is not clearly defined, and has been evaluated only in few small retrospective studies. A retrospective analysis of 7 patients with HIV-1 infection and lymphoma supported the use of PET-CT in patients with suppressed viral loads, with decreased specificity of PET-CT with detectable viral loads [2]. In 45 HIV associated DLBCL patients, FDG PET for interim staging and follow-up had excellent NPV for assessment of response to chemotherapy but PPV was poor due to high rate of HIV-associated reactive changes [3].

Methods: We retrospectively analyzed HIV patients diagnosed with lymphoma, diagnosed between October 1998 and October 2014 at Montefiore Medical Center. Here we present results of our interim analysis.

Results: A total of 132 HIV+ patients with lymphoma were identified. The median age was 46 years. Eighty percent of the patients had NHL (n 105), 16.8% had HD (n 22) and only 3% (n 4) had unique HIV lymphomas (plasmablastic lymphoma). HIV was poorly controlled in the majority of our patients at the time of diagnosis, with 62.3% having a CD 4<200. Only 48% of all patients (n 63) were on HAART at the time of lymphoma diagnosis. 27 patients had a PET-CT and bone marrow biopsy done at the time of initial diagnosis. In 20 patients BM biopsy correlated with PET CT findings, of which 16 cases had both negative PET-CT and BMB, while 4 cases had both positive PET-CT and BMB. In the remaining 7 patients FDG avidity in the bone marrow did not correlate with a positive bone marrow biopsy. Of these seven; 4 had DLBCL, 1 had Burkitt's lymphoma, and 2 had HL. Four of these patients were anemic at the time of PET-CT exam, mean Hgb level being 11.9 gm/dL. Five of the 7 patients had poorly controlled HIV VL (range: 8962->500,000). It is noteworthy that in all but one of these cases, due to extranodal involvement the final stage remained unchanged despite the negative BMB.

Conclusions: It is interesting to note that the bone marrow was negative even in patients with a positive PET uptake, and two of these patients had Hodgkin lymphoma. We recommend that BM biopsy should still be used to accurately stage HIV lymphomas even in patients with positive PET-CT, due to other co-morbidities in this population in contrast to non-HIV lymphomas.

		No of pts (%)
Sex	Male	93 (70.5%)
	Female	39 (29.5%)
CD4+ count	Median	138 cells/μL
	Mean	212 cells/μL
	<200	71 (62.3%)
Stage	I	19 (16.5%)
	II	15 (13%)
	III	25 (21.7%)
	IV	56 (48.7%)

		No of pts (%)
Lymphoma type	Hodgkins	22 (16.8%)
	Low grade NHL	3 (2.3%)
	High grade NHL	106 (80.9%)
Lymphoma outcomes	Remission	53 (52.5%)
	Refractory disease	17 (16.8%)
	Recurrence	31 (39.7%)
Survival	Median OS [months]	18
	Mean OS [months]	38

46. The Potential of Statins to Prevent or Treat AIDS-Lymphoma

Daniel P. Widney, Wen-Ching Tran, Jake Benowitz, Yu Guo, Marta Epeldegui, Otoniel Martinez-Maza

UCLA AIDS Institute, David Geffen School of Medicine at UCLA, Los Angeles, California, USA

Background: Although the incidence of AIDS-lymphoma has decreased in the era of effective combination anti-retroviral therapy (cART), it still remains substantially increased compared to the incidence of lymphoma in the general, HIV-uninfected, population. Similarly, although survival rates for AIDS-lymphoma have improved, many people still die of this disease. Indeed, some recent studies have suggested that, over the long term (i.e., 10 years), a majority of individuals with AIDS-lymphoma will still ultimately die of this disease. Therefore, the development of new methods to prevent or treat this disease remains a priority. HIV infection is associated with B cell hyperactivation, which may be caused, at least in part, by the direct stimulation of B cell activation by CD40L on the surface membrane of HIV virions, by the presence of elevated levels of B cell stimulatory cytokines, and by the loss of immunoregulatory control of the Epstein-Barr virus (EBV) in the context of immunodeficiency. B cell hyperactivation in HIV infection is believed to be directly related to the development of oncogenic mutations mediated by the DNA-mutating enzyme, activation-induced cytidine deaminase (AICDA), which is up-regulated during B cell activation. B cell hyperactivation decreases after cART, but does not fully resolve, which is likely a cause of the continuing increased risk for AIDS-lymphoma in HIV+ subjects taking cART. Statin drugs have been shown to have anti-inflammatory effects that are associated with a reduced incidence of various types of cancers; they have also been shown to inhibit the growth of some cancer cells.

Methods: The ability of statins to inhibit the activation of resting B cells was examined by exposing resting B cells to CD40L-expressing HIV virions or to EBV in vitro in the presence of statins and measuring certain markers of B cell activation, such as Ig production, after 10 days. The effect of statins on the spontaneous production of Ig by B cells in cultures of PBMC from untreated HIV+ subjects was also examined. Next, the ability of statins and also COX-2 inhibitors to inhibit the proliferation/viability of AIDS-lymphoma cell lines was measured using the XTT assay. To determine the ability of statins to inhibit growth of AIDS-lymphoma tumors in vivo, immunodeficient NOD-SCID mice were fed chow containing simvastatin, and then injected with the AIDS-Burkitt cell line, 2F7; time to tumor formation was then measured.

Results: Statins greatly decreased B cell activation induced by CD40L-expressing HIV virions and by EBV in vitro; spontaneous Ig production in cultures of PBMC from untreated HIV+ subjects was also substantially decreased. Simvastatin, lovastatin, and atorvastatin significantly decreased the proliferation/viability of AIDS-lymphoma cell lines in vitro, although pravastatin had little effect. The COX-1/COX-2 inhibitor, indomethacin, also inhibited proliferation/viability of the AIDS-lymphoma cell lines, and had a substantial additive or even synergistic effect when added to cultures simultaneously with simvastatin. There was a trend towards delayed development of AIDS-lymphoma tumors in immunodeficient mice at the relatively low dosage of simvastatin that was tested.

Conclusions: Statins have potential for reducing excess B cell activation in HIV infection, thereby potentially reducing the incidence of AIDS-lymphoma. Statins also have some potential for use as agents to directly treat AIDS-lymphoma. Studies to define the effect of statin use on serum levels of inflammatory cytokines and biomarkers are ongoing in >1000 HIV+ subjects on cART in the Multicenter AIDS Cohort Study (MACS) and should provide novel information on the effects of these drugs on HIV-associated immune activation in those receiving antiretroviral therapy.

47. Prediction of Clinical Outcomes Among Patients With Kaposi Sarcoma Initially Treated With Antiretroviral Therapy Alone: A Systematic Review

Miriam Laker-Oketta[1, 2], Esther Freeman[3], Jeffrey Martin[2]

[1]Infectious Diseases Institute, Kampala, Uganda; [2]University of California, San Francisco, San Francisco, California, USA; [3]Harvard School of Medicine, Boston, Massachusetts, USA

Background: HIV-infected patients with KS who have functionally disabling complications from their KS require urgent chemotherapy to quickly relieve symptoms; treatment with antiretroviral therapy (ART) alone is inadequate. In patients without functionally disabling KS, standard of care is often to initiate ART alone, but, in sub-Saharan Africa, patients treated in this way have ~20% 1-year mortality, which is several-fold higher than in those initiating ART without KS. Initial use of chemotherapy in addition to ART might improve outcomes in these patients but its use in all patients with AIDS-KS in Africa is currently cost-prohibitive. Thus, establishing which factors, if any, predict poor KS outcomes might help to inform which KS patients without an urgent indication for chemotherapy can be safely started on ART alone versus those who might benefit from other concurrent interventions (e.g., chemotherapy).

Methods: We performed a systematic review of the biomedical literature through July 2014 to identify factors predictive of clinical outcomes among patients with KS initially treated with ART alone. To be included, studies needed to include ART-naïve HIV-infected adults diagnosed with KS, who had no urgent indication for chemotherapy, and who were initially treated with combination ART alone. Outcomes included death or the composite of death and/or indication for chemotherapy.

Results: Out of 6267 abstracts reviewed, 6 were ultimately deemed eligible for synthesis (1 RCT research-dedicated cohorts, and 3 clinical practice-based cohorts), of which 3 (Dhrif et al. 2007; Paparizos et al. 2000; and Monticelli et al. 2000) had too few outcomes to yield meaningful information. In the remaining 3 studies, older age, lower education, and ATCG S1 stage were associated with poorer outcomes in at least one study (Table). Only age, however, was reported to be predictive in more than one study, and it was not consistently predictive in all 3 studies.

Author, year	Setting	Design	No. of subjects	Factors associated with death	Factors associated with chemotherapy indication or death
Mosam, 2012	Durban, South Africa; 2003 to 2009	Randomized trial	59	**Education level:** Secondary and beyond vs. primary or none (Ref.) Hazard ratio = 0.26 (95% CI: 0.08–0.9); p=0.03 **ACTG S staging:** S1 vs. S0 (Ref.) Hazard ratio = 3.4 (95% CI: 1.07–11.4); p=0.04	None
Borok, 2010	Harare, Zimbabwe; 2003 to 2005	Research-dedicated cohort	90	**Age**: >45 years vs. ≤45 (Ref.) Odds ratio = 3.45 (95% CI: 0.93-12.9)	Not reported
Lasso, 2003	Santiago de Chile, Chile; 1995 to 2002	Clinical practice-based cohort	13	**Age**: per 1 year increase Odds ratio = 1.3 (95% CI: 0.95–1.67); p=0.08	Not reported

Conclusions: Among HIV-infected patients with KS who had no urgent indications for chemotherapy and who were initially treated with ART alone, a systematic review of the literature found that, other than age, no factor was reported in more than one study to be predictive of death or clinical progression. Why more predictors were not found is not clear, but the formal possibilities include poorly measured exposure variables or the wrong factors evaluated. As the WHO also indicated in its recent guidelines on the management of HIV-associated skin diseases, more research on this question is needed to fill an important gap in our clinical knowledge base.

48. Predictors of Early Mortality Among Patients Initiating Treatment for HIV-Associated Kaposi Sarcoma in Uganda

Warren Phipps[1,4], Elizabeth Krantz[4], George Holoya[5,6], Clement Okello[5,6], James Kafeero[5,6], Ruth Nakuya[6], Ivan Ayebale[6], Jackson Orem[5,7], Corey Casper[1,2,3,4]

[1]Departments of Medicine, [2]Epidemiology, and [3]Global Health, University of Washington, Seattle, Washington, USA; [4]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA; [5]Uganda Cancer Institute, Kampala, Uganda; [6]Hutchinson Center Research Institute-Uganda, Kampala, Uganda; [7]Department of Medicine, Makerere University College of Health Sciences, Kampala, Uganda

Background: To characterize outcomes among patients with HIV-associated Kaposi sarcoma (KS) in Uganda and to identify factors associated with survival.

Methods: We enrolled adult patients with histologically confirmed HIV-associated KS initiating treatment at the Uganda Cancer Institute in Kampala, Uganda, between October 2012 and March 2015. Participants were followed prospectively for up to 1 year from starting treatment with antiretroviral therapy (ART) and chemotherapy with combination bleomycin and vincristine. Plasma and 7 daily oral swabs were collected with each 3-weekly study visit to quantify human herpesvirus-8 (HHV-8) DNA by polymerase chain reaction. Participants were staged using AIDS Clinical Trials Group (ACTG) criteria. Survival estimates used Kaplan-Meier (KM) method, and Cox proportional hazards models were used to estimate associations of baseline variables with survival.

Results: 140 patients with HIV and histologically confirmed KS enrolled in the study. 30 (21%) participants were women, and the median age was 32 years (range 18-75 years). The median baseline CD4 T-cell count was 171 cells/mm^3 (IQR 45, 341 cells/mm^3), and the median baseline plasma HIV-1 RNA level was 5.4 log10 copies/mL (IQR 5.0, 5.8 log10 copies/mL). Among samples tested to date, HHV-8 DNA was detected in 58% (170/291) of baseline oral swabs and 91% (39/43) of baseline plasma samples. 122 patients (87%) had poor risk tumor stage (T1), with lesions involving a median of 8 anatomic sites (range 1-12 sites). 107 (76%) had systemic symptoms meeting poor risk criteria (S1), and 38 (27%) had a Karnofsky performance score under 70. The overall 1-year survival was 52%. Among those who died, 57% (31/54) died within the first 4 months of initiating treatment. In multivariate analysis, risk of death within 4 months of starting chemotherapy was significantly related to CD4 count <200 (HR=3.2, CI 1.4-7.3, p=0.006), Karnofsky score <70 (HR=3.4, CI 1.7-6.9, p=0.001), and each additional anatomic site with tumor involvement (HR=1.2, CI 1.1-1.4, p=0.004). Additional analyses will be presented on the relationship between HHV-8 detection in oral and plasma samples and survival.

Conclusion: Provision of chemotherapy and ART was associated with early mortality in a subset of patients with HIV-associated KS, suggesting a need to better risk-stratify patients most likely to benefit from chemotherapy, to improve supportive care strategies during treatment, and to identify alternative therapeutic approaches to managing KS.

49. Prognostic Model for Patients with Kaposi Sarcoma With Antiretroviral Therapy Alone in Sub-Saharan Africa

Miriam Laker-Oketta[1,2], David Glidden[2], Victoria Walusana[3], Jackson Orem[3], Adrienne Mocello[2], Toby Maurer[2], Peter Hunt[2], Andrew Kambugu[1], Edward Mbidde[1,2] ,Jeffrey Martin[2]

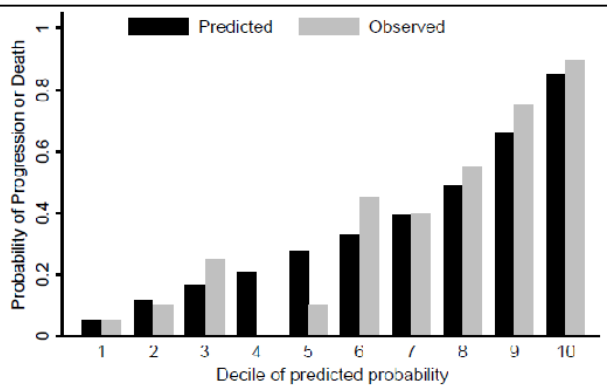
[1]Infectious Diseases Institute, Makerere University, Kampala, Uganda; [2]University of California, San Francisco, San Francisco, California, USA; [3]Uganda Cancer Institute, Kampala, Uganda; [4]Uganda Virus Research Institute, Entebbe,
Uganda

Background: While patients with functionally disabling Kaposi sarcoma (KS) require urgent chemotherapy for rapid reduction in tumor burden, the management of KS which lacks functionally disabling complications is less certain. This is particularly true in sub-Saharan Africa, where the relative high cost of chemotherapy makes decisions about its use difficult and the use of antiretroviral therapy (ART) alone common as initial KS treatment. However, because a substantial fraction of patients with KS in Africa who are treated with ART alone fare poorly, it would be useful to be able to predict which patients with KS will do well on ART alone versus those who might benefit from additional interventions.

Methods: We studied HIV-infected adults in Uganda with KS with no functionally disabling complications who were initially treated with ART alone as part of the AntiRetrovirals for KS (ARKS) trial. We evaluated the predictive value of 57 variables covering clinical history, physical exam, clinically available laboratory characterization, and radiographic findings, each measured prior to ART. Continuous variables were examined both natively with splines and using categories. The outcome was death or KS progression necessitating chemotherapy. The Least Absolute Shrinkage and Selection Operator (LASSO) was used to identify variables with the highest predictive accuracy upon cross-validation.

Results: Among 224 subjects, 44% were women, and median values prior to ART initiation were 34 years old, 119 CD4+ T cells/mm^3, and 5.4 log10 copies/ml of plasma HIV RNA. The final prediction model had 3 continuous variables: number of mucocutaneous anatomic sites with KS, hemoglobin, and Karnofsky performance score. The model showed adequate discrimination: area under the ROC curve = 0.76 (95% CI: 0.73-0.79). Calibration was also good (Figure) through a wide range of predicted probabilities. At lower probabilities of observed outcome, the model tended to overestimate the probability of poor outcome, while at higher ranges of observed outcome, it slightly underestimated outcomes. A model specifying number of anatomic sites with KS, hemoglobin, and Karnofsky performance score in categories, which can allow for readily calculated risk stratification at the bedside using point scores, performed nearly as well: area under the ROC curve was 0.73 (95% CI: 0.68-0.78).

Figure. Calibration of risk prediction model. For each successive decile of predicted probability of progression or death, the mean predicted and observed probabilities for the patients in the decile group are shown.



Conclusions: A prognostic model, consisting of inexpensive measurements, can help to predict which patients in Africa with KS — who present with no functionally disabling complications — will fare poorly when treated with ART alone. The presence of number of KS-containing anatomic sites and hemoglobin in the model suggests the importance of quantitative KS burden in prognosis. The model may help clinicians better advise patients about prognosis and, importantly, inform chemotherapy decisions.

50. Prevalence of Human Papilloma Virus (HPV) in Head and Neck Cancers (HNCs) in Nigeria

Eileen Oga[1,2], Aniefon Umana[3], Imaabasi Bassey[3], Godwin Ebughe[3], S. Alabi[4], Darlington Obaseki[5], L. Schumacker[6], William Blattner[6], Patrick Dakum[2] , Kevin Cullen[6], Clement Adebamowo[1,2,6]*

[1]Department of Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, Maryland, USA; [2]Institute of Human Virology Nigeria, Abuja, FCT, Nigeria; [3]University of Calabar Teaching Hospital, Calabar, Cross River State, Nigeria; [4]University of Ilorin Teaching Hospital, Ilorin, Kwara State, Nigeria; [5]University of Benin Teaching Hospital, Benin, Edo State, Nigeria; [6]Institute of Human Virology and the Marlene and Stewart Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, Maryland, USA

Background: Tobacco smoking and alcohol consumption had for long been implicated as leading risk factors for the development of HNCs. However, recent literature implicates infection with HPV as a main risk factor for the development of HNCs. The burden of HPV-positive HNCs is lower in African Americans than in whites. It is expected that, although rising, the burden of HPV positive HNCs will be low in Nigerians (similar to the African American population). Data from Africa, and specifically Nigeria, on the prevalence of HPV-positive tumors is lacking. The prevalence of HPV in Head and Neck cancers (HNCs) was investigated in this study.

Methods: DNA extraction and HPV analysis of Formalin fixed, Paraffin-Embedded (FFPE) tumor blocks from HNC cancers were carried out on samples from three centers in Nigeria – University of Calabar Teaching Hospital (UCTH), University of Benin Teaching Hospital (UBTH) and University of Ilorin Teaching Hospital (UITH).

Results: In all, 115 tumor blocks of Head and Neck cancers which had been collected over 22 years (1990-2011) were analyzed. 37% were female and 63% were male. Median age was 43 years. Of the 115 blocks, 94 were eligible for DNA core analysis; DNA purification was successful for 84. PCR amplification was successful in 49; Linear Array amplification was successful in 17 cases resulting in an overall success rate of 38%. Of the successful cases, none was found to be positive for HPV of any type.

Conclusion: HPV prevalence appears low in this population, which may be a result of the relative rarity of oral sex practices in this population. However, the poor sample quality and the resultant low overall success rate for PCR and Linear Array amplification means that the results are not conclusive. Focus should be placed on implementing appropriate sample processing and storage methods to ensure that samples are useful for molecular biology work. Also, there is need to carry out larger, prospective studies on the role of HPV in the pathogenesis of Head and Neck cancers in African populations.

51. Recurrence of Cervical Intraepithelial Lesions After Thermo-Coagulation in HIV-Positive and HIV-Negative Nigerian Women

Emmanuel Oga[1,2], Jessica Brown[1], Clayton Brown[1], Eileen Dareng[2,9], Olayinka Olaniyan[3], Richard Offiong[4], Victor Adekanmbi[2], Michael Odutola[2], Kayode Obende[5], Stephen Adewole[6], Peter Achara[7], Patrick Dakum[2], Clement Adebamowo[1,2,8]

[1]Department of Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, Maryland, USA; [2]Institute of Human Virology Nigeria (IHVN), Abuja, Nigeria; [3]National Hospital, Abuja, Nigeria; [4]University of Abuja Teaching Hospital, Gwagwalada, Nigeria; [5]Garki Hospital, Abuja, Nigeria; [6]Mother and Child Hospital, Ondo, Nigeria; [7]Federal Medical Centre, Keffi, Nigeria; [8]Greenebaum Cancer Center and Institute of Human Virology, University of Maryland School of Medicine, Baltimore, Maryland, USA; [9]Department of Public Health and Primary Care, University of Cambridge, United Kingdom

Background: The burden of cervical cancer remains huge globally, more so in sub-Saharan Africa. Effectiveness of screening, rates of recurrence following treatment, and factors driving these in Africans have not been sufficiently studied. The purpose of this study therefore was to investigate factors associated with recurrence of cervical intraepithelial lesions following thermo-coagulation in HIV-positive and HIV-negative Nigerian women using Visual Inspection with Acetic Acid (VIA) or Lugol's Iodine (VILI) for diagnosis.

Methods: A retrospective cohort study was conducted, recruiting participants from the cervical cancer "see and treat" program of IHVN. Data from 6 sites collected over a 4-year period were used. Inclusion criteria were age ≥18 years, baseline HIV status known, VIA or VILI positive, and thermo-coagulation done. Logistic regression was performed to examine the proportion of women who returned for their scheduled follow-up, those with recurrence and factors associated with recurrence. Student's t-test was used to compare continuous variables between HIV-positive and HIV-negative women while Fisher's exact test was performed for categorical variables.

Results: Out of 177 women included in study, 67.8% (120/177) were HIV-positive and 32.2% (57/177) were HIV-negative. Recurrence occurred in 16.4% (29/177) of participants; this was 18.3% (22/120) in HIV-positive women compared to 12.3% (7/57) in HIV-negative women but this difference was not statistically significant (p-value 0.31). Women aged ≥30 years were much less likely to develop recurrence, adjusted OR = 0.34 (95% CI = 0.13, 0.92). Among HIV-positive women, CD4 count <200cells/mm^3 was associated with recurrence, adjusted OR = 5.47 (95%CI = 1.24, 24.18).

Conclusion: Recurrence of VIA or VILI positive lesions after thermo-coagulation occurs in a significant proportion of women. HIV-positive women with low CD4 counts are at increased risk of recurrent lesions and may be related to immunosuppression.

52. Risk Factors for Oropharynx Cancer in a Cohort of HIV-Infected Veterans

Elizabeth Y. Chiao[1], Christine Hartman[1], Jose Zevallos[2]

[1]Baylor College of Medicine, Michael E. Debakey VA Medical Center, Houston, Texas, USA; [2]The University of North Carolina, Raleigh, North Carolina, USA

Background: Since the introduction in 1995 of combined antiretroviral therapy (cART), the mortality rate of HIV-infected individuals from AIDS-associated opportunistic infections has decreased by 70%. The incidence of several cancers caused by infectious agents, such as Kaposi's sarcoma, has also decreased dramatically in HIV-infected individuals in the cART era. However, the incidence of HPV-related cancers has not declined, and in the case of squamous cell cancer of the anus (SCCA), the incidence has increased. Although a recent cohort study of HIV-infected individuals demonstrated that the standardized incidence ratio of oropharynx cancer is approximately 3 compared to HIV-uninfected individuals, the authors found that the incidence of the disease was relatively stable over time (reference).

Methods: We performed a retrospective cohort study among male U.S. veterans diagnosed with HIV infection between 1985 and 2010, using the Veterans Affairs HIV Clinical Case Registry (CCR). We included only those patients who were alive in 1996 (cART era), and those who had at least 1 CD4 count. We calculated Cox proportional hazards ratios of oropharynx cancer in a multivariate model. We adjusted for risk factors, including age, race, year of enrollment into CCR, recent and nadir CD4, and percent time undetectable HIV viral load.

Results: A total of 40,996 HIV-infected male veterans were included in the cohort. There were a total 97 cases of oropharynx cancer. The median follow-up time was 6.9 years. The median age at HIV diagnosis was 45 years of age. Fifty percent of the cohort was black, 14% Hispanic, and 36% white. 80% of the cohort received cART. In multivariable analyses, age >50 (aHR 3.3, CI 1.744, 6.191) and most recent CD4 <200 (aHR5.1, CI2.7, 9.8) were associated with an increased risk of oropharynx cancer. Compared to those diagnosed with HIV infection between 1985 and 1996, those diagnosed with HIV infection between 2001 and 2010 (aHR 0.33, CI 0.17, 0.64) demonstrated a significant decreased risk of oropharynx cancer. Nadir CD4 count, percent of time with undetectable HIV viral load, and utilization of cART were not associated with oropharynx cancer risk.

Conclusion: Older age and having long-term HIV infection appears to increase the risk of oropharynx. In addition, those who had low CD4 counts around the time of diagnosis also had a significantly higher risk of oropharynx cancer. Interestingly, unlike SCCA, which is also a HPV-associated cancer, nadir CD4 count and cumulative viral load control were not associated with oropharynx cancer risk. Further research on risk factors and possible oropharynx cancer prevention is needed for HIV-infected individuals.

Reference

Beachler DC, Abraham AG, Silverberg MJ, et al. Incidence and risk factors of HPV-related and HPV-unrelated Head and Neck Squamous Cell Carcinoma in HIV-infected individuals. *Oral Oncol.* 2014;50(12):1169-1176.

53. Risk of Infection-Related and Non-Infection-Related Cancer in HIV-Positive Adults on ART in South Africa: A Probabilistic Record Linkage Study

Mazvita Sengayi[1], Adrian Spoerri[2], Matthias Egger[2], Janet Giddy[3], Mhairi Maskew[4], Elvira Singh[1], Julia Bohlius[2] for the International Epidemiologic Databases to Evaluate AIDS Southern Africa (IeDEA-SA)

[1]National Cancer Registry, South Africa; [2]University of Bern, Switzerland; [3]McCord Hospital, South Africa; [4]University of Witwatersrand, South Africa

Background: The surveillance of HIV-related cancers in South Africa is hampered by the lack of systematic collection of cancer diagnoses in HIV cohorts and the absence of HIV status in cancer registries. To estimate cancer incidence and explore risk factors for developing infection-related and non-infection-related cancer, we conducted a probabilistic record linkage study of two ART programs providing care for adults and the National Cancer Registry in South Africa.

Methods: We used data for the period 2004-2011 from two ART programs in South Africa (McCord Hospital, KwaZulu-Natal, and Themba Lethu Clinic, Gauteng province) and linked it to the cancer registry data for the same period. We calculated cancer incidence rates and hazard ratios (HR) with 95% confidence intervals (CI) from multivariable Cox regression models for sex, age, CD4 cell counts, and hemoglobin levels at start of ART for infection-related and non-infection-related cancers as defined by the *International Agency for Research on Cancer*.[1]

Table 1: Cancer in HIV-positive men and women on ART stratified by etiology

	Men	Women
	Incidence rate* (95% CI)	Incidence rate*
Total	1240 (1095-1403)	1353 (1242-1474)
Infection cancer	888 (767-1028)	999 (905-1103)
Kaposi sarcoma	437(354-537)	240 (196-294)
Cervical cancer	-	477 (413-551)
Non-Hodgkin's lymphoma	141 (98-204)	126 (96-166)
Lip, oral cavity	58 (33-103)	25 (13-47)
Conjunctiva	58 (33-103)	60 (41-90)
Anorectal	29 (13-65)	10 (4-27)
Penis	39 (19-78)	-
Hodgkin's	34 (16-71)	15 (7-34)
Stomach	24 (10-58)	10 (4-27)
Not infection related	396 (319-493)	420 (361-489)
Breast	-	159 (124-204)
Esophagus	68 (40-115)	18 (8-37)
Prostate	49 (26-91)	-
Colon	15 (5-45)	22 (12-44)

*per 100,000 person-years (95% CI)

Table 2: Adjusted hazard ratios for cancer in HIV- positive adults stratified by etiology

Infection-related		
	Yes	No
	HR (95% CI)	HR (95% CI)
Gender		
Male	1	1
Female	1.17 (0.94-1.45)	1.07 (0.78-1.48)
Age (yrs)		
16-25	1	1
26-35	1.49 (0.98-2.26)	1.02 (0.53-1.94)
36-45	1.52 (0.99-2.23)	1.40 (0.73-2.66)
46-55	1.13 (0.69-1.86)	2.29 (1.17-4.50)
≥56	1.34 (0.64-2.80)	2.63 (1.08-6.39)
CD4 count*		
<100	1	1
100-349	0.91 (0.75-1.11)	1.04 (0.77-1.40)
≥350	0.24 (0.08-0.76)	0.62 (0.19–1.97)
Hemoglobin**	0.92 (0.87-0.96)	0.94 (0.87-1.01)

*cells/µL , **g/dL

Results: We included 23,120 patients in the incidence analysis, 64% were women, at starting ART median age was 36 years (IQR 31-42) and median CD4 cell count was 109 cells/µL (IQR 45-179). During 59,101 person-years (pys) of follow-up, 851 patients developed incident cancer for an overall incidence rate of 1,315/100,000 pys (95% CI 1,225-1,410) (Table 1). Incidence rates were highest for cervix (477; 95% CI 413-551) followed by Kaposi sarcoma (307; 95% CI 266-355). The risk of developing infection-related cancer increased with lower CD4 cell counts at start ART (<100 versus ≥350 cells/µL: HR 0.24, 0.08-0.76) and with lower hemoglobin levels (HR: 0.92, 95% CI 0.87-0.96, Table 2). For cancers not associated with infections, cancer risk increased with higher age at ART start (≥56 versus 16-25 years: HR 2.63, 95% CI 1.08-6.39).

Conclusions: Incidence of cancer in HIV-positive South Africans in the era of potent ART is high, particularly for AIDS-defining cancers and infection-related cancers. There is need to explore and implement known cancer-specific prevention strategies in the HIV population.

54. Screening Interval After Normal Cervical Cytology Among HIV-Infected Women: A Risk-Based Approach

Hilary A. Robbins[1], L. Stewart Massad[2], Christopher B. Pierce[1], Lisa Flowers[3], Teresa M. Darragh[4], Howard Minkoff[5], Lisa Rahangdale[6], Marla J. Keller[7], Joel Milam[8], Margaret Fischl[9], Sadeep Shrestha[10], Christine Colie[11], Howard Strickler[7], Gypsyamber D'Souza[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA; [2]Washington University School of Medicine, St. Louis, Missouri, USA; [3]Grady Memorial Hospital and Emory University School of Medicine, Atlanta, Georgia, USA; [4]University of California, San Francisco, San Francisco, California, USA; [5]Maimonides Medical Center, Brooklyn, New York, USA; [6]The University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA; [7]Albert Einstein College of Medicine, Bronx, New York, USA; [8]University of Southern California, Los Angeles, California, USA; [9]University of Miami Miller School of Medicine, Miami, Florida, USA; [10]University of Alabama at Birmingham School of Public Health, Birmingham, Alabama, USA; [11]Georgetown University Medical Center, Washington D.C., USA

Requested not to post abstract in online publication.

55. Squamous Cell Cancer of Unknown Primary and Primary Breast Cancer in an HIV-Infected Woman: The Importance of Cancer Screening for People Living With HIV/AIDS

Joshua Gulvin[1], David M. Aboulafia[2,3]

[1]Division of Internal Medicine; [2]Section of Hematology and Oncology, Virginia Mason Medical Center, Seattle, Washington, USA; [3]Division of Hematology, University of Washington, Seattle, Washington, USA

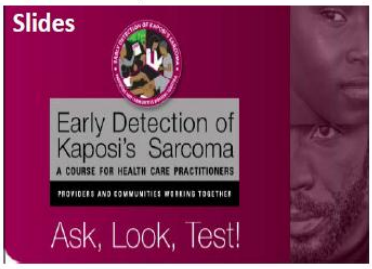
It has been two decades since the implementation of highly active antiretroviral therapy (HAART) and people living with HIV/AIDS (PLWHA) are surviving longer but remain at increased risk of cancer. Evidence-based strategies for cancer screening in this particular population are lacking, although many caregivers are stressing the importance of minimizing alcohol consumption and attempting to offer counseling for smoking cessation. We describe the case of a middle-aged woman with a long history of AIDS and who, while on HAART, had a non-detectable HIV viral load. She is an activist in her own community promoting HIV care for women; however, she herself had not undergone recent and routine cancer screening for breast, cervical, or colonic neoplasia. She presented to medical attention with an enlarged left groin mass, which proved to be a poorly differentiated squamous cell carcinoma, with positive immuno-staining for Human Papillomavirus 16. Anal and cervico-vaginal exams did not show invasive cancer, although high-grade anal dysplasia was identified by high-resolution anoscopy and directed anal biopsies. Through the course of her workup, magnetic resonance imaging demonstrated a right breast mass, which on needle biopsy showed invasive ductal carcinoma. Her breast cancer was treated with lumpectomy, adjuvant intraoperative brachytherapy, and chemotherapy. The left groin tumor was treated with combined chemo- and radiation therapy. We also briefly review the medical literature concerning anal, cervical, breast, colorectal, and lung cancer screening for PLWHA, which we anticipate will be an increasingly important clinical need for our aging population of PLWHA.

56. Training Health Care Providers to Enhance Early Diagnosis of Kaposi Sarcoma in Sub-Saharan Africa

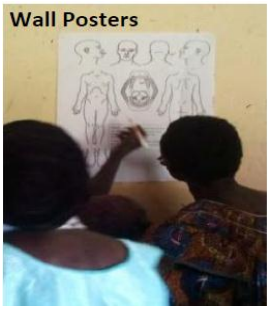
Philippa Kadama-Makanga[1], Miriam Laker-Oketta[1], Lisa Butler[2], Robert Inglis[3], Andrew Kambugu[1], Toby Maurer[4], Edward Mbidde[5], Jeffrey Martin[4]

[1]Infectious Diseases Institute, Kampala, Uganda; [2]Harvard Medical School, Boston, Massachusetts, USA; [3]Jive Media Africa, Pietermaritzburg, South Africa; [4]University of California, San Francisco, San Francisco, California, USA; [5]Uganda Virus Research Institute, Entebbe, Uganda

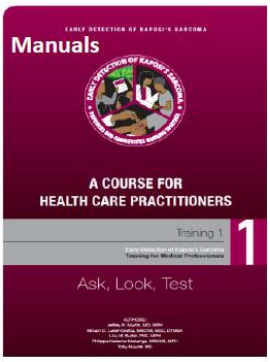
Background: Advanced stage presentation and late diagnosis — when available interventions are typically ineffective — among patients with Kaposi sarcoma (KS) is one of the central problems concerning HIV-associated cancer in sub-Saharan Africa. Several factors are responsible, and we earlier showed that sub-optimal understanding of KS by front-line clinicians and sub-optimal screening practices likely contribute. To address this, as part of our overall "Early Detection of Kaposi's Sarcoma" (EDKS) Program, we set out to develop a training program in KS for health care professionals in Uganda.



Methods: The instructional objective of the EDKS Training Program was to impart setting-specific knowledge regarding the epidemiology, recognition, diagnosis, and initial management of KS. The ultimate clinical goal was to increase early detection of KS. We sought to create transportable materials that could be used to provide efficient and culturally appropriate instruction to the entire spectrum of health care professionals who provide primary care to HIV-infected patients in sub-Saharan Africa.



Results: Three separate training modules were developed. The first was a 1-day session tailored for higher-level professionals including physicians, clinical officers, and nurses; it is intended to be taught by a professional well versed in KS. The second was for less formally trained workers, including nursing assistants, community health workers, and traditional health providers. This session can be taught by either a person well versed in KS or by the supervisor of the trainees (e.g., a nursing supervisor). The third module provided guidance to the aforementioned supervisors on how to implement the second module ("Training the Trainers"). Training emphasized a 3-part theme, relating to the main activities required to enhance early KS detection.



The first is "Ask," meaning that providers should routinely ask HIV-infected patients if they have noticed any skin changes. The second — "Look" — refers to routinely performing skin exams to identify lesions that may have not been noticed by patients. The final theme — "Test" — emphasizes skin biopsy of suspicious lesions for definitive diagnosis of KS. Digital slide sets were developed for the higher level group, and wall posters and handouts were used in the less formally trained group. Pre- and post-training exams were created to evaluate trainees' immediate comprehension.

Conclusion: The EDKS Program has developed a set of materials to train doctors, clinical officers, nurses, community health workers, and traditional health providers in sub-Saharan Africa in recognition, diagnosis, and initial management of KS. The goal is to increase early KS detection. Resources include manuals for training the trainers. The materials are freely available and can be modified to local context.

57. Trends in CNS Lymphoma Incidence and Survival Among Immunocompetent People in the United States

Meredith Shiels[1], Ruth M. Pfeiffer[1], Caroline Besson[2], Christina Clarke[3], Lindsay M. Morton[1], Leticia Nogueira[4], Karen Pawlish[5], Gita Suneja[6], Elizabeth Yanik[1], Eric A. Engels[1]

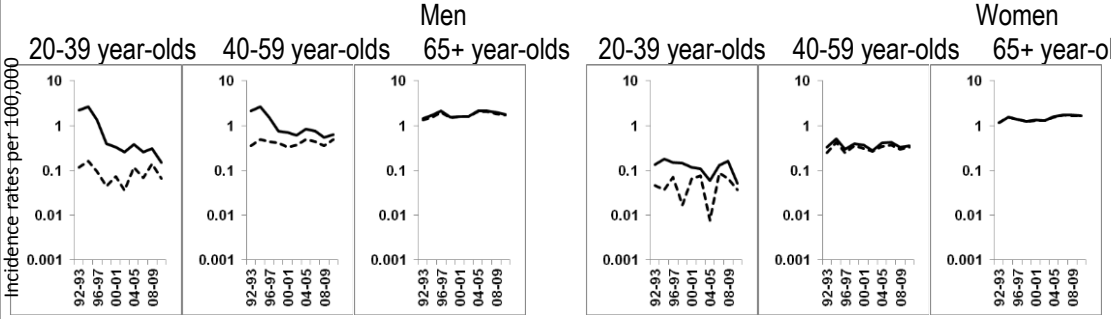
[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, Maryland, USA; [2]Bicêtre University Hospital, Le Kremlin-Bicêtre, France; [3]Cancer Prevention Institute of California, Fremont, California, USA; [4]Texas Department of State Health Services, Austin, Texas, USA; [5]New Jersey Department of Health, Trenton, New Jersey, USA; [6]Department of Radiation Oncology, University of Utah, Salt Lake City, Utah, USA

Background: Central nervous system (CNS) lymphoma rates among HIV-infected people have declined due to effective antiretroviral treatment. In contrast, it is suspected that rates among immunocompetent individuals have increased. Monitoring CNS lymphoma incidence and survival in population-based data is challenging because of the need to separate immunocompetent and immunocompromised individuals. Using data from population-based cancer and solid organ transplant registries, we estimate CNS lymphoma incidence and survival rates among immunocompetent persons by excluding cases occurring among HIV-infected persons and transplant recipients.

Methods: Data on CNS lymphomas were derived from 10 population-based Surveillance, Epidemiology and End Results (SEER) cancer registries from 1992 to 2011. Cases with HIV infection noted at the time of diagnosis or HIV as a cause of death were classified as HIV-infected. Expected cases occurring among transplant recipients were estimated by applying rates of transplant-associated CNS lymphoma from the U.S. Transplant Cancer Match Study to the SEER population. We estimated trends in CNS lymphoma incidence rates overall and among immunocompetent individuals (i.e., without HIV or transplant). We also estimated survival in HIV-infected and HIV-uninfected CNS lymphoma cases.

Results: During 1992-2011, 4,158 CNS lymphomas were diagnosed in SEER areas. 36% of cases (n=1,512) were HIV-infected and an estimated 0.9% occurred in transplant recipients. HIV prevalence in CNS lymphoma cases declined from 64.1% in 1992-1996 to 12.7% in 2007-2011, while the fraction of cases occurring among transplant recipients remained low over time. Immunocompromised CNS lymphoma cases have had a profound impact on general population rates, particularly among younger men (Figure). Among immunocompetent people, rates of CNS lymphoma have significantly increased by 1.7%/year among men and 1.6%/year among women (p-values<0.05) among 65+ year-olds, but remained steady in other age groups (all p-values>0.05). Survival was poor, particularly among HIV-infected cases (5-year survival: 8.3% [HIV-infected]; 26.2% [HIV-uninfected]). However, among HIV-uninfected cases, 5-year survival increased slightly from 20.2% (1992-1996) to 29.2% (2002-2006).

Figure. Age-standardized incidence rates of CNS lymphoma overall (solid line) and after excluding cases occurring in immunocompromised individuals (dashed line) in 10 SEER registries, 1992-2011.



Conclusions: CNS lymphomas arising in HIV-infected people have had a profound impact on general population rates, particularly among young men. Rates of CNS lymphoma among immunocompetent people have increased in the elderly population, but not in younger individuals. Survival remains poor for both HIV-infected and HIV-uninfected CNS lymphoma cases.

58. What Happens to Patients With Kaposi Sarcoma Who Become Lost to Followup? A Qualitative Study From East Africa

Aggrey Semeere[1,2], Mwebesa Bwana[3,4], Michael Kanyesigye[3,4], Merridy Grant[5], Andrew Kambugu[1], Jeffrey Martin[2]

[1]Infectious Diseases Institute, Makerere University College of Health Sciences, Kampala, Uganda; [2]University of California, San Francisco, San Francisco, California, USA; [3]Mbarara University of Science and Technology, Mbarara, Uganda; [4]Immune Suppression Syndrome (ISS) Clinic, Mbarara, Uganda; [5]Centre for Rural Health, University of KwaZulu-Natal, Durban, South Africa

Background: Two years after diagnosis of their cancer, approximately 45% of HIV-infected adults with Kaposi sarcoma (KS) in sub-Saharan Africa have become lost to followup from the clinic where they originally received their KS diagnosis. Why these patients stop returning to their parent clinic and their ultimate outcomes and experiences have heretofore not been examined.

Methods: Among HIV-infected adults diagnosed with KS between January 2009 and July 2012 at the Immune Suppression Syndrome (ISS) Clinic, Mbarara, Uganda, we identified those who became lost to followup as defined by not having a clinic visit for over 3 months. After searching for these lost patients in the community, we obtained consent from either the lost patient him/herself or an informant closest to the patient for an in-depth qualitative interview. Guided by Andersen's Behavioral Model of Health Services Use, we inquired about health care utilization since leaving the ISS Clinic and reasons for the patient's actions. All transcripts were entered into NVivo version 10 qualitative data analysis software. An inductive approach was used and descriptive thematic coding was the primary analytic strategy.

Results: Of 84 patients identified as lost to followup and whom we traced in the community, we found 71 (85%), either the patient him/herself (n=7) or an informant (n=64). Of these, 33 consented to be interviewed, either the patient (n=4) him/herself or an informant (n=29) who believed that they knew the patient well enough to answer our questions. Of the 33 index patients with KS represented in the interviews, 85% were men and had a median age of 38 years (interquartile range: 23 to 65) at the time of KS diagnosis. The qualitative interviews revealed that 19 (58%) of patients died within 2 months of their last recorded ISS Clinic visit. Among the remaining patients (those living beyond 2 months following last ISS Clinic visit), 12 (36%) resumed care at another facility and stayed in care continuously. Only two patients (6%) lived beyond 2 months following their last ISS Clinic visit and dropped out of all "Western" medical care for 2 or more months. Among the 29 patients who ultimately died, 15 (52%) reported compliance with prescribed medications; 8 (28%) admitted to being non-compliant with prescribed medications; and 6 (21%) had difficulty accessing prescribed medications. A variety of reasons were commonly expressed for poor compliance with and/or poor access to KS therapy (Table). Four (14%) of interviewees reported use of traditional healers and/or traditional therapies.

Reasons reported by patients or informants for poor compliance or access to KS treatment		
Patients' Personal Characteristics	**Contextual Environment**	**Health Care Environment**
☐ Self-hate ☐ Self-neglect ☐ Fatalism ☐ Excessive intake of alcohol ☐ Non-disclosure of HIV status	☐ High expense (transport and treatment costs) ☐ Insufficient social support (family neglect) ☐ Cultural beliefs (witchcraft)	☐ Perceived lack of concern from health workers ☐ Distance to health facilities from patients' homes

DAY TWO POSTERS

These highlighted abstracts will be presented on both days. Their abstracts are on pages 47 to 60.

14. A More Representative and Less Biased Approach to Estimating Mortality After Diagnosis of Kaposi Sarcoma Among HIV-Infected Adults in Sub-Saharan Africa in the Era of Antiretroviral Therapy

15. Anti-Tumor Activity of DLX1008, an Anti-VEGF-A Antibody Fragment With Low Picomolar Affinity, in an In Vivo Model of Kaposi Sarcoma

16. Changes in Clinical Context for Kaposi Sarcoma and Non-Hodgkin Lymphoma Among HIV-infected People in the United States

17. Disparities in Cancer Treatment Among HIV-Infected Individuals

18. Does Inflammation at Antiretroviral Therapy Initiation Increase Risk of Early Death and Disease Progression Among HIV-Infected Adults With Kaposi Sarcoma?

19. Expression of HIV-1 Matrix Protein and Correlation With B Cell Lymphoma in HIV-1 Transgenic Mice

20. HO-1 Promotes KSHV Infection of Endothelial Cells Through Inhibition of TLR4 Signaling

21. Immunodeficiency and the Risk of Cervical Intra-epithelial Neoplasia 2/3 and Cervical Cancer: A Nested Case-Control Study in the Swiss HIV Cohort Study

22. HPV16 Infection and Oncogenesis on the Epigenome of Human Tonsil Epithelium

23. Phase I Trial of Cabozantinib (XL184) for Advanced Solid Tumors in Persons With HIV Infection: AIDS Malignancy Consortium (AMC) Trial 087 – Trial in Progress

24. Regulation of p53-Dependent DNA Damage Responses by Hepatitis C Virus Infection

25. Role of Histone H3.3 in the Establishment and Maintenance of KSHV Latency

26. Systematic Analysis of KSHV Specific T Cell Responses in Healthy Seropositive Donors Measured by IFN-γ ELISPOT Using Proteome-wide Overlapping Peptides

14. Targeting an Immune Kinase to Purge KSHV Persistent Infection

59. A Phase 1b/Pharmacokinetic Trial of PTC299, a Novel Post-Transcriptional VEGF Inhibitor, for AIDS-Related Kaposi Sarcoma: AIDS Malignancy Consortium Trial 059

Rachel Bender Ignacio[1,2], Jeannette Lee[3], Michelle Rudek[4], Dirk Dittmer[5,6], Richard Ambinder[4], Susan Krown[7,8] for the AIDS Malignancy Consortium (AMC)-059 Study Team

[1]Division of Allergy and Infectious Diseases, Department of Medicine, University of Washington, Seattle, Washington, USA; [2]Vaccine and Infectious Diseases Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA; [3]Department of Biostatistics, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA; [4]The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, Baltimore, Maryland, USA; [5]Department of Microbiology and Immunology, The University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA; [6]Lineberger Comprehensive Cancer Center, The University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA; [7]Memorial Sloan Kettering Cancer Center, New York, New York; [8]AIDS Malignancy Consortium, New York, New York, USA

Background: Vascular endothelial growth factor (VEGF) plays an important role in Kaposi's sarcoma (KS), a vascular tumor caused by Kaposi's sarcoma-associated herpes virus (KSHV) and the most common malignancy in HIV-infected persons worldwide. Tumor responses to antiretroviral therapy and cytotoxic chemotherapy alone are often incomplete. We conducted a Phase 1b study of PTC299, a novel post-transcriptional inhibitor of VEGF production in HIV-infected KS patients.

Methods: Persons with AIDS-related KS were administered PTC299 at doses of 40mg, 80mg, or 100mg by mouth twice daily in 4-week cycles, for a maximum of 12 cycles. Safety was monitored and pharmacokinetics and clinical and laboratory markers of anti-tumor activity were measured.

Results: Three participants each received twice-daily PTC299 doses of 40mg and 80mg, and 11 received 100mg. PTC299 was generally well tolerated; notably, we did not observe many of the typical adverse events associated with other types of VEGF inhibitors. Three participants (one at the 40mg and two at the 100mg dose level) had partial responses of KS tumors and 11 had stable disease. There were no significant differences in exposure to PTC299 between persons receiving inhibitors or inducers of CYP2C19, the major cytochrome P450 enzyme thought to metabolize PTC299; inhibitors were associated with a 58% decrease in the metabolite C_{max}. Serum (and to a lesser extent plasma) VEGF decreased from baseline, but there were no significant changes in KSHV DNA; plasma or serum IL-6; immunohistochemical staining of tumor biopsies for VEGF, VEGFR 2/3, phospho-Akt, p53, HIF-1α, Ki-67; or viral and cellular gene expression with treatment.

Conclusions: When administered as monotherapy, PTC299 anti-tumor effects on KS were limited but comparable to other anti-VEGF therapies. Given redundancies in the VEGF feedback loop, future trials should consider treatment combinations with anti-tumor, anti-viral, and other targeted agents to inhibit KS and KSHV proliferation. By targeting VEGF post-transcriptional control, this type of molecule may be useful for treatment of KS (and other cancers) without the typical side effects associated with VEGF blockade.

60. Anal HPV16/18-DNA and Serum Free (SF) Testosterone and SF Estradiol in Men Who Have Sex With Men (MSM)

Dorothy J. Wiley[1], Hilary Hsu[1], Todd T. Brown[2], Xiuhong Li[3], Stephen Young[4], Ross D. Cranston[5], Gypsyamber D'Souza[3], Lisa P. Jacobson[3], Otoniel Martínez-Maza[6-8], Eric C. Seaberg[3], Matthew G. Moran[1,9], Joseph B. Margolick[3], Frank J. Jenkins[10], David Elashoff[8], Sitaram Vangala[8], Roger Detels[7]

[1]UCLA School of Nursing, Los Angeles, California, USA; [2]Johns Hopkins University, School of Medicine, Baltimore, Maryland, USA; [3]Johns Hopkins University, Bloomberg School of Public Health, Baltimore, Maryland, USA; [4]Tricore Reference Laboratories, University of New Mexico, Albuquerque, New Mexico, USA; [5]University of Pittsburgh, Pittsburgh, Pennsylvania, USA; [6]UCLA AIDS Institute, Los Angeles, California, USA; [7]Jonathan & Karin Fielding School of Public Health, Los Angeles, California, USA; [8]David Geffen School of Medicine at UCLA, Los Angeles, California, USA; [9]Desert AIDS Project, Palm Springs, California, USA; [10]Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Background: Together, human papillomavirus (HPV) types 16/18 cause most cervical and anal squamous cell carcinomas. HPV16 & HPV18 are 2 of 12 viruses comprising Group 1 high-risk HPVs (hrHPV). Increased estrogen exposure positively influences hrHPV-related cervical malignancies and persistent infections in women. The role of testosterone in hrHPV infection and -related neoplasia is unclear.

Methods: 340 MSM enrolled in the Multicenter AIDS Cohort Study provided serum specimens and were tested for anal HPV16/18 DNA using PCR approximately 36 months later; of these, 37 underwent high-resolution anoscopy (HRA) and biopsy by a single examiner. Total testosterone and estrogens (estrone & 17β-estradiol), and sex-hormone binding globulin were simultaneously measured in cryopreserved serum, and used to estimate free testosterone (FT) and estradiol (FE) levels. Anal histology was classified using the Lower Anogenital Squamous Terminology [1], comparing findings for men with high-grade squamous intraepithelial lesion (HSIL) to those with <HSIL. Poisson regression with robust error variance analyses estimated multivariable-dependent prevalence ratios for HPV16/18 infections, including half-$\log_{10}$-transformed FT and FE, exogenous testosterone use, race, age, other Group 1 high-risk (hr)HPVs, number of anoreceptive intercourse partnerships reported from cohort baseline to HPV-testing, time of blood draw, HIV-infection, and CD4+ T-cell counts among HIV-infected men.

Results: Average total & free 17β-estradiol & estrone levels measured 16.9 & 22.0 pg/mL, & 0.4 & 0.8 pg/mL; total and FT measured 5938.3 & 112.9 pg/mL, respectively. Fully adjusted analyses showed each half-log increase in FT is associated with a 2.83-fold (95% Confidence Interval (CI): 1.58, 5.08) higher prevalence of anal HPV16/18 infection. Additionally, each half-log increase in FE is associated with lower HPV16/18 prevalence (0.62 (0.42, 0.92)). Serum FE & FT were similar for men showing <hHSIL or HSIL: 0.47 (SD:0.34) & 147.1 (SD:78.1) pg/mL, vs. 0.52 (SD:0.3) and 153.6 (SD:105.3) pg/mL, respectively. One or more *other* Group 1 hrHPVs is associated with a 1.75-fold (95% CI: 1.19, 2.57) higher prevalence of HPV16/18 detected in anal swabs.

Conclusions: FT may be more strongly associated with higher prevalence of anal HPV16/18 than previously reported in men, after controlling for the effects of FE and other covariates [2]. Associations between FT, FE, and hHSIL remain unclear and form an important question for future research.

References

1. Darragh, T. M., et al. (2012). J Low Genit Tract Dis 16(3): 205-242.
2. Hsu, H. K., et al., (2015). PLoS One, 10(3), e0119447. doi: 10.1371/journal.pone.0119447.

61. BCL2 and MYC Expression in HIV-Positive Diffuse Large B-Cell Lymphoma

Samantha Kendrick, Melba Jaramillo, Lisa M. Rimsza

Department of Pathology, University of Arizona, Tucson, Arizona, USA

Background: There is an urgent need to characterize HIV-positive (+) associated lymphoma, particularly diffuse large B-cell lymphoma (DLBCL), the most commonly diagnosed lymphoma. Despite ART therapy, HIV-infected DLBCL patients still have a worse prognosis compared to their HIV-negative (-) counterparts, thus raising an important question as to whether the disease in the context of HIV infection utilizes different oncogenic mechanisms. In HIV- DLBCL, over-expression of the *BCL2* and *MYC* oncogenes plays a key role in chemotherapy resistance, but studies in HIV+ DLBCL are limited. In a recent analysis, HIV+ DLBCL displayed higher BCL2/MYC dual and MYC only protein expression and similar levels of BCL2 compared to HIV- DLBCL (Chao et al., Clin Cancer Res, 2015). Here, we further investigate BCL2 and MYC at the protein and gene levels and according to cell-of-origin (COO).

Methods: We performed immunohistochemistry (IHC) for BCL2 (clones 124, Ventana Medical Systems, and SP66, Spring Biosciences) and MYC (clone EP121, Epitomics) on individual HIV+ and HIV- tissue microarrays (TMAs). Positivity for BCL2 and MYC were assessed at previously described cut-offs (Johnson et al. J Clin Oncol 2012). The HIV+ and HIV- DLBCL TMAs were constructed using 72 and 38 formalin-fixed paraffin-embedded tissues (FFPET), respectively, and those FFPET with additional material were also evaluated for *BCL2* and *MYC* gene expression using the PanCancer Pathways Panel and COO using the Lymph2Cx assay on the nCounter Nanostring platform. The HIV+ TMA and FFPET from available cases for RNA isolation were kindly provided by the AIDS Cancer Specimen Resource (San Francisco, CA). The University of Arizona Institutional Review Board in accordance with the Declaration of Helsinki approved the use of human tissues for this study.

Results: Overall, there were fewer BCL2+ cases in the post-ART HIV+ cohort compared to HIV- DLBCL when detected with clone 124 (42% vs. 74%; Fisher's exact test: $P<0.01$) and SP66, although not as significant (65% vs. 80%; $P=0.16$). The HIV+ DLBCL cases consisted of a similar percentage of MYC+ cases as HIV- DLBCL (52% vs. 64%; $P=0.83$). Gene expression of *BCL2* and *MYC* paralleled these observations in that the HIV+ cases displayed a 3-fold decrease in *BCL2* mRNA (FDR<0.01) and no difference in *MYC* mRNA (FDR=0.20) compared to HIV- DLBCL. Due to the prognostic value of double-expressers, we also determined the percent cases positive for both oncoproteins in HIV+ and HIV- DLBCL using SP66 to detect BCL2, which reportedly is more sensitive for IHC (Kendrick et al. Hum Pathol 2014); however, there was no difference (43% vs. 49%; $P=0.66$). When BCL2 and MYC expression were evaluated in the context of COO, there were significantly fewer BCL2+ cases in GCB-DLBCL HIV+ cases when detected with 124 (24% vs. 77%; $P<0.01$); however, this difference was negligible when using SP66 (67% vs. 85%; $P=41$). To further characterize HIV+ DLBCL, we examined BCL2 and MYC expression according to EBV status. The 34% EBV+ cases tended to be slightly more BCL2+ and less MYC+ than EBV-/HIV+ DLBCL ($P=0.12$ and $P=0.07$). Notably, the ABC COO were enriched with EBV+ cases with respect to the GCB COO (75% vs. 19%; $P=0.05$), which was statistically significant when considered along with the unclassifiable and non-GCB (Hans algorithm) cases (50% vs. 19%; $P=0.03$).

Conclusion: Our study demonstrates HIV+ DLBCL consists of fewer BCL2+ cases and a similar number of MYC+ cases compared to HIV- DLBCL. However, there are still a substantial number of BCL2+ and/or MYC+ cases suggesting that HIV+ DLBCL also utilizes these oncogenic pathways that can serve as therapeutic targets in the HIV setting where new treatments are needed to limit toxicity. The association of EBV with the nonGCB phenotype and BCL2 expression supports previous findings (Chao et al. Clin Cancer Res 2012 and Blood et al. Arch Virol 2004) and may contribute to the aggressiveness of HIV+ DLBCL. Ongoing research in our group is aimed at fully characterizing the molecular nature of HIV+ DLBCL including *MYC* amplification to facilitate the discovery of novel therapies to improve patient outcome.

62. Cancer Presentation and HIV Persistence in a Cohort With No Measurable Plasma or CSF Viral Load at Autopsy

Susanna Lamers[1], Rebecca Rose[1], David J. Nolan[1], Debra Garcia[2,3], Bruce Shiramizu[4], Ekaterina Maidji[3], Cheryl Stoddart[3], Elyse J. Singer[5], Michael S. McGrath[2,3]

[1]Bioinfoexperts, LLC, Thibodaux, Louisiana, USA; [2]The AIDS and Cancer Specimen Resource, San Francisco, California, USA; [3]The University of California, San Francisco, San Francisco, California, USA; [4]The University of Hawaii, Mānoa, Hawaii, USA; [5]The National Neurological AIDS Bank and the University of California at Los Angeles, Los Angeles, California, USA

Background: Combined antiretroviral therapy (cART) can reduce plasma HIV to undetectable levels. Although resting blood T cells may harbor HIV, there are limited studies demonstrating cART's ability to clear HIV from anatomical sites and almost no information is available concerning how residual HIV at anatomical sites could contribute to AIDS associated pathologies. Current cART regimens incorporate a variety of drugs that are focused on preventing viral replication and are generally effective in allowing for the recovery of the CD4 T-cell population. Given that chronic diseases including cancer are frequent in HIV+ patients on cART, the goal of this study was to test whether persistent tissue-based HIV might play a role in these diseases. We obtained frozen autopsy specimens from the National Neurological AIDS Bank (NNAB) through the AIDS and Cancer Specimen Resource (ACSR) from patients who died with no measurable plasma viral load at death. Detailed histologic evaluations were performed and cART and medical histories were available for review on the 19 cases studied, 10 of which were from patients who died with cancer.

Methods: A 19 patient, cART-treated, plasma and CSF HIV-negative cohort was obtained from the ACSR. Blood viral loads were assessed at autopsy by cardiac aspiration and CSF viral loads were measured using RT-PCR. Patient medical histories and pathology reports were compiled and over 200 tissues were histologically evaluated. All tissues were also assessed for the presence of HIV using digital drop PCR (ddPCR). Some tissues were evaluated using in situ HIV amplification and single-genome HIV sequencing.

Results: Subject demographics were diverse with 16 males and 3 females enrolled in the study. 12 subjects contracted HIV through male-to male contact, 4 through intravenous drug use, and 5 through heterosexual transmission. The median patient age and length of HIV infection were 46.5 years and 12 years, respectively. Ten subjects were on ARV until death, while 9 others stopped ARV within weeks prior to death. The average post mortem interval was 7.75 hours. Fifty-three percent of patients in the cohort had developed some form of cancer (lymphoma, Kaposi's sarcoma, anal carcinoma, lung cancer, and renal carcinoma) with most patients demonstrating atherosclerosis and multi-organ disease. Infections were noted in 6 patients. Abnormal histological findings were identified in liver, kidney, lung, heart, lymph nodes, and spinal cord. All patients had some brain abnormalities that ranged from mild to severe. No correlation between age, length of infection, disease, or abnormal pathology was observed. Overall, HIV infection was identified in 61% of brain tissues and 69% of peripheral tissues with >200 HIV copies/million cell equivalents. HIV RNA (RNAscope) was identified within lymphoma tissue macrophages and in the brain. When present, HIV-infected macrophages were surrounded by numerous CD68+ uninfected macrophages. Full-length HIV env-nef RNA and DNA could be sequenced from cases containing ddPCR detected HIV DNA.

Conclusions: This study highlights the importance of understanding HIV infection at anatomical sites and its contribution to co-morbidities during cART. HIV replicating in organs and vessels is likely to result in chronic disease, which could be due to low-levels of HIV-infected tissue macrophages that are capable of signaling other immune cells to sites of infection and damage. When properly activated, macrophages are a well-known supplier of tumor promoting factors. This ACSR/NNAB cohort, and others like it, will be useful in future studies of cancer and HIV reservoirs in HIV-infected patients.

63. Cellular Consequences of Epigenetic Reprogramming of Immortalized Epithelial Cells Following Epstein-Barr Virus Infection

Christine Birdwell, Joseph Guidry, Rona Scott

Department of Microbiology and Immunology, Center for Molecular and Tumor Virology and Feist-Weiller Cancer Center, Louisiana State University Health Sciences Center, Shreveport, Shreveport, Louisiana, USA

Background: The oral cavity is the persistent reservoir for Epstein-Barr virus (EBV) with lifelong infection of resident epithelial and B cells. Infection of these cell types results in distinct EBV gene expression patterns that are regulated by epigenetic modifications involving DNA methylation and chromatin structure. Viral exploitation of the host epigenetic machinery for its own lifecycle can inadvertently result in long-lasting, oncogenic epigenetic changes to the host as is seen in EBV-associated cancers. To test this hypothesis in the context of EBV infection of epithelial cells, we established a transient infection model to identify the epigenetic consequences after EBV infection of immortalized oral keratinocytes and subsequent viral loss.

Methods: Clonally derived, human telomerase-immortalized normal oral keratinocytes (NOK) were infected with a recombinant EBV Akata. Transiently-infected EBV negative NOK were single-cell cloned following removal of selection pressure for 10 passages. EBV-positive, transiently infected clones and controls were analyzed by reduced representation bisulfite sequencing and microarray gene expression profiling to identify epigenetic effects that occurred following EBV infection. Virally induced changes to epithelial differentiation were measured by transelectrical epithelial resistance and suspension in differentiation media, methylcellulose. Epithelial motility and invasion were examined using wound healing and chemotactic transwell assays.

Results: Reduced representation bisulfite sequencing identified DNA methylation changes in EBV-positive and EBV-negative transiently infected cells compared to uninfected controls. CpG island hypermethylation was evident in EBV-positive cells and retained in cells that lost EBV, reflecting the CpG island hypermethylator phenotype of EBV-associated carcinomas. Functionally, passage of EBV through keratinocytes resulted in delayed differentiation and increased invasiveness compared to uninfected controls. Differential gene expression of cells exposed to EBV compared to uninfected controls showed a significant correlation to genes associated with cancer and cell movement. Altered expression of the WNT signaling pathway was noted. Specifically, the transcription factor lymphoid enhancer factor (LEF) 1 and the Wnt5A secreted ligand were increased in cells exposed to EBV compared to uninfected controls. SiRNA knockdown of LEF1 in EBV-positive and EBV-negative transiently infected clones reduced the invasive phenotype to that of uninfected control. Ectopic expression of LEF1 in parental uninfected cells did not increase invasion to that observed following EBV infection.

Conclusions: Our data indicate that epigenetic reprogramming following EBV infection conferred oncogenic features observed in EBV-associated carcinomas. Increased LEF1 levels were necessary but not sufficient for the invasive phenotype following EBV infection of immortalized epithelial cells. The ability of EBV to induce cellular epigenetic changes defines a new mechanism for EBV's oncogenic effects with long term consequences towards tumor progression in the absence of the viral genome or viral gene expression.

This work was supported by grants from the National Institute of Dental and Craniofacial Research (R01DE025565), Louisiana Board of Regents (LEQSF2012-15-RD-A-15), and Feist-Weiller Cancer Center predoctoral fellowship to CEB.

64. Correlation of KSHV MicroRNA Sequence Polymorphisms With Levels of Mature MicroRNA in Kaposi Sarcoma Lesions

Vickie Marshall[1], Nazzarena Labo[1], Elena M. Cornejo Castro[1], Joanna Sztuba-Solinska[3], Kathleen M. Wyvill[2], Karen Aleman[2], Lynne McNamara[4], Stuart F.J. Le Grice[3], Thomas S. Uldrick[2], Robert Yarchoan[2], Patrick MacPhail[4], Mark N. Polizzotto[2], Denise Whitby[1]

[1]Viral Oncology Section, AIDS and Cancer Virus Program, Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, Inc., Frederick, Maryland, USA; [2]HIV and AIDS Malignancy Branch, National Institutes of Health, Bethesda, Maryland, USA; [3]HIV Drug Resistance Program, National Cancer Institute, Frederick, Maryland, USA; [4]Clinical HIV Research Unit, Department of Internal Medicine, University of the Witwatersrand, Johannesburg, South Africa

Background: We previously reported KSHV microRNA sequence variations in clinical samples and PEL cell lines and correlated specific combinations of polymorphisms with increased risk of MCD. We demonstrated using in vitro systems that microRNAs with variant sequence have different secondary structure, maturation, and mature microRNA expression. In this study, we illustrated the association between microRNA sequence variation and changes in microRNA expression within KS lesions.

Methods: T0.7, and microRNA cluster region sequences were determined from 24 KS and 5 control skin biopsies from KSHV positive individuals. KS cases and controls were recruited from clinic attendees in South Africa (5 cases and 1 control) and the United States (13 AIDS KS, 6 HIV negative KS, and 4 controls). Mature KSHV microRNA expression was evaluated using 21 custom small RNA qPCR assays. KSHV microRNA sequence observed in BCBL-1 cells was defined as wild type. The human small nuclear RNA RNU6B was used as endogenous control and for normalization. KSHV viral load was determined by qPCR.

Results: As previously reported, KSHV viral load was not associated with mature microRNA expression. Thirteen KSHV encoded microRNAs were overexpressed in KS lesions compared to control biopsies. MicroRNA K12-9-5p was strongly downregulated in South African vs. U.S. biopsies. Low expression of K12-9-5p was associated with a SNP in miR-K12-9-5p but also with SNPs in miR-K12-4-5p, 7-3p, and 3-5p. The SNP in miR-K12-3-5p also resulted in downregulation of miR-K12-12-5p, 6-3p, and 8-3p and the upregulation of 5-5p. Additional sequence variation was further associated with changes in mature microRNA expression.

Conclusions: The levels of mature KSHV encoded microRNAs in KS lesions correlate with sequence variation. This relationship is complex and likely reflects changes in secondary and tertiary RNA structure.

65. Diagnosis of HIV-Related Malignancies: Cutaneous and Non-Cutaneous (Brain, Liver, Nodes, and Nasopharynx) and Therapeutic Potential With a Novel CD206-Receptor Agent

B. Abbruzzese[1], F.O. Cope[1], J. Zhang[2], T. Maurer[2], S. Behr[2], J. Sanders[1], P. Braccib[3], M. McGrath[2,3]

[1]Navidea-Macrophage Therapeutics, Dublin, Ohio, USA; [2]University of California, San Francisco, San Francisco, California, USA; [3]AIDS & Cancer Specimen Resource, San Francisco General Hospital, San Francisco, California, USA

Background: Malignant lesions associated with HIV are most often expressed on the lower extremities, face, trunk, etc., e.g., Kaposi's sarcoma (KS). Extracutaneous sites of malignancy include the oral cavity, GI tract, respiratory tract, nasopharynx, and brain. Our recent data show that macrophages (MOs) play a significant role in the tumorigenesis specifically of numerous tumors and are an integral part of KS. MOs appear to confer the expression of the mannose receptor (CD206) to the KS parenchyma and other possible malignant foci. Manocept (MC), a molecular targeting agent, binds and enters CD206+ KS and MOs via pinocytosis of holo-CD206, providing a portal for the evaluation of MC as a MO and KS imaging and targeting agent. We sought to evaluate malignant lesions by exploiting the localization of MC that was isotopically labeled with Tc99m.

Methods: MC is a synthetic molecule with a dextran backbone, and 12-20 mannose CD206-targeting moieties. Ex vivo CD206 analysis was conducted with a Cy3 Manocept (C3M) on and in MOs and cKS. In cKS lesions, binding/localization of C3M was assessed through intracellular and flow cytometric quantitation of C3M binding and uptake using in vitro CD206+ MOs. The evaluation of cKS tumor tissue was by immunofluorescence staining and confocal imaging to confirm C3M uptake in cKS and TAMs. Tc99m tilmanocept (TMC) was used to detect tumors and involved eKS. Patients received one SC injection of 50µg TMC w/ 2.0 mCi Tc99m w/ SPECT/CT imaging at 4 hours post-injection to visualize localization of cKS and suspected eKS (Clinicaltrials.gov NCT02201420).

Results: Imaging results for 5 KS patients (5 HHV8+à 4HIV+; 1 HIV-) indicate that not only do multiple KS lesions localize TMC to KS CD206 and that KS lesions are anatomically linked in chains by and within the lymphatic ducts of the extremities, these lesions being further anatomically linked to regional lymph nodes that possibly harbor KS (based on localization signals in imaging). Additionally, MC identifies multiple lesions in the brain, head/neck, and liver that are CD206-expressing and differentiated from the normal parenchyma.

Conclusions: Both ex vivo and in vivo data suggest that KS in both HIV+ and HIV- patients have pan-tumor expression of CD206. TMC localizes not only to cKS but to suspected eKS lesions of the nasopharynx, lymph nodes, and the brain. TMC may be easily visualized, mapped, and potentially used routinely to assess suspected eKS via SPECT/CT imaging. The imaging in these patients suggests that KS tumor dissemination may be opportunistic relative to the anatomic linkage of KS lesions with the lymphatic ducts and the nexus to the blood vascular system. Our data also strongly suggest that (1) TMC crosses the blood/brain barrier, (2) low dose and low exposure may provide for use as routine cKS and eKS assessment, (3) MC be employed to deliver a therapeutic moiety with high target effect and low off-target concerns in cKS and eKS, and (4) MC be employed to deliver a therapeutic moiety with high target effect and low off-target concerns in other solid tumors with MO constituents.

66. EBV EBNA3A Mediates Survival at the Mitochondria Differentially at Early and Late Times Post EBV Infection of Primary B Cells

Alexander Price[1], Joanne Dai[1], Pavel Nikitin[1], Luv Patel[2], Martin Allday[3], Anthony Letai[2], Micah Luftig[1]

[1]Department of Molecular Genetics and Microbiology, Center for Virology, Duke University, Durham, North Carolina, USA; [2]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, USA; [3]Molecular Virology, Imperial College London, London, United Kingdom

Epstein-Barr virus (EBV) is a a-herpesvirus that infects 90% of the world's adult population. Despite its high prevalence, EBV-associated malignancies are largely kept in check by a strong cytotoxic T-cell immune response. However, EBV causes lymphoproliferative disease and lymphoma in immune-deficient individuals following transplant and in HIV-infected individuals. In vitro, EBV infection of primary human B cells results in proliferation and outgrowth of indefinitely proliferating lymphoblastoid cell lines (LCLs), which represent a viable model for the pathogenesis of EBV-associated malignancies in the presence of AIDS.

It has long been known that early after infection EBV expresses a set of latency-associated genes that mimic normal B-cell maturation and aid in immortalization of the infected B cell including the EBV Nuclear Antigens (EBNA) and Latent Membrane Proteins (LMP). In particular, Latent Membrane Protein 1 is a constitutively active version of the host CD40 receptor that signals chronically through the downstream NFkB pathway. This LMP1-induced NFkB signaling is critical for the generation and survival of LCLs. Indeed, inhibition of NFkB in LCLs induces apoptosis. As such, it was quite surprising when recent work from our laboratory identified a period early after infection where EBV-infected B cells proliferated in the absence of LMP1 and were immune to apoptosis induced by NFkB inhibition.

To ascertain how these cells survive in the absence of LMP1-induced NFkB activation, we performed BH3 profiling to query the state of mitochondrial priming of apoptosis at different times post infection. These data support a model where uninfected B cells are characterized by BCL-2, suggesting dependence on this anti-apoptotic molecule. As proliferation commences early after EBV infection, the cells are characterized by combined BCL-2 and MCL-1 dependence. Finally, the resultant LCLs are characterized by BFL-1/A1 dependence. This model has been further validated using small molecule inhibitors of BH3-only anti-apoptotic molecules including ABT-737, a potent inhibitor of BCL-2, BCL-xL, and BCL-w.

While B-cell mitogens such as CpG or CD40L/IL-4 can induce B-cell proliferation, only EBV infection induces potent resistance to ABT-737. Furthermore, when primary B cells were infected with an EBNA3A-, but not EBNA3C-, deleted virus the proliferating B cells were no longer resistant to ABT-737. This lack of ABT-737 resistance carried over to spontaneous EBNA3A KO LCLs that grew out from our primary infections. Further inquiry showed that EBNA3A deletion impairs MCL-1's ability to associate with the mitochondria early after infection and subsequently fails to induce BFL-1/A1 expression in the resultant LCL. Overall, these data aim to provide insight into how EBV prevents apoptosis and how this knowledge might be used to treat EBV-associated malignancies in the setting of HIV/AIDS.

67. Effect of Fatty Acid Supplementation on Immunological and Inflammatory Plasma Markers Among HIV and HHV8 Co-Infected Ugandan Adults

A.E. Coghill[1], J. Schenk[2], W. Phipps[2,3], J. Orem[3], C. Casper[2,3]

[1]Infections and Immunoepidemiology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA; [2]Fred Hutchinson Cancer Research Center, Seattle, Washington, USA; [3]Uganda Cancer Institute/Hutchinson Center Cancer Alliance, Kampala, Uganda

Background: Long-chain polyunsaturated fatty acids have a variety of immune-modulating and inflammatory effects. In a preliminary study of HIV-infected Ugandan adults, the ω-6: ω-3 fatty acid ratio was associated with increased shedding of human herpes virus-8 (HHV8), the etiologic agent of Kaposi sarcoma (KS). We conducted a randomized clinical trial to determine whether ω-3 fatty acid supplementation could alter immunological status, systemic inflammation, and HHV8 viral load in HIV and HHV8 co-infected Ugandan adults.

Methods: HIV and HHV8 co-infected adults were recruited from the Uganda Cancer Institute/Hutchinson Center Cancer Alliance in Kampala, Uganda and were randomized to receive either ω-3 or placebo. Participants in the ω-3 arm received a daily fatty acid dose of 3 grams, while placebo arm participants received a comparable daily dose of safflower oil. Plasma concentrations of fatty acids (eicosapentaenoic acid (EPA), decosahexaenoic acid (DHA), docosapentaenoic acid (DPA)), immune cells (CD4+ and CD8+ T-cells), and inflammatory cytokines (C-reactive protein (CRP), interleukin-6 (IL-6)) were measured in blood collected at baseline (week 1) and study end (week 13). Post-treatment HHV8 viral loads were measured at weeks 11-13.

Results: A total of 69 eligible HIV and HHV8 co-infected Ugandans (58 KS patients; 11 non-KS patients) were enrolled and randomized to receive either ω-3 fish oil or placebo for 12 weeks. The weight of EPA, DHA, and DPA as a percentage of total fatty acids increased significantly in the ω-3 arm (+4.0, +2.8, and +1.3, respectively) compared to the placebo arm (P <0.001). The increase in CD4+ T-cell count was also larger among ω-3 arm participants (+66 cells/mm^3) compared to placebo (+32 cells/mm^3), although the intervention effect was not statistically significant (P=0.31). In contrast, we observed a significant difference in IL-6 concentrations by study arm (P=0.04), with a modest decrease in in the ω-3 arm (-0.78 pg/mL) compared to a near doubling above baseline in the placebo arm (+3.2 pg/mL). There was no significant difference in post-treatment HHV8 viral load between ω-3 and placebo arms (1.2 log cml ± 1.6 vs. 1.4 log cml ± 1.4; P=0.30).

Conclusions: We observed excellent compliance in our double-blind, placebo-controlled trial of ω-3 supplementation for HIV and HHV8 co-infected Ugandan adults. Supplementation with ω-3 fatty acids significantly increased plasma concentrations of ω-3 long-chain polyunsaturated fatty acids but decreased concentrations of the inflammatory cytokine IL-6. However, supplementation did not significantly alter immune cell counts or post-treatment HHV8 viral load, providing limited data to support supplementation as a viable treatment option for HIV-infected KS patients in Uganda.

68. Gammaherpesvirus ORF10 Selectively Inhibits Cellular mRNA Nuclear Export

Danyang Gong[1], Yong Hoon Kim[1], Yuchen Xiao[2], Kevin Lee[3], Yushen Du[1], Yafang Xie[1], Jun Feng[1], Nisar Farhat[1], Novan J. Krogan[4], Ren Sun[1], Ting-Ting Wu[1]

[1]Department of Molecular and Medical Pharmacology; [2]Department of Microbiology, Immunology, and Molecular Genetics, [3]School of Dentistry, University of California, Los Angeles, Los Angeles, California, USA; [4]Department of Cellular & Molecular Pharmacology, University of California, San Francisco, San Francisco, California, USA

Background: Infections of two human gammaherpesviruses, Epstein-Barr virus (EBV or HHV-4) and Kaposi sarcoma-associated herpesvirus (KSHV or HHV-8), are associated with several malignancies. Central to pathogenesis and spread of gammaherpesvirus is its lytic replication, the process that begins with cascade expression of viral genes and ends with assembly and release of infectious virions into extracellular space for viral propagation. Indubitably, this cycle requires precise control of cellular pathway in favor of successful production of progeny virus. Through co-evolution with hosts, viruses have acquired many strategies to modulate cellular environment to facilitate their own replication. Nuclear export of mRNAs has been found to be an important cellular pathway targeted by numerous viruses. Here, we present that ORF10, a lytic protein conserved among gamma-herpesviruses, can block cellular mRNA exports.

Methods and Results: We observed nuclear accumulation of poly(A) RNA in cells infected with a gamma-herpesvirus expressing ORF10 and in cells expressing ORF10 alone. To elucidate the molecular mechanism of this observation, we performed a mass-spec (MS) analysis, from which we identified the interaction between ORF10 and a cellular mRNA export protein, Rae1. Furthermore, we performed site-directed mutations on ORF10 and found several mutations that greatly diminished its interaction with Rae1. Interestingly, these mutations also abolished its ability to inhibit mRNA export, thus confirming the pivotal role of its interaction with Rae1 in this inhibitory process. Next, we performed RNA deep sequencing analysis, which showed that ORF10 selectively blocks the nuclear export of only a subset of cellular mRNAs with short RNA and protein half-lives. To further determine the biological significance of such inhibition in the context of infection, we constructed KSHV ORF10 mutants that contain either a premature stop codon (10S) or site-specific mutations that diminish its interaction with Rae1, and examined the replication kinetics of these mutant viruses. Surprisingly, all mutant viruses exhibited marked reductions in late gene expression and more than 10-fold decrease in virion productions without affecting their DNA replication or immediate-early and early gene expressions. Moreover, both KSHV wild type virus in the absence of Rae1 and ORF10 loss-of-interaction mutants showed similar impacts on late gene expression as KSHV 10S virus, thus unveiling for the first time an unrecognized connection between cellular mRNA export and viral late gene expression mediated by ORF10.

Conclusion: ORF10, conserved among gammaherpesvirus family, is identified as a new viral inhibitor of cellular mRNA nuclear export, and it enhances viral lytic replication using its host mRNA export inhibition function.

69. Gammaherpesvirus Rhesus Monkey Rhadinovirus Inner Tegument Protein ORF52 Induces Microtubule Bundling Alone, and in the Context of Lytic Infection

Matthew S. Loftus[1,2], Dean H. Kedes[1,2,3]

[1]Myles H. Thaler Center for AIDS and Human Retrovirus Research, University of Virginia Health Systems, Charlottesville, Virginia, USA; [2]Department of Microbiology, Immunology, and Cancer Biology, University of Virginia Health Systems, Charlottesville, Virginia, USA; [3]Department of Internal Medicine, University of Virginia Health Systems, Charlottesville, Virginia, USA

Background: Rhesus monkey rhadinovirus (RRV), a gamma-2 herpesvirus, is a homolog of the human oncogenic pathogen, Kaposi sarcoma associated herpesvirus (KSHV/HHV8). RRV can infect primary or immortalized Rhesus monkey fibroblasts (RhF) and replicates to high viral titer relative to KSHV. This quality, along with the high levels of conservation in their genomic sequence and organization make RRV a useful model to study the structure and lytic (productive) replication of KSHV and other gammaherpesviruses. The tegument layer of herpesviruses comprises a collection of proteins that is unique to each viral species. Our previous work demonstrated that RRV ORF52 is a highly abundant tegument protein that is tightly associated with the capsid of the virus and essential for the final envelopment stage of virion maturation. The crystal structure of its murine homolog, MHV68 ORF52, readily forms dimers and possibly tetramers in solution. In light of its abundance within the virion and high level of expression during lytic replication, we asked whether RRV ORF52 might affect the cytoskeleton, focusing particularly on microtubules (mt) since previous work has demonstrated their role in herpesvirus intracellular transport.

Methods: RhF cells were infected with RRV at different MOIs (5 to 50), depending on the experiment. Cells were then incubated for 6 to 48 hours and subsequently analyzed by western blot or immuno-confocal microscopy with antibodies to α-tubulin, acetylated α-tubulin, as well as to structural proteins anti-RRV SCIP (ORF65) and anti-RRV ORF52. RhF cells were also transfected, following siRNA control or siRNA ORF52 knockdown, with plasmids containing siRNA-resistant RRV wt ORF52, mutant RRV ORF52, or KSHV ORF52. Morphologic changes consistent with mt bundling, including levels of acetylated α-tubulin were then assessed by confocal microscopy and western analyses.

Results: Confocal microscopy revealed that high MOI RRV infection led to mt thickening, particularly at the microtubule organizing center (MTOC) as early a 6 h p.i., well before a significant contribution from newly synthesized ORF52. The overall bundling then progressed throughout the cytoplasm by 24 hours and increased further by 48 hours. We also noted that the thickened mt structures co-localized with acetylated tubulin staining, consistent with mt bundling and stabilization. In contrast, siRNA knock down of ORF52 prior to infection blocked these effects. In addition, we found that over-expression of RRV ORF52 alone in RhF cells was sufficient to induce high levels of mt bundling and acetylated tubulin as well as disruption of cytokinesis, leading to multinucleated cells. In contrast, transfection with ORF52 mutated at a highly conserved residue, Arg^{103} (R→A), abrogated both mt bundling and any increase in acetylated tubulin levels. Transfection with KSHV ORF52 showed no change in mt phenotype or acetylated tubulin expression.

Conclusions: Our results indicate that RRV ORF52 interacts with mt either directly or, possibly, through mt associated proteins (MAPs), causing mt bundling and acetylation and that intravirion ORF52 is sufficient to initiate this process. Our results further indicate that ORF52 can induce these cytoskeletal changes even in the absence of other viral proteins. Finally, the mutation of the conserved Arg^{103} residue to an alanine abolishes the ability of ORF52 to affect mt structure. This is of interest because the analogous site in gamma68 ORF52 appears to be critical for function and tetramerization of the protein. We are actively investigating the mechanisms underlying these effects that we hope will generate insights into their potential function during RRV infection.

70. HCV and HIV Dynamics and Gene Expression in Liver Tissue Studied at the Single Cell Level

David R. McGivern, Michael Chua, Oksana Zakharova, Julie Nelson, Monica Schmidt, Kevin Greene, Jama Darling, Joseph J. Eron, Stanley M. Lemon

Department of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Background: Despite wide use of highly active antiretroviral therapy in persons infected with human immunodeficiency virus (HIV), the consequences of chronic co-infection with hepatitis C virus (HCV), including cirrhosis and hepatocellular carcinoma (HCC), have become leading causes of morbidity and mortality in persons with HIV/AIDS, in both Europe and the United States. How HCV causes hepatocellular carcinogenesis is poorly understood, in part due to the paucity of model systems for the study of co-infection. Human tissue banks represent a rich source of biological material for the study of disease progression but detection of HCV antigen is challenging. Greater knowledge of the types and numbers of cells infected by each virus in the liver in HCV mono-infection vs. HCV/HIV co-infection would enhance understanding of how co-infection enhances liver disease and the risk of liver cancer and may also give insight into HIV persistence.

Methods: An in situ hybridization (ISH) approach was used to detect viral RNA in sections of formalin-fixed, paraffin-embedded liver biopsy specimens from HCV mono- and HCV/HIV co-infected patients. Patient isolate-specific ISH probe sets were designed based on bulk consensus sequence of HCV RNA from plasma drawn within 3 months of biopsy. Biopsy sections were incubated with target-specific probe sets comprised of 20 oligonucleotide pairs. Amplification and detection were achieved by secondary hybridization of fluorescently labeled, branched DNA probes to adjacent pairs of oligonucleotide probes. Fluorescent signal was visualized by confocal microscopy.

Results: RNA ISH using isolate-specific probes was successfully applied to detect HCV in mono- and co-infected liver. Specificity was confirmed by using HCV-specific probes to interrogate biopsy specimens from HCV-negative liver and HCV non-specific probes (e.g., against hepatitis A virus) to probe HCV positive liver. HIV RNA was also detected in liver biopsy specimens from co-infected patients who were not on ART at the time of sampling. In liver tissue, HIV-infected cells were non-parenchymal and very rare.

Conclusions: Application of this technique will facilitate comparison of intrahepatic infection dynamics of HCV in mono-infected and HIV co-infected liver. Separation of HCV-infected and uninfected cells in liver will allow comparison of gene expression in virus-infected versus uninfected bystander cells. Co-staining with cell-type specific markers will identify the subset of cells in liver that can support HIV replication.

71. HHV-8 Infection Is Associated With Increased Circulating Cytokine Levels and Elevated Serum PSA in Men From Tobago

Jaideep Karamchandani[1], Jill Henning[2], Luis Bonachea[2], Clareann Bunker[3], Aaron Patrick[4], Frank Jenkins[1,5]

[1]Infectious Diseases and Microbiology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; [2]Department of Biology, University of Pittsburgh, Johnstown, Johnstown, Pennsylvania, USA; [3]Department of Epidemiology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; [4]Tobago Regional Health Authority, Scarborough, Tobago, Trinidad and Tobago, West Indies; [5]Department of Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Background: Prostate specific antigen (PSA) is a serine protease glycoprotein produced by prostate epithelial cells. Increased serum levels of PSA have been used extensively as a marker for prostate cancer but this use has become controversial due to a significant number of false negatives. Elevated serum PSA is seen in a majority of prostate cancer cases, but these levels can also be increased due a number of conditions outside of cancer including prostatic infections, benign prostatic hyperplasia, and injury to the prostate gland. We have previously demonstrated that human herpesvirus 8 (HHV-8) is capable of establishing chronic, latent infections in the prostates of seropositive men in both the United States as well as the Caribbean island of Tobago. In this study we tested the hypothesis that HHV-8 infection is associated with elevated plasma PSA levels in Tobago men. We also compared circulating cytokine profiles between HHV-8 seropositive and seronegative men with elevated and normal PSA levels.

Methods: HHV-8 serostatus and circulating cytokine levels were measured in Tobago men with an elevated PSA (>4ng/ml) and non-cancerous biopsy and healthy controls (normal digital rectal exam (DRE) and PSA <4 ng/ml).

Results: Men with elevated PSA (n=168) were significantly more likely to be HHV-8 seropositive than controls (n=140; OR 2.51; 95% CI 1.48 – 4.29; p=0.000). Cytokine analyses demonstrated a strong pro-inflammatory response (significantly elevated levels of IL-1β, IL-6, and IL-8) and a mixed Th_1 and Th_2 response (significantly elevated levels of IFNγ, IL-2, IL-4, IL-10, and IL-13). This cytokine profile was due in part to HHV-8 infection as comparisons of cytokine levels among HHV-8 seropositive and seronegative men in the control group also demonstrated significantly increased levels of IL-12p70, IL-10, and IL-13. Logistic regression analyses demonstrated that a model with IL-12p70, TNFα, IL-4, IL-6, IL-8, and IL-13 correctly predicted elevated PSA cases from controls in 88.9% of the cases. Among HHV-8 seropositive men, a model with TNFα and IL-4 correctly predicted elevated PSA cases in 94.5% of the cases.

Conclusions: These results demonstrate that HHV-8 infection is significantly associated with elevated PSA levels and elicits a strong pro-inflammatory, mixed Th_1 and Th_2 immune response. Our data suggest that men with elevated PSA levels should be screened for HHV-8 infection.

72. HIV-Associated Kaposi Sarcoma Is Characterized by Mast Cell Activation That Reflects as Elevated Plasma Levels of Tryptase and Histamine

Arturo Barbachano-Guerrero[1], Leona W. Ayers[2], Jeffrey Martin[3], Rosemary Rochford[1], Christine A. King[1]

[1]Department of Microbiology and Immunology, SUNY Upstate Medical University, Syracuse, New York, USA; [2]Department of Pathology, The Ohio State University, Columbus, Ohio, USA; [3]Department of Epidemiology and Biostatistics, University of California, San Francisco, California, USA

Background: Kaposi sarcoma herpes virus (KSHV) is an oncogenic herpesvirus and etiologic agent of the "hemorrhagic" endothelial cell neoplasm Kaposi sarcoma (KS). Chronic inflammation and latent KSHV infection of endothelial cells are commonly implicated but we have demonstrated that central players of chronic inflammation, mast cells (MCs), are specifically localized to KS lesions, activated, and degranulated. We hypothesized that because these KS associated MCs are activated to degranulate in close association with KSHV infected endothelial cells and spindle cells, MC granule-derived tryptase and histamine should be reflected systemically in plasma. Both tryptase and histamine have a short half-life with rapid removal from the bloodstream, so that measured tryptase and/or histamine in plasma would represent the dynamics between tissue MC degranulation and removal of the absorbed MC products from the blood stream.

Methods: Plasma samples from African, antiretroviral (ARV) therapy naïve, HIV+ patients with an average of 15 cutaneous KS lesions, viral load averaging 339,194, and CD4 counts ranging from 4 to 609 were obtained from the AIDS and Cancer Specimen Resource (ACSR). Quantification of MC-specific granule-associated tryptase and histamine was by commercial ELISA.

Results: Plasma levels of MC-granule products from 25 KS patients with multiple KS lesions indicated that KS patients have significantly higher levels of tryptase 9.55±1.99 vs. 1.64±0.69 ng/ml, and histamine 723.10±144.62 vs. 101.10±31.96 pg/ml, mean ± SEM, as compared to healthy controls. Tryptase average levels of 20 ng/ml were associated with average CD4 111 count, 4 ng/ml with 455 CD4 and 1.50 ng/ml with 70 CD4 counts. Histamine averaged 673 pg/ml with average 111 CD4 count, 836 pg/ml with 455 CD4 and 1183 with average 70 CD4 count.

Conclusions: HIV-associated KS patients with multiple cutaneous lesions have extensive MC activation with degranulation and concomitant release of tryptase and histamine that reflects systemically in plasma. Highest levels of tryptase in plasma are associated with CD4 counts associated with greatest vulnerability for extensive new KS lesion development. Histamine levels appear to have an inverse relationship with CD4 counts with highest histamine levels associated with lowest average CD4 counts. Plasma tryptase may provide a clinically useful measure of KS disease extent and level of new lesion production.

73. HPV16 E6 Seropositivity Elevated in Subjects With Oral HPV16 Infection

Yuehan Zhang[1], Tim Waterboer[2], Michael Pawlita[2], Dorothy Wiley[3], Howard Minkoff[4], Susheel Reddy[5], Lisa Jacobson[1], Joseph Margolick[1], Ross Cranston[6], Howard Strickler[7], Elizabeth Sugar[1], Kathleen Weber[8], Maura Gillison[9], Gypsyamber D'Souza[1]

[1]Johns Hopkins School of Public Health, Baltimore, Maryland, USA; [2]German Cancer Research Center (DKFZ), Heidelberg, Germany; [3]University of California, Los Angeles, Los Angeles, California, USA; [4]Maimonides Medical Center, Brooklyn New York, USA; [5]Northwestern University, Chicago, Illinois, USA; [6]University of Pittsburgh, Pittsburgh, Pennsylvania, USA; [7]Albert Einstein College of Medicine, Bronx, New York, USA; [8]CORE Center at John H. Stroger, Jr. Hospital of Cook County, Chicago, Illinois, USA; [9]Ohio State University Comprehensive Cancer Center, Columbus, Ohio, USA

Background: HPV16 E6 serum antibodies are common in people diagnosed with HPV-related oropharyngeal cancers (HPV-OPC), but not in the general population (reference). Given oropharyngeal pre-cancerous lesions are difficult to detect, HPV16 E6 antibodies have been suggested by some as a potentially specific marker for HPV-OPC screening. To evaluate the potential utility of HPV16 E6 antibodies we explored their prevalence among a high risk cohort of participants with and without oral HPV16 DNA detected, the presumed cause of HPV-OPC.

Methods: Oral rinse samples were collected every 6 months in the Multicenter AIDS Cohort Study (MACS) and Women's Interagency HIV Study (WIHS) and tested for 36 types of HPV DNA by polymerase chain reaction and type-specific hybridization for HPV. HPV16 E6 serum antibodies were tested at the visit of first oral HPV detection in 54 participants with prevalent, 39 participants with incident, and 154 participants with no oral HPV16 infection using the glutathione S-transferase multiplex assay. Available cancer registry, anal and cervical cytology, and histology data in these cohorts were also evaluated. HPV16 E6 seroprevalence was calculated overall and in subgroups, and predictors of seropositivity were examined using multivariate logistic regression.

Results: There were 10 HPV16 E6 seropositive participants identified among the 247 participants tested. These participants were primarily male (90%), HIV positive (80%), had >500 lifetime oral sexual partners (80%), and had an oral HPV16 infection (90%). Median age and CD4 T cell count of seropositive participants was 53 years and 784 cells/µl, respectively. None of the seropositive participants had been diagnosed with HPV-OPC, anal cancer, or invasive cervical cancer, and only 1 had a known history of HPV-related dysplasia (a man with a biopsy confirmed anal high-grade squamous intraepithelial lesion). Among the remaining 9 seropositive participants, 2 were unscreened and 7 had recent screening suggesting no HPV-related anogenital dysplasia, including multiple recent normal anal cytology among the 6 men and multiple negative cervical cytology among the 1 seropositive woman. HPV16 E6 seroprevalence was 4.5% among 177 HIV-infected participants and 2.9% among 70 uninfected participants; substantially higher than previous studies reporting 0.6% prevalence in the general population without cancer (reference). HPV16 E6 seroprevalence was significantly more common in those with than without oral HPV 16 infection (9.7% vs 0.7%, $p<0.001$; OR=16.4, 95%CI=2.0-131.6). However, seroprevalence was similar in those with prevalent (9.3%, 5/54) and incident (10.3%, 4/39) oral HPV16 infection ($p=0.87$). Oral HPV16 infection remained a strong predictor of HVP16 E6 seropositivity after adjustment for other risk factors (aOR=32.5, 95%CI=3.5-298.9).

Conclusions: HPV16 E6 seropositivity was higher among people with than without oral HPV16 infection, despite no known anogenital disease in these participants, suggesting seropositivity might represent undetected oropharyngeal pre-cancer in some of these participants.

Reference

Kreimer AR, Johansson M, Waterboer T, et al. Evaluation of human papillomavirus antibodies and risk of subsequent head and neck cancer. *Journal of Clinical Oncology,* 2013;31:2708-2715.

74. Hypoxia-Inducible Factor-1 Is Important for the Replication of Kaposi Sarcoma-Associated Herpesvirus and the Proliferation of Primary Effusion Lymphoma Cells

Prabha Shrestha, David A. Davis, Ravindra P. Veeranna, Robert F. Carey, Robert Yarchoan

HIV and AIDS Malignancy Branch, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland, USA

Background: Primary effusion lymphoma (PEL) is an aggressive non-Hodgkin lymphoma caused by Kaposi sarcoma-associated herpesvirus (KSHV). KSHV is maintained in most infected cells predominantly in a latent state with a small fraction of the cells undergoing lytic replication. Cells activated to lytic replication express a number of genes that help maintain the malignant phenotype. Hypoxia-inducible factor 1 (HIF-1), the primary mediator of the cellular response to hypoxia, can induce KSHV to undergo lytic reactivation and also directly activate certain KSHV genes. In addition, it has been shown that KSHV increases the level of HIF-1 upon infection and that PEL and other KSHV-infected cells have enhanced aerobic glycolysis, which is mediated by genes regulated by HIF-1. Since KSHV can increase the levels of HIF-1, which in turn modulates KSHV gene expression and tumor cell metabolism, we hypothesized that interfering with HIF-1 would have substantial effects on KSHV-infected cells and that this pathway may be a target for the treatment of PEL or other KSHV-induced tumors.

Methods: In this study we suppressed expression of HIF-1 in two PEL cell lines, BCBL-1 and BC-3, using lentivirus encoding shRNA to HIF-1 and directly investigated the roles of HIF-1 in the replication of KSHV and the proliferation of PELs.

Results and Conclusions: We found that suppression (knocking down) of HIF-1 leads to reduced lytic replication of KSHV, as shown by down-regulation of the lytic switch gene RTA as well as reduced amount of virus particles released from the cells. This decrease was observed under conditions of both normoxia and hypoxia, suggesting that the involvement of HIF-1 in the regulation of KSHV lytic replication extends beyond conditions of low oxygen. We also observed that HIF-1 knockdown leads to a dramatic reduction in both intracellular and secreted levels of viral interleukin-6 (vIL-6), a KSHV homolog of cellular IL-6, that plays important roles in the survival and proliferation of KSHV-infected cells and the pathogenesis of KSHV-induced tumors. Additionally, knocking down HIF-1 led to reduced glycolysis as shown by a decrease in lactate production. This decrease appears to be caused, at least in part, by reduced expression of glucose transporters GLUT-1 and GLUT-3. Finally, knocking down HIF-1 led to reduced viability and proliferation of PELs. These findings demonstrate that HIF-1 is a key factor in the maintenance of KSHV infection and the survival of infected PEL cells. Also, they provide a rationale to explore the use of inhibitors of HIF-1 in the treatment of PEL and other KSHV-related tumors.

This work was supported by the intramural research program of the National Institutes of Health, National Cancer Institute.

75. Immunological Determinants of AIDS-Associated Kaposi Sarcoma in Africa

Helen Byakwaga[1,2], Peter W. Hunt[2], Miriam O. Laker-Oketta[2,3], Yong Huang[2], David V. Glidden[2], Conrad Muzoora[1], A. Rain Mocello[2], Tricia H. Burdo[4], Russell P. Tracy[5], Albert R. Davalos[6], David R. Bangsberg[7], Edward K. Mbidde[8], Sheila C. Dollard[9], Michael M. Lederman[10], Jeffrey N. Martin[2]

[1]Mbarara University of Science and Technology, Uganda; [2]University of California, San Francisco, San Francisco, California, USA; [3]Infectious Diseases Institute, Kampala, Uganda; [4]Boston College, Chestnut Hill, Massachusetts, USA; [5]University of Vermont, Burlington, Vermont, USA; [6]Buck Institute for Research on Aging, Novato, California, USA; [7]Massachusetts General Hospital, Boston, Massachusetts, USA; [8]Uganda Virus Research Institute, Entebbe, Uganda; [9]Centers for Disease Control and Prevention, Atlanta, Georgia, USA; [10]Case Western Reserve University, Cleveland, Ohio, USA

Background: KSHV infection is highly prevalent in sub-Saharan Africa, as is HIV infection. Among HIV-infected persons, those with low CD4+ T cell counts are at highest risk for Kaposi sarcoma (KS); yet, only a minority of individuals in Africa with AIDS develop KS. Thus, KSHV and CD4 lymphopenia are insufficient to cause KS, and additional mechanisms must exist that either promote or prevent KS in KSHV/HIV co-infected individuals. These additional mechanisms are poorly understood, and few have been directly studied in humans. We investigated the role of several biomarkers — already recognized for their role in HIV pathogenesis — for their specific role in the occurrence of KS in HIV-infected adults.

Methods: In a case-control study among untreated HIV-infected Ugandan adults, cases had biopsy-confirmed KS and were derived from the AntiRetrovirals for KS (ARKS) study; controls were without KS and were sampled from the Uganda AIDS Rural Treatment Outcomes (UARTO) cohort. All participants were evaluated for sociodemographic and clinical characteristics using the same instruments and had routine laboratory characterization at the same clinical laboratory. Nine soluble biomarkers were measured in cryopreserved plasma, collected prior to any therapy, using separate dedicated assays.

Results: We studied 224 KS cases and 450 non-KS controls (41% KSHV-antibody-positive). In multi-variable regression, lower levels of HMGB1, IP-10, and I-FABP and higher levels of IL-6 and D-dimer were independently associated with higher risk of KS (Table). Of note, higher plasma HIV RNA and low CD4 count remained independently associated with KS (both p<0.001), even after controlling for all the biomarkers. Inferences remained unchanged when restricting analyses to KSHV-antibody-positive controls. Among the cases with KS, the number of KS-containing mucocutaneous anatomic sites was associated with higher D-dimer (as a dose-response relationship) and higher KT ratio (as a threshold effect) levels after adjustment for age, sex, CD4 count, and plasma HIV RNA. In the non-KS controls, there was no association between any of the nine biomarkers and other non-KS opportunistic infections.

Plasma Biomarker	Unadjusted		Adjusted†		Adjusted‡	
	Odds Ratio* (95% CI)	P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value
Kynurenine:Tryptophan (KT ratio)	0.63 (0.39-1.0)	0.057	0.60 (0.34-1.1)	0.073	0.52 (0.19-1.4)	0.21
High Mobility Group Box 1 (HMGB1)	0.10 (0.06-0.18)	<0.001	0.09 (0.05-0.17)	<0.001	0.06 (0.03-0.15)	<0.001
Soluble CD14	1.7 (1.1-2.6)	0.027	1.6 (0.94-2.9)	0.08	1.4 (0.54-3.7)	0.48
Soluble CD163	0.52 (0.33-0.82)	0.005	0.64 (0.38-1.1)	0.086	1.5 (0.69-3.4)	0.30
D-dimer	8.2 (4.8-13.8)	<0.001	11.6 (6.3-21.4)	<0.001	11.4 (4.9-26.8)	<0.001
Interleukin (IL)-6	4.7 (2.8-7.8)	<0.001	4.1 (2.3-7.4)	<0.001	3.7 (1.5-9.2)	0.005
Interferon Inducible Protein (IP)-10	0.11 (0.06-0.19)	<0.001	0.05 (0.03-0.11)	<0.001	0.02 (0.01-0.05)	<0.001
Soluble CD27	0.57 (0.36-0.90)	0.017	0.65 (0.38-1.1)	0.12	2.4 (0.94-6.0)	0.066
Intestinal Fatty Acid Binding Protein (I-FABP)	0.24 (0.15-0.38)	<0.001	0.17 (0.10-0.29)	<0.001	0.13 (0.06-0.28)	<0.001

* For each biomarker, odds ratio compares 4th quartile (highest levels) to the 1st quartile (lowest levels; reference category);
†Adjusted for age, sex, plasma HIV RNA and CD4 count; ‡Adjusted for age, sex, HIV RNA, CD4 count, and all variables in the table

Conclusions: Several biomarkers that are generally known to play a role in HIV pathogenesis are also specifically associated with occurrence of KS. With the exception of D-dimer, which may be a marker of KS burden, the lack of correlation between extent of KS and biomarker level supports a causal role of the biomarker in the development of KS. Interestingly, some biomarkers are related to KS in an unexpected indirect direction. While inflammation might promote KS pathogenesis, some consequences of HIV-associated immune activation might actually decrease the risk of KS in this setting.

76. Increases in Epigenetic Marks Contribute to Reactivation of Epstein-Barr Virus by Metabolic End Products of Anaerobic Oral and Gut Bacterial Pathogens

W.T. Seaman, R. Rothwell, T. Morris, R. Arnold, J. Webster-Cyriaque

The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Background: Epstein-Barr virus (EBV) is a large double-stranded gamma herpesvirus associated with several human oral and gastric cancers. EBV plays a major role in many AIDS-associated malignancies. Lytic reactivation from latency contributes to the spread of EBV. Determining in vivo mechanisms of viral reactivation is essential to combat EBV associated disease. Increased EBV viral load in the oral cavity is correlated with the severity of periodontitis suggesting bacterial pathogens contribute to viral reactivation. This study sought to determine if anaerobic oral and gut pathogens produce metabolic end products that reactivate the EBV lytic cycle.

Methods: Spent media from anaerobic bacterial cultures were used to treat the latently infected EBV+ human gastric carcinoma cell line, AGS-EBV. ChIP and reporter assays were performed to assess transcriptional activation of viral lytic promoters. Dose response and time course experiments were performed to characterize viral reactivation following treatment of cells with bacterial spent media. Immunoblots were used to detect lytic viral proteins after treatment. Inhibitors of p38 MAPK, AKT, and NF-kappaB were used to determine if these pathways were involved in lytic reactivation. Epigenetic marks associated with regulation of gene expression were analyzed by western blot. Intracellular viral DNA levels following activation of lytic viral gene expression were assessed by qPCR.

Results: Spent media from late log phase bacterial cultures resulted in efficient reactivation of temporal lytic gene expression, while mid-phase did not. Treatment of latently infected EBV+ cells with bacterial spent media resulted in acetylated histone 3 association with EBV lytic gene promoters. Histone H3 acetylation was associated with expression of lytic viral genes. Specifically, acetylation occurred at lysine 9 (H3K9) and lysine 27 (H3K27) of H3, known to by epigenetic marks associated with activated gene expression. Additionally, H3 methylation at known transcription activator epigenetic marks at lysine 4 (H3K4) and lysine 36 (H3K36) were also detected. Activation of lytic gene expression was followed by increasing intracellular viral DNA.

Conclusion: The ability of bacterial spent media to induce EBV gene expression suggested that bacterial metabolic end products reactivated the viral lytic cycle. H3 acetylation and methylation suggests that epigenetic factors induced by the interaction of bacterial end products with latently infected cells are involved in regulating the reactivation of lytic gene expression through HDAC inhibition and lysine methyltransferase (KMT) activation, respectively. Decreased viral gene expression with p38 MAPK and NF-kappaB inhibitors suggested that activation of these pathways was critical for increased epigenetic marks required for EBV lytic reactivation. We conclude that oral and gut anaerobic pathogens induce EBV lytic reactivation, contributing to the spread of viral infection, and increase the risk of EBV-associated neoplasms.

77. Kaposi Sarcoma-Associated Herpesvirus Latently Infected Cells With Inducible Caspase 9

Kazushi Nakano, Michiko Shimoda, Chie Izumiya, Feng Zhou, Yuanzhi Lyu, Mel Campbell, Yoshihiro Izumiya

Department of Dermatology, University of California, Davis Medical Center, Sacramento, California, USA

Background: Kaposi's sarcoma-associated herpesvirus known as HHV-8 is the causative agent of primary effusion lymphoma, multicentric Castleman disease, and Kaposi sarcoma. Because of low infectivity and replication of KSHV following de novo infection, primary infection studies often require collecting KSHV particles from a large quantity of stimulated latently infected cells. It is known that KSHV can be effectively transmitted to neighboring cells, when KSHV lytic replication-induced cells are co-cultured with target cells. However, it is not very clear how KSHV infects by a cell-associated manner. To study such mechanisms and evaluate the efficacy of cell-to-cell transmission, it is important to have a system to conveniently eliminate viral donor cells from target cells. Accordingly, we have established a caspase 9-activatable KSHV infected B-cell line and examined its efficacy of the infection.

Methods: KSHV immediate-early protein (K-Rta) inducible BCBL-1 cells were transduced with a caspase 9 expression cassette. Dimerization of caspase 9 was then induced with a chemical; this triggers cell apoptosis and eliminates "KSHV donor cells" from co-cultures. The efficacy of KSHV infection was examined with immunofluorescence analyses.

Results: Co-culturing 293T target cells with the suicide-BCBL-1 cells allow KSHV to be efficiently transmitted from BCBL-1 to 293T cells, and 99% of the K-Rta inducible BCBL-1 cells underwent apoptosis after administration of 10 nM of chemical inducer of dimerization (CID). Flow cytometer analyses showed that the CID specifically killed co-cultured suicide-BCBL-1 but not 293T cells and approximately 70% of 293T cells were infected in a single "infection."

Conclusions: Our co-culture system provides a convenient KSHV infection method, which may allow us to study not only the cell-associated transmission of KSHV but also relationship between KSHV replication and cell apoptosis.

78. Kaposi Sarcoma(KS) Is a Macrophage (MO) Tumor Resulting From a Phenotypic "Fusion" CD206+MOs and Lymphatic Endothelium

Frederick Cope[1], J. Zhang[2], T. Maurer[2], Bonnie Abbruzzese[1], J. Sanders[1], S. Behr[2], P. Bracci[2,3], M. McGrath[2,3]

[1]Navidea-Macrophage Therapeutics, Dublin, Ohio, USA; [2]University of California, San Francisco, [3]AIDS & Cancer Specimen Resource, San Francisco General Hospital, San Francisco, California, USA

Background: The origin of KS allegedly lies in HHV-induced transformation of lymphatic endothelial cells (EC). Signature elements of both ECs and transformed spindle ECs (tEC) are the expression of VECGFr-3 and/or D2-40 (1). Our recent data show that KS tumor parenchyma overlays are also concordantly positive for macrophage CD163, CD68, and CD206 (mannose binding receptor; MBR). In order to establish concordance of these elements in HHV8+ (HIV+ and HIV-) patients, we mapped the localization of a novel CD206-targeted agent in KS+/HHV8+ patients with multiple lesions using Manocept-Tc99m (TcT). The mapping of these patients was correlated with pre-imaging assessment of expression markers of the KS lesions.

Methods: Manocept is a synthetic molecule with a dextran backbone, and 12-20 mannose CD206-targeting moieties. Ex vivo CD206 analysis was conducted with a Cy3 fluorescent version of Manocept on and in MOs and KS. In lesions, binding/localization of Cy3-Manocept (C3M) was assessed through intracellular visualization and flow cytometric quantitation of C3M. The fresh KS tumor tissue culture followed by immunofluorescence staining and confocal imaging was performed to confirm C3M uptake in KS and TAMs and other KS markers (LANA, etc.). TcT was used to detect tumors and involved lymph nodes in KS patients. Patients received a SC injection of 50µg TcT w/ 2.0 mCi Tc 99m w/ SPECT/CT imaging at 4 hours post-injection to visualize localization of KS in the patients (Clinicaltrials.gov NCT02201420).

Results: Pre-imaging biopsy of KS lesions revealed the co-localization of KS and macrophage markers including the mannose binding receptor, CD206. Imaging results for 5 KS patients (5 HHV8+à 4HIV+; 1 HIV-) indicate that not only do multiple KS lesions localize TcT to KS CD206, but that KS lesions are anatomically linked in chains by and within the lymphatic ducts of the extremities, these lesions being further anatomically linked to regional lymph nodes that likely harbor KS (based on localization signals in imaging). In addition, recently completed cytokine profiling of macrophage behavior under similar conditions suggests that this phenotypic fusion is driven by altered expression of specific cytokine sets.

Conclusions: Both ex vivo and in vivo data suggest that KS is a fusion tumor arising from the formation of HHV8-processing MOs entering the lymphatic terminuses and forming neo-stromal junctions with the lymphatic endothelium and producing multifocal disease within anatomic linked lymphatic ducts. These data also support further studies regarding HHV8+/HIV+ patients with CD206 variant alleles that progress significantly slower (x) with the development of KS (2).

References

1. Douglas J et al. 2009. Characterization of c-Kit expression & activation in KSHV-infected ECs. Virology, 390:174-85.
2. Maas J et al. 1998. Presence of the variant mannose-binding lectin alleles associated with slower progression to AIDS. AIDS. 12:2275-80.

79. KSHV Infection Alters microRNA Biogenesis Factors to Promote Viral microRNA Biogenesis

Christine Happel, Joseph Ziegelbauer

HIV and AIDS Malignancy Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

Requested not to post abstract in online publication.

80. KSHV Infection of Endothelial Cells Induces Cytokines That Promote Differentiation and Polarization of Monocytes Into Tumor-Associated Macrophages

Fengchun Ye, Natarajan Bhaskaran, Sanhai Qin, Aaron Weinberg, Pushpa Pandiyan

Department of Biological Sciences, School of Dental Medicine, Case Western Reserve University, Cleveland, Ohio, USA

Background: Tumor-associated macrophages (TAMs) promote tumor angiogenesis, proliferation, metastasis, suppression of anti-tumor innate immunity, and development of resistance to chemotherapy. Originating from blood monocytes, TAMs express common monocyte/macrophage markers CD14 and CD68 and unique markers such as CD163, CD206, and legumain, an asparaginyl endopeptidase that degrades fibronectin to activate a number of pro-oncogenic proteases. The conversion of monocytes into M1 macrophages, the effector cells to clear up infection, or M2 macrophages, which are involved in wound healing and tissue repair, depends on the local tissue environment that contains specific cytokines. M-CSF, IL-4, IL-6, IL-10, and IL-13 promote monocytic differentiation into the "pro-tumor" M2 macrophages. Interestingly, these cytokines are present in high levels in the plasma of patients with Kaposi sarcoma (KS), a malignancy of endothelial cell origin that results from KSHV infection. In the present study, we investigated if KSHV infection of endothelial cells plays a role in the conversion of monocytes into TAMs to promote KS tumor growth.

Method: Human umbilical vein endothelial cells (HUVECs) were infected with mock and KSHV for 48 hours. Cytokines from mock and KSHV-infected cells were analyzed by real-time RT-PCR and Western blot analysis. Purified human CD14+ monocytes were incubated with culture supernatants from mock and KSHV-infected HUVECs for 3 days. TAM-specific transcripts and proteins in the monocytes after incubation were analyzed by real-time RT-PCR, fluorescence antibody staining, and flow cytometry. To test if KSHV-induced TAMs indeed promote tumor growth, equal numbers of KSHV-infected and telomerase-immortalized HUVECs (TIVE-KSHV) were mixed with equal numbers of monocytes that had been incubated with supernatant from mock or KSHV-infected HUVECs and then injected into nude mice. Tumor volume was measured weekly with a caliper.

Results: We found a strong presence of legumain-expressing TAMs in KS tumors. We previously showed that KSHV infection of endothelial cells induces expression of angiopoietin-2 (Ang-2), which not only promotes angiogenesis but also plays a role in the recruitment of monocytes into the infected sites by interacting with Tie-2 expressing monocytes. Here we report that KSHV infection of HUVECs also induces expression of IL-6, IL-10, and IL-13, which are known to promote monocytic differentiation and polarization into "pro-tumor" M2 macrophages. Indeed, incubation of monocytes with supernatant from KSHV-infected HUVECs significantly increases the levels of TAM-specific transcripts CD163, CD206, and legumain, and results in higher numbers of cells expressing the TAM-specific marker legumain. Data from the in vivo study indicate that the KSHV-induced TAMs enhance tumor growth in nude mice.

Conclusion: KSHV infection of endothelial cells induces multiple cytokines that promote differentiation and polarization of monocytes into TAMs to promote KS tumor growth. Therefore, therapies earmarked for TAM inhibition should be studied in ameliorating KS.

81. KSHV microRNAs Deregulate the STAT3 Interaction Network, Inhibit STAT3 Activation, and Promote Lytic Reactivation

Dhivya Ramalingam, Joseph Ziegelbauer

HIV and AIDS Malignancy Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

Background: MicroRNAs (miRNAs) are small ~22-nt long RNAs that regulate gene expression post-transcriptionally. Kaposi sarcoma-associated herpesvirus (KSHV) encodes 12 pre-miRNAs during latency and the cellular targets of these miRNAs as well as their functions are beginning to emerge. We have previously reported the identification of the signal transducer and activator of transcription 3 (STAT3) as the cellular target of KSHV miRNAs. STAT3 levels were repressed with several KSHV miRNAs and during de novo KSHV infection of endothelial cells.

Methods: To understand the functional roles of STAT3 repression during KSHV infection, we employed network-building strategies to search for pathways containing multiple miRNA targets. One network of validated miRNA target genes of KSHV was constructed using only published direct interactions between proteins. We validated these individual targets using luciferase assays and also ensured target suppression using transient miRNA transfections into endothelial cells. We also studied the effect of miRNAs on STAT3 activation using cytokine activators like IL6 and interferon-alpha A (IFN-aA). To understand the significance of their repression, we also infected endothelial cells that were transiently transfected with siRNAs against various KSHV miRNA targets and measured infection rates and levels of various apoptosis-related genes. In addition, the effect of target suppression on cell cycle progression was also measured using flow cytometric methods. Finally, to understand the significance of STAT3 repression in the context of KS, we electroporated siRNAs against STAT3 and other related targets into the latently infected BCBL-1 cells and measured levels of lytic reactivation.

Results and Conclusions: We observed that four miRNA targets, Met, IRAK1, PKCD, and EPOR, stimulate STAT3 activity in normal cells. KSHV miRNAs also suppressed the STAT3-regulated gene, survivin. KSHV miRNAs strongly repressed the levels of the activated form of STAT3 (pTyr705-STAT3), upon IL6 and IFNaA-treatments. Knockdown of survivin in endothelial cells enhanced KSHV infection, increased p21 levels, and arrested the cells at the G2/M phase of the cell cycle. Finally, we observed a robust lytic reactivation of KSHV upon STAT3 knockdown in BCBL-1 cells, as measured by the levels of immediate-early (RTA) and lytic (ORF57, ORF59, K8) miRNAs of KSHV. These results could also be recapitulated using the STAT3-inhibitor, Stattic. Together, our results show that KSHV miRNAs suppress STAT3 activation to increase KSHV infection of endothelial cells, deregulate the cell cycle, and facilitate the transition to the lytic phase of its life cycle in PEL cells.

82. Latent EBV Infection Impairs Epithelial Differentiation in an Oral Keratinocyte Model

Mark Eichelberg, Ben Korte, Kyle McChesney, Kathleen Makielski, Dhananjay M. Nawandar, Makoto Ohashi, Paul F. Lambert, Shannon C. Kenney, Eric C. Johannsen

McArdle Laboratory for Cancer Research and Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA

Background: Epstein-Barr virus (EBV), like all herpesviruses, establishes both lytic and latent infections in cells. In normal epithelial cells, EBV infection is lytic and restricted to the differentiated layers. In nasopharyngeal carcinoma (NPC) EBV infection is latent; nearly all cases of undifferentiated NPC are EBV associated. Persons with AIDS are less able to control both latent and lytic EBV infections and have increased incidence of EBV associated malignancies, including NPC. Enrichment for genetic lesions affecting chromatin modification and differentiation has been observed in studies of NPC genetic landscape, suggesting they play a critical role in NPC pathogenesis. Investigation of the interaction between differentiation and EBV infection state has previously been limited by the lack of a physiologic model of EBV epithelial infection.

Methods: We established an h-tert immortalized, normal oral keratinocyte (NOK) line that can be latently infected with EBV (NOK-EBV). NOK can be grown in raft cultures and undergo organotypic differentiation. Importantly, in NOK-EBV, infection switches from latent to lytic in the differentiated layers. Using this highly physiologic model, we investigated the effect of EBV infection on epithelial differentiation and the mechanisms that link differentiation to the EBV latent/lytic switch.

Results: By RNA-seq, we observed a striking decrease in the extent of differentiation induced gene expression changes in NOK-EBV compared to NOK. In NOK cells, calcium induced differentiation resulted in 1828 upregulated and 1628 downregulated genes. By contrast, in NOK-EBV only 684 genes were significantly upregulated and 695 downregulated. This effect was also apparent in raft cultures, where spinous and granular layers were less well defined compared to NOK. By immunohistochemistry, NOK-EBV raft cultures had decreased differentiation marker expression compared to NOK, including Involucrin, keratin10, filaggrin, and loricrin. Pathway analysis comparing RNA-seq data from NOK to NOK-EBV in the undifferentiated states found enrichment for gene changes in multiple pathways including the Wnt, p53, TGF-beta, and interferon signaling pathways. Our preliminary hydroxymethylcytosine profiling suggests that this epigenetic mark is also influenced by EBV infection as has been previously demonstrated for methylcytosine.

Conclusions: NOK and NOK-EBV represent an important model for studying EBV infection of epithelial cells in vitro. This model recapitulates the dependence of EBV lytic infection on differentiation. Surprisingly, we find EBV infection of NOK disrupts their normal differentiation, suggesting that EBV latent infection in itself may contribute to the differentiation arrest observed in nasopharyngeal carcinoma. We are currently using the NOK-EBV model to determine the extent to which specific EBV gene products, particularly the BART microRNAs, are responsible for this effect.

83. Modulation of Human β-defensin 3 (hBD3) Expression by Human Papillomavirus (HPV) Oncogenic E6 Protein: Role of the Tumor Suppressor Protein TP53 Family

Twishasri DasGupta[1], Emeka I. Nweze[1], Hong Yue[1], Liming Wang[4], Jessica Jin[5], Santosh Ghosh[1], Hameem I. Kawsar[1], Chad Zender[2], Elliot J. Androphy[6], Aaron Weinberg[1], Thomas S. McCormick[3], Ge Jin[1]

[1]Department of Biological Sciences, Case Western Reserve University School of Dental Medicine; [2]Department of Otolaryngology-Head & Neck Surgery; [3]Department of Dermatology, Case Western Reserve University School of Medicine, University Hospitals Case Medical Center, Cleveland, Ohio, USA; [4]Center for Molecular Cancer Diagnosis Inc., Twinsburg, Ohio, USA; [5]Human Developmental and Regenerative Biology, Harvard University, Cambridge, Massachusetts, USA; [6]Department of Dermatology, Indiana University School of Medicine, Indianapolis, Indiana, USA

Requested not to post abstract in online publication.

84. A Novel Approach to Visually Characterize Persistent Cellular Reservoirs of HIV in Tissues From Individuals on Antiretroviral Therapy

J.J. Vásquez[1], R. Hussien[1,2,3], C. Bacchus-Souffan[1,2], J. Estes[1,3,4], M. McGrath[1,2], P. Hunt[1,2], J. McCune[1,2]

[1]University of California, San Francisco, San Francisco, California, USA; [2]AIDS Cancer Specimen Resource, San Francisco, California, USA; [3]Delaney AIDS Research Enterprise to Cure HIV, San Francisco, California, USA; [4]Leidos Biomedical Research, Inc., San Francisco, California, USA; [5]Frederick National Laboratory for Cancer Research, Frederick, Maryland, USA

Background: Cellular reservoirs of HIV persist despite suppressive antiretroviral therapy (1). While circulating memory CD4+ T cells have been well established as important persistent HIV reservoirs, the anatomic localization of infected CD4+ T cell subsets in lymphoid tissues and the extent to which tissue macrophages contribute to the persistent HIV reservoir remain unclear (2). Since the vast majority of persistently infected cells reside in tissues, highly sensitive and specific methods to characterize cellular reservoirs of HIV in situ are urgently needed to advance HIV cure research (3). We propose a novel approach to visually assess the tissue reservoir of HIV in formalin-fixed paraffin-embedded tissues (FFPE).

Methods: Probing of HIV sense sequences by DNAscope, a highly sensitive method of in situ hybridization (ISH) using branched-DNA technology (Advanced Cell Diagnostics), has made it possible to visually detect single copy intra-nuclear HIV-DNA within cells from FFPE tissues (4). Fixed tissues and bronchoalveolar lavage (BAL) from HIV-infected donors were assessed by HIV-DNAscope and immunofluorescence (IF). Negative controls included HIV-negative tissue and scrambled sequence HIV probes. Positive controls included ACH2 cells, known to harbor a single integrated copy of HIV DNA.

Results: Staining conditions were optimized in BAL and other sample types to reliably detect nucleus-associated HIV DNA in positive controls with minimal background signal in negative controls. Using these methods, we can visualize nucleus-associated HIV DNA within alveolar macrophages from HIV-infected individuals. We can also detect infected T cells and macrophages within tissues.

Conclusion: Single-cell analysis using IF and ISH by DNAscope is a novel approach to characterize the tissue reservoir of HIV. More work is required to assess the quantitative potential of this approach in diverse human tissues.

References

1. Furtado MR, Callaway DS, Phair JP, Kunstman KJ, Stanton JL, Macken CA, et al. Persistence of HIV-1 transcription in peripheral-blood mononuclear cells in patients receiving potent antiretroviral therapy. N Engl J Med. 1999 May 27;340(21):1614–22.
2. Calantone N, Wu F, Klase Z, Deleage C, Perkins M, Matsuda K, et al. Tissue Myeloid Cells in SIV-Infected Primates Acquire Viral DNA Through Phagocytosis of Infected T Cells. Immunity. Elsevier; 2014 Sep 18;41(3):493–502.
3. Blankson JN, Persaud D, Siliciano RF. The Challenge of Viral Reservoirs in HIV-1 Infection. Annual Review of Medicine. 2002 Feb;53(1):557–593.
4. Wang Z, Portier BP, Gruver AM, Bui S, Wang H, Su N, Vo H-T, Ma X-J, Luo Y, Budd GT, Tubbs RR. Automated quantitative RNA in situ hybridization for resolution of equivocal and heterogeneous ERBB2 (HER2) status in invasive breast carcinoma. J Mol Diagn. 2013 Mar;15(2):210–219. PMID: 23305906.

85. Pathology External Quality Assurance Program for Kaposi Sarcoma International Clinical Trials AMC-067/A5264 and AMC-066/A5263

Scott Ely[1], Sharon Barouk[1], Morgan Gapara[2], Katie Lammersen[2], Janice Darden[2], Margaret Borok[3], Mina C. Hosseinipour[4], Susan Krown[5], Thomas Campbell[6], Neal Wetherall[7], Robert W. Coombs[8], Ethel Cesarman[1], Catherine Godfrey[7]

[1]Weill Cornell Medical College, New York, New York, USA; [2]AIDS Clinical Trials Group, Silver Spring, Maryland, USA; [3]University of Zimbabwe, Harare, Zimbabwe; [4]UNC Project Malawi, Lilongwe, Malawi; [4]AIDS Malignancy Consortium, New York, New York, USA; [6]University of Colorado, Denver, Colorado, USA; [7]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA; [8]University of Washington/AIDS Clinical Trials Group, Seattle, Washington, USA

Background: Histopathology services in resource limited settings (RLS) face many challenges, especially in Africa, with few pathologists per population and few internationally accredited laboratories.[1,2] External quality assurance (EQA) is an accepted tool for ensuring the validity of test results across sites in clinical practice, and is useful in clinical trials.[3] Most EQA programs for histopathology are disease specific or occur in the context of continuing professional development and there are no programs for the diagnosis of Kaposi sarcoma (KS). Immunohistochemistry (IHC; LANA) is used to confirm the diagnosis of KS in resource rich environments but is not generally available in areas of the world where KS is prevalent. The AIDS Clinical Trials Group (ACTG) and the AIDS Malignancy Consortium (AMC) have implemented two clinical trials that will establish treatment standards for KS in RLS. Biopsy proven KS is required for entry in both studies. We report the prequalifying assessment and the first round of EQA.

Methods: ACTG and AMC international sites participating in A5263/AMC066 and A5264/AMC067 proposed a laboratory and pathologist to interpret biopsies obtained for KS diagnosis. Laboratories were visited in order to evaluate their suitability and assess their needs for performing the protocol. A quality assurance program comprising two schemes was established to improve diagnostic capabilities. Scheme 1 confirmed the site pathologists' abilities to accurately diagnose KS and consists of both KS and non-KS specimens for review and interpretation. Scheme 2 consists of a blinded review of study entry biopsy material consisting of one hematoxylin and eosin (H&E) stained slide and one paraffin block or tissue sections for all cases diagnosed as KS at entry, which were sent to the central lab. Sites were asked to also submit screening specimens that were interpreted as negative for KS. IHC was performed in all cases at the central lab, but was not available locally at most site labs or required to confirm the diagnosis. Discordant samples were read by a third pathologist at the central lab.

Results: Scheme 1 standards were established by U.S. based and clinical trial site pathologists. Five of eight labs successfully completed scheme 1. For scheme 2, biopsies from 234 patients from 11 sites were submitted to the central lab through June 2015. Tissue quality and H&E were adequate to good in 232/234 specimens. Immunohistochemistry for LANA was successful confirming good tissue quality and fixation. There were 14/234 discordant cases (6%) including 3 read as positive by local pathologists and 11 read as negative. Investigation of discordant specimens revealed that the wrong specimen was sent in 7 cases from a single lab; in all other cases a consensus diagnosis was reached after review.

Conclusions: Central review of patients enrolled in these trials showed generally good quality and accurate diagnosis, even without immunohistochemistry. Good clinical lab practice including specimen tracking, labeling, and chain of custody remains a challenge but likely will be improved by the rigor required by ACTG/AMC clinical trials and through the education provided to the labs through this program.

86. Potential for Therapeutic Targeting of Kaposi Sarcoma (KS) and the Macrophage of HIV With the CD206-Receptor Targeting Agent: Localization in HIV Patients, Macrophage Reservoirs, and Off-Target Risks

M. McGrath[1,2], J. Zhang[1], T. Maurer[1], S. Behr[1], B. Abbruzzese[3], J. Sanders[3], P. Bracci[1,2], F.O. Cope[3]

[1]University of California, San Francisco, [2]AIDS & Cancer Specimen Resource, San Francisco General Hospital, San Francisco, California, USA; [3]Navidea-Macrophage Therapeutics, Dublin, Ohio, USA

Background: Inflammation appears to play a critical role in tumor development of Kaposi sarcoma (KS). Specifically, emerging data show that KS tumor cells that co-express various macrophage (MO) antigens become resistant to current anti-viral therapies used to treat KS and AIDS. MOs also are a known source of KS tumor cell growth factors and further evidence suggests that tumor associated MOs (TAMs) may represent a reservoir for HIV and its evolved variants. The MO pool driving these two pathological pathways share a common element rooted in the MOs, the CD206 MO mannose receptor. Manocept, a molecular targeting agent, binds and enters MOs via pinocytosis of holo-CD206, providing a portal for the evaluation of Manocept as a MO and KS targeting agent.

Methods: Manocept is a synthetic molecule with 12-20 mannose CD206-targeting moieties. CD206 targeting assays were conducted using both in vitro monocyte-derived CD206+ MOs and ex vivo fresh KS tumor tissue (provided by the AIDS and Cancer Specimen Resource [ACSR]). Manocept-Cy3 with/without a chemo-therapeutic agent attached (Mano-Cy3-CTA or Mano-Cy3) interactions with cellular and tumor targets were tracked by flow cytometry and immuno-histochemistry to evaluate Manocept uptake and targeting capability for delivery of drug into KS tumor cells and TAMs. In addition, Technetium Tc99m tilmanocept (TcT) was used to detect tumors and involved lymph nodes in HIV KS patients. Patients received one SC injection of 50µg TcT w/ 2.0 mCi Tc 99m w/ SPECT/CT imaging at 4 hours post-injection to visualize localization of KS in the patients (Clinicaltrials.gov NCT02201420).

Results: In vitro studies showed that the CD206+ MO uptake of Mano-Cy3 and Mano-Cy3-CTA was time- and dose-dependent. Confocal microscopy evaluation of fresh KS organ culture confirmed the uptake of Manocept into both KS tumor cells and CD206+ TAMs. Apoptosis induction after exposure to Mano-Cy3-CTA was confirmed by increased Annexin-V expression on MOs and in tumor tissue. This was coupled by loss of CD206 MOs and by loss of HHV8+ spindle cells overnight. There was less effect on cells exposed to CTA alone. Pre-imaging biopsy of KS lesions revealed the co-localization of KS markers and macrophage markers including the mannose binding receptor, CD206. Imaging results for 5 KS patients (5 HHV8+ 4HIV+; 1 HIV-) indicates that not only do multiple KS lesions localize TcT to KS CD206, but that KS lesions are anatomically linked in chains by and within the lymphatic ducts of the extremities, these lesions being further anatomically linked to regional lymph nodes that likely harbor KS (based on localization signals in imaging).

Conclusions: Both the ex vivo and in vivo data suggest that KS in both HIV+ and HIV- patients have the pan-tumor parenchyma that expresses CD206 and that KS lesions can easily be easily visualized, mapped potentially routinely assessed via SPECT/CT imaging. Moreover, the imaging in these patients suggests that KS tumor dissemination may be opportunistic relative to the anatomic linkage of KS lesions with the lymphatic system and its nexus to the blood vascular system. Our data also suggest that the TcT may be employed to deliver a therapeutic moiety with high target effect and low off-target concerns.

87. Potential New Therapy in Kaposi Sarcoma: Checkpoint Inhibitor Immunotherapy

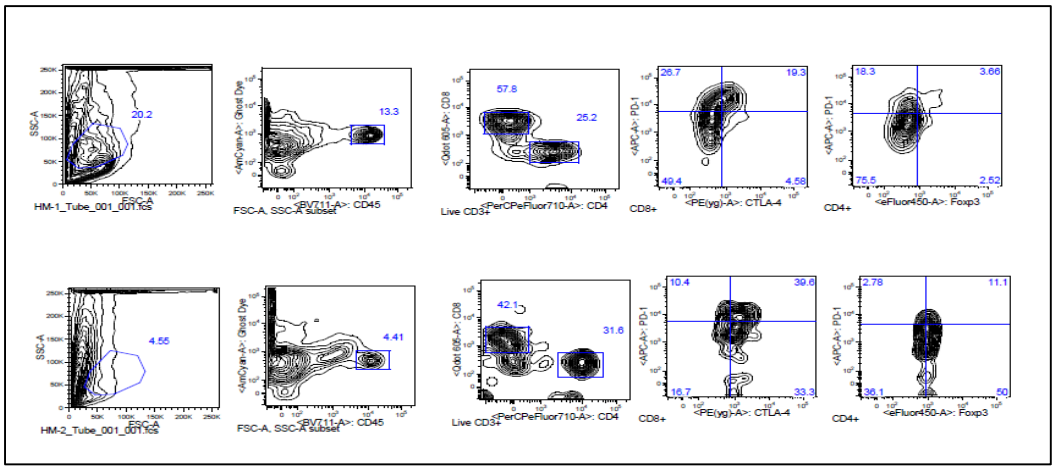
T. Maurer, K. Leslie, G. Isaza-Gonzalez, M. Rosenblum

Department of Dermatology, University of California, San Francisco, San Francisco, California, USA

Background: The programmed death 1 protein (PD-1) is an immune checkpoint receptor expressed by activated T cells. Tumor cells that express its ligand (PD-L1) can evade key immune responses, thereby preventing the immune system from rejecting the tumor. Accordingly, researchers have identified the PD-1 pathway as an appealing opportunity for therapeutic intervention in human cancers. Various clinical trials and investigations have shown promising results with the use of PD-1 and PD-L1 antibodies as possible therapeutic modalities in a variety of malignancies, such as melanoma. However, the efficacy of PD-1 and PD-L1 inhibitors in Kaposi sarcoma (KS) is under investigation.

Methods: Four mm punch biopsies were obtained from lesional skin of 2 subjects: an African-American male with a 14 year history of KS (subject 1) and a Caucasian male with a 15 yr history of KS (subject 2) . Both subjects had progression of KS despite trials of doxorubicin, bleomycin, vincristine, and paclitaxel (Subject 1) and liposomal doxorubicin (subject 2). Both subjects had been on stable ART regimens with CD4 counts of 800-900 cells/mm^3 and sustained virilogic suppression for over 10 years. Freshly harvested KS tissue from skin was subjected to multi-parameter (15 color) flow cytometry using a unique method of cell stimulation, allowing for analysis of the functionality of the immune microenvironment.

Results: A clear population of exhausted tumor-infiltrating lymphocytes (Live CD45+CD8+PD-1+CTLA-4+ cells) was observed. Increased percentages of CD8+ T cells and reduced percentages of Tregs (Live CD45+CD4+Foxp3+ cells) were found when comparing his KS tissue to normal skin (from historical controls) as shown in the figure below. This population is identical to the population observed in patients with metastatic melanoma that respond to immune checkpoint blockade (i.e., anti-PD-1 therapy).



Conclusions: Based on our findings, we believe that there is a need for further investigation of the efficacy of immune checkpoint inhibitors in the treatment of KS. New therapeutics such as Anti-PD/Anti-PD-L1 antibodies could have a significant impact on patients with KS who have failed multiple therapeutic modalities.

88. Propranolol for the Treatment of Kaposi Sarcoma and Primary Effusion Lymphoma

Shane C. McAllister[1,2], Ryan S. Hanson[1,2], Rory D. Manion[1,2], Dylan B. Zawilla[1,2]

[1]Division of Pediatric Infectious Diseases, University of Minnesota Medical School, Minneapolis, Minnesota, USA; [2]Center for Infectious Diseases and Microbiology Translational Research, Minneapolis, Minnesota, USA

Background: Kaposi sarcoma (KS) and primary effusion lymphomas (PEL) are two common tumors associated with immune suppression in HIV-positive individuals infected with KS Herpesvirus KSHV. Both tumors can be extremely difficult to treat, even in the setting of favorable virological and immunological responses to antiretroviral therapy. Furthermore, clinical outcomes for patients with KS in sub-Saharan African countries, where the highest KS burden has been documented, are even poorer given the paucity of anti-cancer agents that are affordable in limited resource settings. Therefore, we have sought out generic medications with in vitro activity against these neoplasias in order to identify additional agents that may improve the treatment response of patients with KS and PEL in a variety of economic settings.

Methods: Given the histological and pathophysiological similarities between KS and another vascular lesion infantile hemangioma (IH), and the known exquisite sensitivity of IH cells to the generic b-adrenergic receptor antagonist propranolol, we assessed the effect of this drug using an established in vitro model of KS as well as the PEL cell line BCBL-1. Proliferation of cells was analyzed by colorimetric measurement of XTT metabolism, and cell cycle sub-populations were enumerated by BrdU and 7-AAD staining. Expression of cyclins, cyclin-dependent kinases (CDKs), and the retinoblastoma protein was assessed by immunoblotting, and knockdown of specific cell cycle regulators was achieved using siRNA. Finally, RT-PCR was used to assess viral gene expression.

Results: Proliferation of both KS and PEL cells was reduced in cultures treated with propranolol. In both cell types, drug treatment was associated with a reduction in expression of CDK1 and cyclin A2, cellular proteins required for mediating the completion of S phase and driving the G_2-M transition. Knockdown of CDK1 and cyclin A2 using siRNA recapitulated the proliferation defect seen in cells treated with propranolol. Propranolol was also associated with induction of expression of viral lytic genes in both cell types, a phenotype associated with decreased expression of CDK6.

Conclusions: Our data demonstrate that the generic drug propranolol has activity against two KSHV-associated tumors in vitro and suggest that autocrine β-adrenergic signaling contributes to tumor cell proliferation. Given the long history of clinical experience with this medication and others in the same class, further evaluation of β-adrenergic antagonists for the treatment of KSHV-associated neoplasias is warranted

89. Recently Approved Kinase Inhibitors for the Treatment of B-Cell Malignancies Block B-cell–receptor Mediated Epstein-Barr virus (EBV) Lytic Activation

Jaeyeun Lee, John G. Kosowicz, Richard F. Ambinder

Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Background: Epstein-Barr virus (EBV) is a human gammaherpesvirus that is associated with a variety of B-cell malignancies. In vitro, a variety of agents including phorbol esters, calcium ionophores, and activation of B cell receptor signaling are all associated with activation of lytic infection.[1] Recently two kinase inhibitors that interfere with B cell receptor signaling have been approved for the treatment of chronic lymphocytic leukemia and some other B cell malignancies. These are ibrutinib, an inhibitor of Bruton's tyrosine kinase (BTK), and idelalisib, a PI3-kinase β inhibitor.[2-3] We evaluated the impact of these agents on EBV lytic activation.

Methods: We studied the Akata Burkitt lymphoma cell line and some derivative cell lines: the parent EBV(+) cell line, a derivative EBV(-) Akata cell line, and an engineered derivative cell line (BX1) that carries a recombinant EBV that constitutively expresses a green fluorescent protein (GFP). Lytic induction leads to GFP signal in the BX1 Akata cell line and fluorescence microscopy was used to determine the number of lytic cells expressing GFP. Quantitative PCR assay of the Bam W repeat region of the EBV genome was performed for measuring viral load and western blotting and qRT-PCR were performed to examine gene expression on protein and mRNA levels. Cell viability was assessed by CellTiter Glo.

Results: Anti-Ig treatment on Akata cell line induced expression of EBV ZTA as assessed by fluorescence microscopy, immunoblot, and qRT-PCR. Both ibrutinib and idelalisib treatment blocked anti-Ig lytic induction in a dose dependent manner. These agents were more toxic to EBV(+) Akata cells than EBV(-) Akata cells. Neither ibrutinib nor idelalisib interfere with other EBV lytic inducing stimuli studied (TPA, NaB, ionomycin).

Conclusion: These results demonstrate that in a Burkitt lymphoma cell line, BCR-driven EBV lytic expression is blocked by these kinase inhibitors and that in these isogenic cell lines, EBV(+) cells are more sensitive to killing than EBV(-) cells. Further studies with these agents may help provide insights into the role of BCR activation of EBV lytic cycle in vivo and clarify the mechanisms underlying increased sensitivity to cell killing in the EBV(+) cells.

References

1. Murata T et al., 2014. Switching of EBV cycles between latent and lytic states. Rev. Med. Virol.
2. Brown J, 2013. PCI-32765, the First BTK (Bruton's tyrosine kinase) Inhibitor in Clinical Trials. Curr Hematol Malig Rep.
3. Ikeda H, Hideshima T, Fulciniti et al. 2010. PI3K {delta} is a novel therapeutic target in multiple myeloma. Blood.

90. Reversine Blocks EBV Activation by Multiple Lytic Inducers

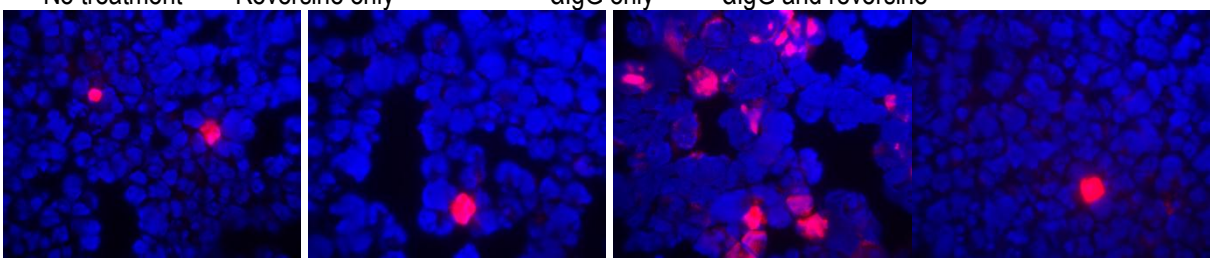
John G. Kosowicz, Jaeyeun Lee, Gangling Liao, Diane Hayward, Richard F. Ambinder

Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Background: Epstein–Barr virus (EBV) is a human gammaherpesvirus that is associated with a variety of B-cell malignancies. In vitro, a variety of agents including phorbol esters, calcium ionophores, and activation of B cell receptor signaling are all associated with activation of lytic infection (1). Reversine is a small molecule with the properties of a mitotic spindle checkpoint (MPS) inhibitor, an AURORA B kinase inhibitor, and a dedifferentiation agent (2,3). We set out to determine its effect on EBV activation.

Methods: Cell imaging and immunofluorescent staining was carried out using a Burkitt lymphoma cell line (Akata) that carries recombinant EBV (BX1-Akata), which constitutively expresses a green fluorescent protein (GFP). In this cell line, lytic induction leads to GFP signal and EBV ZTA protein expression. Lytic cycle was induced by treatment with αIgG, phorbol ester, ionomycin, or bortezomib. Fluorescence microscopy was used to determine the number of lytic cells expressing GFP. Quantitative PCR assay of the Bam W repeat region of the EBV genome was performed for measuring viral load.

Results: Reversine blocked lytic induction by αIgG, but was ineffective when washed out prior to αIgG treatment or when added 1 hour after αIgG treatment. Reversine was partially effective in blocking bortezomib lytic induction but not at all effective in blocking lytic induction by ionomycin or TPA.



αIgG lytic induction is blocked by the addition of reversine. EBV ZTA in BX1 Akata cells was imaged after treatment by reversine and αIgG for 24h.

Conclusions: Reversine is a small molecule that blocks αIgG lytic induction in Akata cells. The ability to block αIgG lytic induction but not phorbol ester or calcium ionophore lytic induction suggests that reversine may act early in the B-cell receptor pathway.

HIV+/EBV- (3)	20%	75%	0%	9%	0%	12%	18%
HIV+/EBV+ (28)	17%	35%	0%	6%	2%	7%	7%

91. Serum Biomarkers of Inflammation and Immune Activation and HIV-Associated Non-Hodgkin Lymphoma in HAART-Exposed Men in the Multicenter AIDS Cohort Study

Shehnaz K. Hussain[1,2], Solomon B. Makgoeng[2], Elizabeth Crabb Breen[3,4], Jay Bream[5], Giovanna Rappocciolo[6], Sudhir Penogonda[7], Lisa Jacobson[5], Roger Detels[2], Otoniel Martínez-Maza[2,3,4]

[1]Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, California, USA; [2]Department of Epidemiology, Fielding School of Public Health, University of California, Los Angeles, Los Angeles, California, USA; [3]David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California, USA; [4]UCLA AIDS Institute, Los Angeles, California, USA; [5]Johns Hopkins University, Baltimore, Maryland, USA; [6]Department of Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; [7]Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA

Background: Non-Hodgkin lymphoma (NHL) risk remains elevated in the era of effective multi-agent antiretroviral treatment (HAART). We have previously shown that there is a prolonged and heightened degree of immune activation and inflammation prior to NHL diagnosis in HAART naïve men, and that HAART dampens but does not normalize immune activation/inflammation. It is unknown whether elevation in immune biomarkers precedes NHL in HAART treated men.

Methods: We sought to examine this question in a subcohort of participants from the Multicenter AIDS Cohort Study (MACS) who were recently longitudinally characterized for immune biomarkers. This study included 1,282 HAART-exposed men with biomarkers characterized following HAART initiation, including 17 participants who subsequently developed NHL. Multiplex assays were used to quantify 23 immune biomarkers in sera (IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, TNF-α, GM-CSF, IFN-γ, CCL2, CCL4, CCL11, CCL13, CCL17, CXCL10, sCD14, sCD27, sgp130, sIL-2Rα, sIL-6R, sTNF-R2, BAFF, and CXCL13). We used multivariate Cox-proportional hazards regression to calculate hazard ratios representing the risk of AIDS-NHL associated with log-transformed continuous biomarker levels. Risk time began at HAART initiation and ended at NHL diagnosis, death, or the date of last contact.

Results: For those men who developed NHL, the median time to NHL diagnosis from HAART initiation was 5.3 years (range: 1.2 to 15.4 years), and the median number of serial biomarker measurements for each case was 4 (range 1 to 14). Increased concentrations of several markers of immune activation and inflammation were significantly associated with NHL in HAART-exposed men including sIL-2Rα (HR=5.7, 95% CI=2.6-12.7), sTNFR2 (HR=2.8, 95% CI=1.3-5.9), sCD27 (HR=2.3, 95% CI=1.2-4.1), and CXCL10 (HR=2.0, 95% CI=1.1-3.6). Although the majority of NHLs diagnosed in these HAART-exposed men occurred during intervals of high HIV load, there appears to be a greater strength of association between several biomarkers and NHLs diagnosed during intervals of viral suppression. For comparison, these same markers (sIL-2Rα, sTNFR2, sCD27, and CXCL10) and two additional markers (sCD14 and CXCL13) were significantly associated with NHL in MACS participants who developed NHL prior to the availability and/or initiation of HAART.

Conclusions: Heightened immune activation and inflammation precedes the diagnosis of HIV-associated NHL in HAART-exposed men, and may serve as biomarkers for risk stratification or early detection. Furthermore, therapeutic adjuvants that could further dampen immune activation and inflammation may prevent NHL development in this high risk population.

92. Shotgun Next Generation Sequencing of Whole g-Herpesvirus Genomes From Clinical Samples

Nazzarena Labo[1], Vickie Marshall[1], Elena M. Cornejo Castro[1], Priyanka Vengurlekar[2], Kathleen M. Wyvill[3], Karen Aleman[3], Mark N. Polizzotto[3], Thomas S. Uldrick[3] , Brandon F. Keele[2], Robert Yarchoan[3], Denise Whitby[1]

[1]Viral Oncology Section and [2]Retroviral Evolution Section, AIDS and Cancer Virus Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, Maryland, USA; [3]HIV and AIDS Malignancy Branch, National Cancer Institute, Bethesda, Maryland, USA

Background: Studies of KSHV diversity have been based on the variable K1 gene, the polyallelic K15 gene, and less variable genes such as T0.7/Kaposin, ORF75, and, recently, the microRNA encoding region. These, however, comprise less than 5% of the ~137 kb KSHV genome. Only few full viral genomes have been fully sequenced, nearly all derived from PEL cell lines. We recently developed a NGS approach to sequence KSHV, which we apply in this study to clinical samples.

Methods: We selected pleural effusion samples with high or very high KSHV viral load from patients with KSHV associated malignancies. Pair ended 251bp Illumina libraries were generated from each sample using total DNA and sequenced; reads were mapped and assembled on reference sequences.

Results: We sequenced 11 clinical specimens, pleural effusions, or ascites from 9 PEL patients and two AIDS KS patients. Libraries contained from ~40000 to ~50 million KSHV genome equivalents (GE). For inputs greater than 5 million genomes, KSHV coverage was 100%; mean coverage depth was ~50x to ~600x. We also sought to sequence EBV; however, EBV content was lower in each library, varying from ~740000 genomes to undetectable. For samples with over 100000 GE coverage was over 90% and mean coverage depth varied from 4x to 40x.
Sequence variance analysis showed that K1 and ORF73 were most variable; however, there was also subtle diffuse variability across all ORFs. Phylogenomic analysis indicated that most but not all KSHV "isolates" were closely related to one another, and to cell line derived viruses. However, K1 genotype did not determine overall genetic distance between strains.

Conclusion: We successfully applied a NGS shotgun approach to quickly and efficiently obtain whole a-herpesviruses sequences from clinical samples, increasing by three times the number of whole KSHV sequences available to date. This capability is particularly important for KSHV, which is extremely difficult to isolate, and will be critical to studying virus transmission and the association of sequence variation with disease risk in clinical and field settings.

93. STAT3 Regulates Lytic Activation of Kaposi Sarcoma-Associated Herpesvirus

Christine A. King[1], Xiaofan Li[2], Arturo Barbachano-Guerrero[1], Sumita Bhaduri-McIntosh[2,3]

[1]Department of Microbiology and Immunology, Upstate Medical University, Syracuse, New York, USA; [2] Division of Infectious Diseases, Department of Pediatrics, Stony Brook University School of Medicine, Stony Brook, New York, USA; [3] Department of Molecular Genetics and Microbiology, Stony Brook University, Stony Brook, New York, USA

Requested not to post abstract in online publication.

94. Suppression of Glycolysis by KSHV Promotes Cell Survival and Oncogenic Transformation

Ying Zhu[1], Suzane Ramos da Silva[1], Qiming Liang[1], Chun Lu[2], Pinghui Feng[1], Jae Jung[1], Shou-Jiang Gao[1]

[1]Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, California, USA; [2]Department of Microbiology and Immunology, Nanjing Medical University, Nanjing, People's Republic of China

Cancer cells undergo metabolic reprogramming to sustain cell proliferation and survival under diverse proliferative and stress conditions. Glycolysis, particularly aerobic glycolysis, is essential for supporting the fast growth of a number of cancers. However, the role of glycolysis in the survival of cancer cells under stress conditions is unclear. Here, we show that oncogenic Kaposi sarcoma-associated herpesvirus (KSHV) suppresses aerobic glycolysis and oxidative phosphorylation to promote cell survival and oncogenic cellular transformation under nutrient stress. Specifically, KSHV-encoded microRNAs and vFLIP suppress glycolysis by activating the NF-κB pathway to downregulate the expression of glucose transporters, GLUT1 and GLUT3. While overexpression of either GLUT1 or GLUT3 in KSHV-transformed cells rescues the glycolytic activity, it induces apoptosis, and reduces the efficiency of colony formation in softagar under glucose deprivation. GLUT1 and GLUT3 inhibit constitutive activation of the AKT and NF-κB pathways, implicating the existence of a negative feedback loop mediated by the glucose transporters. These results reveal a novel mechanism by which an oncogenic virus targets a key metabolic pathway to adapt to the stress in the tumor microenvironment. Our findings illustrate the importance of fine-tuning of the metabolic pathways for sustaining the proliferation and survival of cancer cells, particularly under stress conditions.

95. Unexpected Absence of Epstein-Barr Virus in Diffuse Large B-Cell Lymphoma Among HIV+ Patients in Malawi

Y. Fedoriw[1], N. Montgomery[1], J. Parker[1], S. Salahuddin[2], C. Kampani[4], S. Kamiza[3], T. Tomoka[3], R. Krysiak[1], N.G. Liomba[4], K.L. Richards[2], S. Gopal[1,3,4]

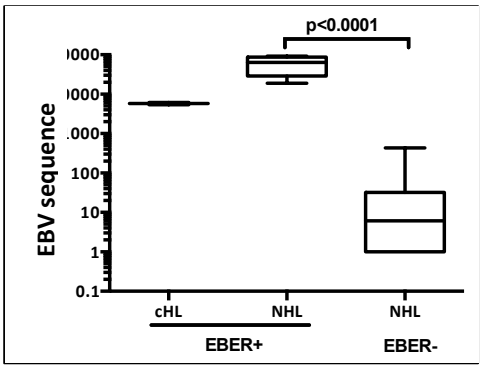
[1]The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; [2]Cornell University, Ithaca, New York, USA; [3]University of Malawi College of Medicine, Blantyre, Malawi; [4]UNC Project, Lilongwe, Malawi

Background: Lymphoma incidence in sub-Saharan Africa (SSA) is increasing due to HIV, population growth, and aging. While antiretroviral therapy (ART) is associated with decreased HIV+ lymphoma incidence, there are scarce HIV+ lymphoma data from SSA in the era of widespread ART scale-up. Epstein-Barr virus (EBV) is a known etiologic factor in lymphomagenesis, and can be identified in approximately half of HIV+ diffuse large B cell lymphomas (DLBCL) and Burkitt lymphomas (BL), and nearly all cases of HIV+ classical Hodgkin lymphoma (cHL) and primary central nervous system lymphoma in the Western world. However, the etiologic contribution of EBV to HIV+ lymphomas in SSA is not known. Elucidating this can help inform lymphoma prevention and treatment strategies in the region. Here we describe the association of EBV with lymphoma samples from the ongoing Kamuzu Central Hospital (KCH) Lymphoma Study prospective cohort previously described.

Methods: From June 2013 to January 2015, 63 patients with histologically confirmed lymphoproliferative disorders were enrolled at KCH in Lilongwe. CD4, HIV RNA, and ART status were documented for all HIV+ patients, as well as lymphoma-related clinical and laboratory data. Formalin fixed and paraffin embedded (FFPE) tissue was submitted to the University of North Carolina (UNC) for additional assessment and WHO classification. Tissue sections were stained for EBV using in situ hybridization for EBER (EBER-ISH), with appropriate RNA control. The whole transcriptome of 36 FFPE cases, including 27 DLBCL was sequenced and aligned with published EBV sequences. Clinical, laboratory and pathologic data from 16 consecutive HIV+ DLBCLs diagnosed in the United States at UNC were reviewed for comparison.

Results: 57 cases with available FFPE tissue have been reviewed at UNC, including 29 cases of DLBCL (19 HIV+, 10 HIV-), 8 cHL, 5 multicentric Castleman disease, 5 NK/T-cell neoplasms, 2 plasmablastic lymphomas, 4 high-grade B-cell lymphomas not otherwise specified, 1 BL, 2 low-grade non-Hodgkin lymphomas (NHLs), and 1 plasmacytoma. EBER-ISH was negative in 28 of 29 Malawi DLBCLs, including all 19 HIV+ cases, while 8 of 16 HIV+ DLBCL from UNC were EBV-positive by EBER-ISH (p=0.0005). EBER-ISH was positive in all cases of plasmablastic and Burkitt lymphomas in Malawi, as well as in the malignant cells of HIV+ cHL. Of 36 samples with complete transcriptome data, 22 were HIV+ on ART at lymphoma diagnosis (DLBCL: n=15, BL: n=1, cHL: n=2, PL: n=1, other: n=3), 9 of whom were diagnosed with lymphoma within 6 months of starting ART. Three were HIV+ but ART-naïve (DLBCL: n=3), and 11 were HIV- (DLBCL, including 1 case of EBV+ DLBCL of the elderly: n=9, other: n=2). Comparison of normalized aligned EBV sequence reads for cHL, EBER+/- NHLs are shown in the figure.

Conclusions: Consistent with previous studies from SSA, HIV+ lymphomas in Malawi were predominantly high-grade B-lineage lymphomas. EBV presence as assessed by EBER-ISH is less common than prior reports from HIV+ DLBCL studies in the United States. This finding is unexpected, given that SSA is enriched overall for infection-related cancers and EBV acquisition has been reported to be nearly universal during childhood. More detailed, larger studies are needed. However, our results suggest that etiologic mechanisms other than EBV (e.g., chronic immune activation) may also be important for HIV+ lymphomagenesis in SSA, particularly for DLBCL in the current ART era.



96. Virally Suppressed Cancer Patients Demonstrate Consistent HIV Evolution in Lymphoid Tissues

Rebecca Rose[1], Susanna L. Lamers[1], David J. Nolan[2], Ekaterina Maidji[3], Marco Salemi[2], Cheryl Stoddart[3], Michael S. McGrath[3]

[1]Bioinfoexperts, LLC, Thibodaux, Louisiana, USA; [2]University of Florida, Gainesville, Florida, USA; [3]University of California, San Francisco, San Francisco, California, USA

Background: While the use of antiretroviral therapy (ART) by HIV+ patients is nearly universally effective in reducing plasma viral load to undetectable levels and restoring immunity, HIV-associated cancers remain a significant co-morbidity. Current chemotherapeutic regimes are often ineffective or poorly tolerated, necessitating better treatment options, although development is hindered by the incomplete understanding of causal mechanisms. Our past research has indicated the presence of genetically and structurally distinct sub-populations of tumor-specific virus in several ART-naïve lymphoma patients, leading to the hypothesis that HIV evolves to either initiate or benefit from the tumor environment. However, whether this population structure is retained through ART is unclear. Here, we investigate HIV RNA and DNA evolution in multiple tissues obtained post mortem from two HIV+/ART+ lymphoma patients (C02, C04) and one HIV+/ART+ lung cancer patient (C05) with known dates of infection. All died with undetectable plasma viral load and had consistent ART use for at least 1 year prior to death. All three patients had at least one tumor-positive tissue included in the analysis, as confirmed by a pathologist at autopsy. Tissues were stored with the ACSR and patients gave appropriate consent.

Methods: We performed viral RNA and DNA isolations from a total of 27 post mortem tissues. The HIV *env* and *nef* genes were amplified and sequenced from all HIV positive tissues using a single-genome sequencing approach. We determined patterns of HIV evolution among tissues by inferring maximum-likelihood phylogenies and performing multiple statistical tests for viral compartmentalization. We measured the viral evolutionary rate and population genetic diversity over time in each patient using a Bayesian time-associated framework and the known date of infection. We compared these results with those from two additional HIV+/ART-naïve Kaposi sarcoma patients to directly assess the impact of ART. Finally we used an RNA signal detection technique (RNAscope) combined with histological staining to visualize the cellular location of replicating HIV.

Results: Across the three patients, we obtained viral RNA from 5 tissues and viral DNA from 17 tissues, including from the tumor lymph node, kidney, and spleen in C02. Maximum-likelihood phylogenies showed little evidence of viral compartmentalization among tissues/pathologies, confirmed by statistical testing (all $p<0.01$). Two patterns of branching were evident in all three patients: clades with long terminal branches indicative of population expansion and ongoing evolution, and clades with little genetic diversity consistent with clonal expansion. Population genetic diversity in the two patients without detectable tumor virus was constant over time, while diversity actually *increased* over time in C02, contrary to the expectation that ART *reduces* population genetic diversity. The distribution of evolutionary rates of tissue virus for the two ART+ cancer patients without detectable tumor virus overlapped those from two ART-naïve cancer patients. Surprisingly, the rate for C02 was significantly *higher* than the ART-naive and ART+ cancer patients. We used RNAscope combined with immunohistochemistry to simultaneously identify HIV+ cells in the tumor lymph node and non-tumor cerebellum from C02. CD163+/CD68+ macrophages co-localized with RNA expression of HIV *gag-pol* in lymph node, and infected cells were spatially distinct. In contrast, HIV RNA in the cerebellum appeared in clusters and was surrounded by infiltrating macrophages, consistent with clonal expansion of HIV+ cells.

Conclusions: Our results suggest that tumor tissue may offer a privileged environment for persistent HIV replication during ART, which may impact tumor growth and metastasis and further point towards potential locations for the elusive viral reservoir during ART.

97. Development of a Point-of-Care Device for Detection of Oral Pathogens and Oncogens

Andy Teng[1], Jozelyn Pablo[1], Christopher Hung[1], Douglas Molina[1], Xioawu Liang[1], Abraham Lee[2], Philip Felgner[1,2], David Camerini[1,2]

[1]Antigen Discovery Incorporated, Irvine, California, USA; [2]University of California, Irvine, Irvine, California, USA

Background: Human immunodeficiency virus (HIV), herpes simplex virus (HSV) and human papilloma virus (HPV) are three of the most significant viral pathogens that may be found in the oral cavity. HIV can be transmitted by blood present in the oral cavity and both HSV and HPV are readily transmitted by saliva or blood. HIV infection can be controlled by lifelong treatment, but causes AIDS when treatment fails or is absent. HSV infection is very prevalent; it causes painful recurrent lesions in healthy individuals with more severe symptoms in immunocompromised hosts. HPV infection is also very prevalent and is the leading cause of cervical cancer, anal cancer, and oropharyngeal cancer in the United States. We are developing a tool for rapid, cost-effective, point-of-care detection of HIV, HSV, HPV, and antibodies directed against them in saliva.

Methods: Initial development is being done with a protein microarray bearing all proteins and some protein fragments and epitopes derived from all relevant types of HIV, HSV, and HPV. Proteins and protein fragments were produced by coupled in vitro transcription and translation or, for glycoproteins, in eukaryotic cells, printed on nitrocellulose coated slides and used to assay reactivity of patient antibodies in blood and saliva. Antigen-capture will be used for direct detection of HIV, HSV, and HPV using the same format. A microfluidic device will be developed for implementation of the assay at the point of care.

Results: We can readily detect antibodies directed to viral antigens in blood and saliva of infected or previously infected individuals. The most reactive antigens of each virus will be used in further steps of development of a point-of-care device. We are currently optimizing the collection and treatment of saliva samples to enhance the sensitivity and specificity of detection.

Conclusions: We have achieved several important first steps in the development of a point-of-care device for detection of HIV, HSV, HPV, and antibodies to each virus in saliva. Future steps will include optimization, simplification, and addition of direct antigen capture assays for each virus. A microfluidic device will be used for implementation at the point of care. The end result will aid in the detection of three important viral pathogens in the oral cavity: HIV, the leading cause of fatal infection worldwide, HSV, the most prevalent cause of recurring oral lesions, and HPV, the leading cause of cervical, anal, and oropharyngeal cancers in the United States.

Acknowledgment: This project is supported by SBIR grant R43DE025440 from the NIDCR.

98. T-Cell Functional Subsets in HIV-Positive and Immunocompetent (IC) Classical Hodgkin Lymphoma (cHL): Evidence for BLIMP1 Dysregulation in EBV-Positive Cases

Amy Chadburn[1], Ethel Cesarman[1], Yifang Liu[1], Ingrid Sumpter[2], Kelly Petrowski[3], Paul Rubinstein[3]

[1]Weill Cornell, Pathology and Laboratory Medicine, New York, New York, USA; [2]Northwestern Memorial Hospital, Chicago, Illinois, USA; [3]Cook County Hospital, Hematology-Oncology, Chicago, Illinois, USA

Background: The immune microenvironment (ME), including T cell composition, and the presence of viruses impact lymphoma development and behavior. Although mechanistically not clearly defined, T cells differentiate into functional subsets with preferential transcription factor (TF) expression. BCL6 and BLIMP1 play a role in this process including directing T cells to memory (BCL6; MC) or effector (BLIMP1; EC) cells. Chronic viral infections, such as HIV, can cause EC exhaustion, poor function and altered TF expression, including high BLIMP1 levels. We studied T cells and viral status in cHL to evaluate the ME's impact on disease pathobiology.

Methods: TMAs of 31 HIV (28 EBV+; NS-14, MC-14 or NOS-2) and 40 IC (8 EBV+; NS-25; MC-13; LR-2; NOS-1) cHLs were studied for the presence of Th1 (Tbet), Th2 (GATA3), Th17 (RORgT), Treg (FOXP3), TFH (PD1/BCL6), CD4, CD8 T cells; EC (BLIMP1) and MC (BCL6) regulators, NK (CD56) cells, B cells (CD20) and macrophages (CD68) by IHC. Average percent (%) positive cells per hpf containing $\geq$2 Reed-Sternberg cells was manually determined (<5% = 0).

Results: T Cell Transcription Factor Expression in cHL Based on HIV/EBV Viral Status

Group (#pt)	T bet	GATA3	RORgT	FOXP3	PD1	BCL6	BLIMP1
IC/EBV- (32)	11%	51%	2%	8%	3%	8%	14%
IC/EBV+ (8)	19%	53%	1%	8%	7%	6%	4%
HIV+/EBV- (3)	20%	75%	0%	9%	0%	12%	18%
HIV+/EBV+ (28)	17%	35%	0%	6%	2%	7%	7%

The CD56+ (NK) and CD20+ (B) cells were similar in all groups except for fewer CD20 cells in the HIV+/EBV- group. The average The CD4/CD8 in HIV+/EBV+, HIV+/EBV- and IC/EBV+ cHLs was 0.5, 0.5, 0.8 respectively. In IC/EBV- cHL, the CD4/CD8 was higher (2.3) with less CD68+ macrophage inflitration than in other groups. In HIV+/EBV+ and IC/EBV+ cHL, BLIMP1 expression was less, correlated inversely with macrophage infiltration (p=0.07), but did not correlate with survival.

Conclusions: Although the ME CD4/CD8 ratio was less in HIV+ and/or EBV+ cHL, the functional T cell groups, based on viral status, were not significantly different except for (1) a low percentage of GATA3/Th2 cells in HIV+/EBV+ cHL, presumably lowering Th2 mediated humoral response and (2) low BLIMP1 expression in EBV+ cHL, regardless of HIV status, which was associated with a larger infiltrate of macrophages, a known poor prognostic indicator. The low expression of this T-effector cell regulator in a viral-associated neoplasm suggests the possibility of differentiation dysregulation of this cell population by a mechanism other than exhaustion, such as modulation by macrophages and/or EBV leading to nonproductively activated T cells.

AUTHOR INDEX