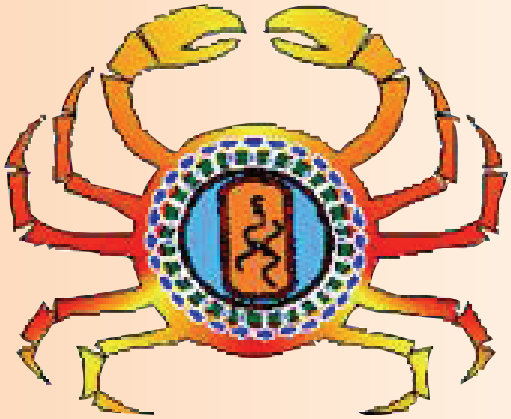
SEPTEMBER 26–27, 2005

MARRIOTT BETHESDA NORTH HOTEL & CONFERENCE CENTER, NORTH BETHESDA, MARYLAND

9th International Conference on Malignancies in AIDS and Other Acquired Immunodeficiencies: Basic, Epidemiologic and Clinical Research



Presented by the Office of International Affairs

DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health
National Cancer Institute

PROGRAM

MONDAY, SEPTEMBER 26

8:00 – 6:30 PM **Poster Presentations**

8:30 AM **Opening Remarks and Welcome**
Jodi Black, PhD, MMSc
Director Research Administration
TGen Research Institute and NCI special volunteer
Phoenix, Arizona, USA

8:35 **AIDS, Cancer and the International Arena**
Joe Harford, PhD
Director
Office of International Affairs, National Cancer Institute, NIH
Bethesda, Maryland, USA

8:45 AM **AIDS Malignancies and NCI Strategic Priorities**
Mark Clanton, MD, MPH
Deputy Director
National Cancer Institute, NIH
Bethesda, Maryland, USA

9:00 - 10:00 AM **Plenary Lectures Session 1**
AIDS and the Spectrum of Cancers in Resource Limited Settings
Moderator: *Jodi Black, PhD, MMSc*

9:00 S1 The President's Emergency Plan and Scaling up Anti-retroviral Treatment
Reuben Granich, MD, MPH
Office of the US Global AIDS Coordinator
Washington DC, USA

9:20 S2 Anti-Retroviral Therapy in India,
N. Kumarasamy, MD
Chief Medical Officer
YRG Care (Y.R. Gaitonde Centre for AIDS Research and Education)
Chennai, India.

9:40 S3 Spectrum of Cancers in AIDS in Uganda
Edward Katongole-Mbidde, MD
Uganda Cancer Institute
Kampala, Uganda

10:00 S4 Spectrum of AIDS Related Cancers in Brazil
Eduardo Netto, MD, MPH

Federal University of Bahia
Salvador de Bahia, Brazil

10:20 Break

10:40 - 11:40 AM **Plenary Lectures Session 2**
Emerging Cancers in the post-HAART Era
Moderator: *Andrew Grulich, PhD*

10:40 S5 HIV-related Malignancies in Developing Countries
Valerie Beral, MBBS
University of Oxford
Oxford, England

11:00 S6 HCV Infection as a Risk Factor for Non-Hodgkin Lymphoma
Silvia de Sanjose, PhD
Institute Català d'Oncologia
Barcelona, Spain

11:20 S7 Temporal Trends in Risk of Non-AIDS Cancers in People with AIDS (PWAs) in the HAART Era
Eric Engels, PhD
National Cancer Institute, NIH
Bethesda, Maryland, USA

11:40 AM Lunch

1:15 - 2:30 PM **Abstract Presentations Session 1**
Epidemiology of AIDS-Associated Cancers
Moderator: *Denise Whitby, PhD*

1:15 1 Immunesuppression and Cancer Risks: An Epidemiological Study in HIV-Positives and Transplant Persons in Italy and France
Diego Serraino, MD, MsC
Dipartment Epidemiologia, INMI "L Spallanzani"
Rome, Italy

1:30 2 HIV Is Not a Risk Factor for Lung Cancer in Women: Data from the Women's Interagency HIV Study (WHIS)
Alexandra Levine, MD
University of Southern California
Los Angeles, California, USA

1:45 3 Spectrum of Cancers Among HIV-Infected Persons in Africa: The Uganda AIDS-Cancer Registry Match Study
Sam Mbulaiteye, MD
National Cancer Institute, NIH

Bethesda, Maryland, USA

2:00 4 Hodgkin Lymphoma and Immunity in Persons with AIDS
Robert Biggar, MD
National Cancer Institute, NIH
Bethesda, Maryland, USA

2:15 5 Human Herpesvirus 8 in American Adolescents: Evidence for Early Infection Among Adolescents: Evidence for Early Infection Among Persons at Risk for HIV Acquisition
Corey Casper, MD, MPH
University of Washington
Seattle, Washington, USA

2:30 - 3:30 PM **Plenary Lectures Session 3**
Genetic and Epigenetic Changes and Viral Associated Cancers
Moderator: *Joel Palefsky, MD*

2:30 S8 HHV-8 Infection and Chromosome Instability
Diane Hayward, PhD
Johns Hopkins School of Medicine
Baltimore, Maryland, USA

2:50 S9 Genetic and Genomic Analysis of KSHV Infection and Pathogenesis
Shou-Jiang Gao, PhD
University of Texas Health Science Center at San Antonio
San Antonio, Texas, USA

3:10 S10 The Role of HPV DNA Methylation in Cervical and Anal Neoplasia
Hans-Ulrich Bernard, PhD
University of California, Irvine
Irvine, California, USA

3:30 PM Break

3:50 - 4:50 PM **Abstract Presentations Session 2**
Advances in Clinical and Molecular HPV Research
Moderator: *Elizabeth Read-Connole, PhD*

3:50 6 AMC 035: A Phase I/II Trial of SGN-0101 in the Treatment of High-Grade Anal Intraepithelial Neoplasia (AIN) in HIV-Positive Individuals
Joel Palefsky, MD
University of California San Francisco
San Francisco, California, USA

4:05 7 Lack of Concordance Between Oral and Genital Human Papillomavirus (HPV) in Women's Interagency HIV Study (WHIS) Cohort

		Carole Fakhry, MD *Johns Hopkins School of Medicine* *Baltimore, Maryland, USA*
4:20	8	Papillomavirus Infection Requires Furin Cleavage on the Viral Capsid Protein, L2 *Patricia Day, PhD* *National Cancer Institute, NIH* *Bethesda, Maryland, USA*
4:35 – 6:30 PM		Adjourn to posters
7:00 PM		Dinner

TUESDAY, SEPTEMBER 27

8:00 AM – 6:00 PM **Poster Presentations**

8:40 AM **Introductory Remarks**
Elliott Kieff, MD, PhD
Harvard Medical School
Boston, Massachusetts, USA

8:45 - 9:45 AM **Plenary Presentations Session 4**
Immune Evasion
Moderator: *Cliona Rooney, PhD, Kevin Howcroft, PhD*

8:45 S11 Regulation of the Immune Response in Hodgkin's Lymphoma and Hodgkin's Disease
Sibrandes Poppema, MD, PhD
University Medical Center Groningen
Groningen, The Netherlands

9:05 S12 The Influence of HLA Class I on HIV-1 Disease Progression
Mary Carrington, PhD
SAIC Frederick, National Cancer Institute, NIH
Bethesda, Maryland, USA

9:25 S13 Interferon Regulatory Factors, Oncogenesis and Immune Evasion
Joseph Pagano, MD
University of North Carolina at Chapel Hill
North Carolina, North Carolina, USA

9:45 S14 Hepatitis C Virus in the HIV Infected Patient
Andrea Cox, MD
Johns Hopkins University
Baltimore, Maryland, USA

10:05 AM Break

10:30 - 11:30 AM **ROUNDTABLE: CONTROVERSIES IN THE MANAGEMENT OF AIDS LYMPHOMA**

- Role of Rituximab in Combination with Chemotherapy
  - **R1.** AIDS Malignancy Consortium Experience
    The Role of Rituximab in HIV-Associated Lymphoma: The AIDS Malignancies Consortium Experience
    Lawrence Kaplan, MD
    University of California San Francisco
    San Francisco, California, USA

- **R2.** Italian Experience
  Rituximab Plus Cyclophosphamide, Doxorubicin and Etoposide (R-CDE) in HIV-NHL: Pooled Results from Three Phase II trials
  Michele Spina, MD
  National Cancer Institute
  Aviano, Italy

- **R3.** Appropriate Timing of HAART when Used Together with Chemotherapy
  Use of Highly Active Antiretroviral Therapy (HAART) and Chemotherapy (CT) for AIDS-Related Lymphoma (ARL): Concomitant or Sequential?
  Richard Little, MD
  National Cancer Institute, NIH
  Bethesda, Maryland, USA

- **R4.** AIDS-Lymphoma: Prognostic Factors for Survival in the Era of HAART
  Alexandra Levine, MD
  University of Southern California School of Medicine
  Los Angeles, California, USA

- Roundtable Discussion

11:30 AM - 12:30 **Abstract Presentations Session 3**
Lymphoma Related Therapy
Moderator: *Richard F. Ambinder, MD, PhD*

11:30 9 In Vivo Imaging of Primary Effusion Lymphomas in Mice, and Effective Treatment by Inhibition of NF-κB
Ethel Cesarman, MD, PhD
Cornell University
New York, New York, USA

11:45 10 Targeted Killing of KSHV-Infected PEL Cells with Ganciclovir and Zidovudine Via Hypoxic Activation of ORF 21 and ORF 36
David Davis, PhD
National Cancer Institute, NIH
Bethesda, Maryland, USA

12:00 11 Activation of LMP1- and LMP2-Specific T-Cells for the Immunotherapy of EBV Positive Malignancies With an Adenoviral Vector Encoding Full Length LMP1 and LMP2
Stephen Gottschalk, MD
Baylor College of Medicine
Houston, Texas, USA

12:15 12 Tymic Output After Autologous Bone Marrow Transplantation (ASCT) HIV-Related Lymphoma
Michele Spina, MD
National Cancer Institute
Aviano Italy

12:30 PM Lunch

1:30 - 2:30 PM **Plenary Presentations Session 5**
Treatment: Small Molecule Inhibitors
Moderator: *T-C Wu, MD, PhD*

1:30 S15 Inhibition of Human Papillomavirus DNA Replication by Small Molecule Antagonists of the E1-E2 Protein Interaction
Jacques Archambault, PhD
Institut de recherches cliniques de Montreal,
Montreal, Québec, Canada

1:50 S16 Inhibitors of EBNA-1 of EBV
Elliott Kieff, MD, PhD
Harvard Medical School
Boston, Massachusetts, USA

2:10 - 3:10 PM **Abstract Presentations Session 4**
KSHV Molecular Biology
Moderator: *Douglas Lowy, MD*

2:10 13 Regulation of KSHV Latent Gene Expression
Dirk Dittmer, PhD
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina, USA

2:25 14 KSHV Fusion/Entry Receptor: Functional cDNA Cloning of a Specific Amino Acid Transporter
Jonathan Kaleeba, PhD
National Institute of Allergy and Infectious Diseases, NIH
Bethesda, Maryland, USA

2:40 15 Long-Term Infected Telomerase-Immortalized Endothelial Cells: A Tool to Study KSHV Latency In Vitro and In Vivo
Rolf Renne, PhD
University of Florida
Gainesville, Florida, USA

2:55 16 Latent KSHV Infection Converts Primary Vascular Endothelial Cells From a Functional Metabolic Vessel Wall Phenotype Into an Angiogenic Phenotype

Gary S. Hayward, PhD
Johns Hopkins University
Baltimore, Maryland, USA

3:10 PM Break

3:30 - 4:30 PM **Plenary Presentations Session 6**
Translational investigations
Moderator: *Kishor Bhatia, PhD*

3:30 S17 Cytokines and Primary Effusion Lymphoma
Giovanna Tosato, MD
National Cancer Institute, NIH
Bethesda, Maryland, USA

3:50 S18 Cytokines and Development of AIDS Related Lymphoma (Longitudinal MACS Data)
Otoniel Martinez-Maza, PhD
UCLA School of Medicine
Los Angeles, California, USA

4:10 - 4:55 PM **Abstract Presentations Session 5**
Viral Associated Carcinogenesis
Moderator: *Robert Yarchoan, MD*

4:10 17 Cell and Animal Model of KSHV-Induced Kaposi's Sarcoma
Enrique A. Mesri, PhD
University of Miami
Miami, Florida, USA

4:25 18 Etiopathogenetic Mechanisms of Conjunctival Squamous Cell Carcinoma Epidemic Uganda
Luigi Buonaguro, MD
National Cancer Institute, "Fond Pascale"
Naples, Italy

4:40 19 Cervical Stem Cells: Isolation, Characterization, and Potential Role in Human Papillomavirus (HPV)-Induced Cervical Carcinogenesis
Astrid Baege, MD
National Cancer Institute, NIH
Bethesda, Maryland, USA

4:55 – 6:00 PM Adjourn to posters

Continuing Medical Education

The National Institutes of Health/Foundation for Advanced Education in the Sciences (NIH/FAES) is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

The NIH/FAES designates this educational activity for a maximum of 13 category 1 credits toward the AMA Physician's Recognition Award. Each physician should claim only those credits that he/she actually spent in the activity.

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National Institutes of Health
National Cancer Institute

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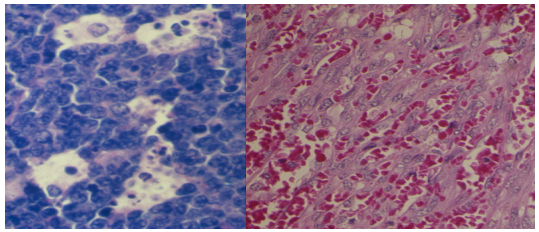


AIDS Lymphoma **AIDS Kaposi's Sarcoma**

The AIDS and Cancer Specimen Resource contains more than 100,000 individual specimens and associated clinical information.

Examples of fresh, frozen, or fixed specimens include:

- Kaposi's sarcoma
- Non-Hodgkin's lymphoma
- Hodgkin's lymphoma
- Genito-urinary system dysplasia
- Non-HIV malignancy controls

Linkages with repositories of:

- Women's Interagency HIV Study (WIHS)
- Multicenter AIDS Cohort Study (MACS)
- National NeuroAIDS Tissue Consortium (NNTC)
- Specialized international collections

ACSR Specimen Use

ELISA Immunologic Studies

Epidemiology Studies

Flow Cytometry

Gene Micro-Array

Immunohistochemistry

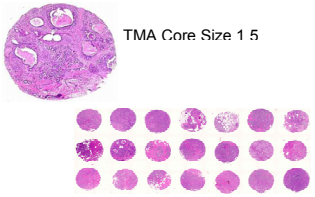
Quantitative Proteomics

Viral Genetics

http://acsr.ucsf.edu

ACSR Advanced Technologies

The ACSR offers tissue micro-arrays (TMA)



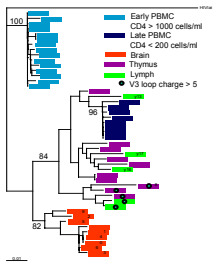
- Hundreds of tissue samples can be assembled into a single TMA
- Opportunity for examination of large numbers of tumors
- HIV infected tissues and related malignancies along with non-HIV related controls

Tissue Resource/Informatics Interface

HIVbase® *ACSResource*

Software designed to store, link and manipulate many HIV data types.

- Biological data integration
- Modeling of biological systems from tissues to genes
- High-speed data analysis
- Examine HIV sequence variation in tissues and cancers
- Easily provide for large-scale sequence analysis studies
- Eliminate repetitious HIV sequence editing



S1

THE PRESIDENT'S EMERGENCY PLAN AND SCALING UP ANTI-RETROVIRAL TREATMENT

Reuben Granich, MD, MPH, Office of the US Global AIDS Coordinator, Washington DC

According to the World Health Organization, only 50,000 of the 4.1 million sub-Saharan Africans who could benefit from anti-retroviral drugs were receiving them in 2002. In 2003 the President's Emergency Plan for AIDS Relief was launched. The Plan represents the largest international health initiative in history dedicated to a single disease undertaken by a government. The Emergency Plan is a five-year, $15 billion, multifaceted approach to combating HIV/AIDS. In addition to intensive programs in15 focus countries, the Plan includes efforts in more than 100 countries around the world and support for multilateral organizations such as the Global Fund to Fight AIDS, Tuberculosis and Malaria. Emergency Plan goals include supporting treatment for 2 million people infected with HIV/AIDS, preventing 7 million new infections, and supporting care for 10 million people infected and affected by HIV/AIDS.

The Emergency Plan is implemented through a coordinated inter-agency effort involving the Department of State, Department of Health and Human Services, United States Agency for International Development, Department of Defense, Department of Commerce, Department of Labor, and Peace Corps. The Emergency Plan is committed to supporting national HIV/AIDS strategies and is focused on capacity building. U.S. government field staff work closely with international and national partners to implement each host nation's vision for fighting HIV/AIDS. With Emergency Plan support, host nations are providing services while at the same time building local, sustainable capacity that is required to build long-term national response to HIV/AIDS. Over 80% of Emergency Plan partners are indigenous organizations.

By September 30, 2004, the Emergency Plan supported care (e.g., treatment of opportunistic infections) for more than 1,727,000 adults and children. Of those receiving care during the first 9 months, 630,000 were orphans and vulnerable children. As of March 31, 2005, the President's Emergency Plan has supported anti-retroviral treatment for more than 235,000 men, women, and children through programs in 15 of the most afflicted countries in Africa, Asia, and the Caribbean. More than 230,000 of those receiving ARVs live in sub-Saharan Africa. These numbers exceeded the goal set forth in January 2004, to support treatment for more than 200,000 people by June 2005. This represents important progress towards scaling-up to meet the Emergency Plan's goal of supporting treatment for two million people in five years.

Emergency Plan support for capacity development includes the training of clinical staff, laboratory personnel, and counselors. Additionally, the Emergency Plan supports the improvement of physical infrastructure including clinics, laboratories, and the distribution, logistics and management systems for drugs and other commodities. In fiscal year 2004, the Emergency Plan supported training for more than 312,000 service providers in 15 focus countries including: 202,600 trained in prevention services, 24,600 health workers trained in services working to prevent mother-to-child HIV transmission, 14,100 trained to provide counseling and testing, 36,700 trained to care for people living with HIV/AIDS, 22,600 trained to care for orphans and vulnerable children and 12,200 health workers trained to provide anti-retroviral treatment.

The Emergency Plan is committed to an integrated HIV/AIDS prevention, treatment and care program. Realization of Emergency Plan objectives will require increased collaborative efforts to meet the tremendous need for support.

S2

ANTIRETROVIRAL THERAPY IN INDIA

N.Kumarasamy., MBBS.,PhD, Chief Medical Officer
YRG Centre for AIDS Research and Education, VHS
Chennai 600113 India

Currently it is estimated that there are 5.13 million persons living with HIV in India. Majority of HIV transmission in India is through heterosexual route. Transmission through sharing of needles is reported from North East India. Tuberculosis is the most common opportunistic infection in India. More than 80% of the persons who are diagnosed with HIV have one or more opportunistic infections. Antiretroviral drugs were first introduced in India by the generic manufacturers in the year 1994. Combination fixed dose antiretroviral drugs were launched in 1999. Studies conducted in India using generic ARVs showed that these drugs are safe, tolerable, efficacious and effective. Cost and stigma are the major cofactors for poor adherence to ARVs in India. During 2003-2004, the cost of these generic ARVs drastically came down which lead to increased access to ARVs in India and in other developing countries. Recent studies have shown the generic HAART has made a huge impact on changing the natural history of HIV disease in India and also has lead to dramatic reduction in HIV related mortality. Adverse events due to Immune reconstitution syndrome are a major issue among persons who are co-infected with Tuberculosis and initiating on HAART. ARV resistance strains are reported from ARV naive population recently in India. Due to increased numbers of HIV infected persons needing HAART and also due to increased access to ARVs, immediate efforts should be made to train physicians on antiretroviral therapeutics.

S4

SPECTRUM OF AIDS RELATED CANCERS IN BRAZIL

Eduardo M Netto [1,2], Estela M Luz[2], Carlos Brites [1,2] & William Harrington [3].1 - Universidade Federal da Bahia, Brazil; 2 - Fundação Bahiana de Infectologia, Salvador – Bahia- Brazil; 3 - University of Miami, Florida

In Brazil, the AIDS epidemic is starting to plateau although there remain a high number of new cases per year. HAART therapy and the preventive measures have cut the estimated number of cases in a half. The impact of these strategies is seen in the overall trend of AIDS malignancies, the incidence of Kaposi's sarcoma decreased from 11.4% ((1980-88) to 2.1% (1998-1999). The access to antiretroviral therapy and OI prophylaxis may partially explain these results. HHV-8 has a relatively low seroprevalence (1-4.1%) among healthy children and young adults but was highly prevalent in men who have sex with men (32.6%) and AIDS patients with KS (98.7%). In the last 6 years a surveillance system of viral associated malignancies was implemented in Bahia, a state populated principally by descendants of West Africans. The most important known diseases (KS, NHL [including ATLL], Cervical Cancer, and other lymphoproliferative conditions) associated with viruses are being surveyed. Overall, up to May 2005, 2240 cases were enrolled in the databank. 52% of the patients enrolled were with Cervical Cancer (1161), of the 169 tested for HIV, 5 were positive (3%). The second group more frequent report was the NHL, excluding ATLL, with 763 patients, of the 118 tested for HIV, 32 (27%) were positive, this percentage is obvious biased. NHL were younger with extranodal involvement, morphology of large cells B phenotype and high cellular proliferation among the HIV infected patients. 0/43 ATLL and 44/44 KS had HIV infection. The pathogenesis of lymphomas diagnosed in Northeastern Brazil differs from those seen in Europe and the US as they much more associated with oncogenic herpes viruses, 87%-93% associated with EBV of Burkitt Lymphomas from Bahia, associated with low long-term survival (39%). The majority of HIV related lymphomas are also associated with EBV, although this is likely to vary according to region.

S5

HIV-RELATED MALIGNANCIES IN DEVELOPING COUNTRIES

Valerie Beral[1], Harold Jaffe[2] on behalf of the International Collaboration on HIV and Cancer.
Cancer Research UK Epidemiology Unit[1] and Department of Public Health,[2] Old Road Campus, University of Oxford, Oxford OX4, UK.

Background: The International Collaboration on HIV and Cancer was set up in 1998, to bring together worldwide data on the risk of specific cancers in HIV-infected individuals, from both developed and developing countries.

Results: The risk of the AIDS-defining cancers was increased in HIV-infected people in both settings, but for each cancer type the relative risk was lower in developing than in developed countries. The respective relative risks (RR) and 99%CI in developing and developed countries were: 29.6(15.7-56.0) and 150(146.3-154.5) for Kaposi's sarcoma; 5.1(3.3-7.7) and 65.5(63.2-68.0) for non Hodgkin's lymphoma; and 2.3(1.7-3.0) and 7.5(5.9-9.6) for cervical cancer. The risk of certain non-AIDS-defining cancers was also increased in HIV-infected people in both settings: cancer of the oral cavity (RR and 99%CI: 3.3, 1.7-6.3; and 2.1, 1.5-2.9, respectively); vulval cancer (RR and 99%CI: 3.4, 1.6-7.1; and 14.2, 7.4-27.0, respectively); cancer of the eye - largely conjunctival cancer (RR and 99%CI 4.9, 2.1-11.8; and 2.7, 1.0-7.8, respectively). There was no increase in the risk of cancer of the oesophagus, stomach, colo-rectum, breast, ovary or prostate in either setting.

Conclusion: Overall, HIV-infected people are at an increased risk of a small number of specific cancer types, and the cancers are similar in developing and developed countries. However, the relative risks in HIV-infected people tend to be lower in developing than developed countries and the reason for this is not clear.

S6

HCV INFECTION AS A RISK FACTOR FOR NON-HODGKIN LYMPHOMA

Silvia de Sanjose, Catalan Institute of Oncology, Barcelona, Spain

Hepatitis C virus is a well-established risk factor in the etiology of liver cancer and of mixed cryoglobulinemia type II., a condition that can evolve to malignant lymphoma in 8-10% of affected cases. HCV has also been suggested to play a role in the etiology of malignant lymphoma not related to cryoglobulinemia. Data from case-case comparisons and case-control studies indicate several fold higher prevalence of HCV infection among B-cell lymphoma patients compared to control populations. Data from prospective studies are more limited and show contradictory observations: Prospective studies in Japan, France and in Sweden have shown an increased risk for non Hodgkin's lymphoma among anti-HCV carriers while follow up of hemophilic patients has not identified an increased risk for lymphomas associated with HCV, either with or without human immunodeficiency virus (HIV) co-infection. Further, HCV persistent infection has been associated to posttransplantation lymphomas in some series but not others. While methodological issues may be responsible for these inconsistencies, recent data on HCV intervention support that HCV may be a risk factor inducing lymphomagenesis. Several case reports from different settings have identified an association between marginal zone lymphomas and HCV and have shown that effective viral treatment may, in some cases, lead to tumor regression.

The mechanism of how HCV could induce lymphomagenesis are unclear but virological data suggest that HCV may interfere in the host immuneresponse by increasing the hypermutation activity of the immunoglobulin gene of the B lymphocytes leading to activations of certain protooncogens or tumor suppressor genes. The ability of HCV to infect B-cells is under evaluation.

The objective of this presentation is to summarize the epidemiological data on the association between hepatitis C and lymphomagenesis.

S7

TEMPORAL TRENDS IN RISK OF NON-AIDS CANCERS IN PEOPLE WITH AIDS (PWAs) IN THE HAART ERA

E.A. Engels, R.M. Pfeiffer, J..J. Goedert, R.J. Biggar. Division of Cancer Epidemiology and Genetics, National Cancer Institute.

Background: Availability of HAART since 1996 has decreased AIDS morbidity and prolonged survival. PWAs are at heightened risk for non-AIDS cancers due to the high prevalence of other risk factors (e.g., tobacco, hepatitis C virus). Data are lacking on the changing incidence of non-AIDS cancers as PWAs live longer.

Methods: The AIDS Cancer Match Registry study linked AIDS and cancer registries in 11 U.S. regions. To measure cancer risk in PWAs, we calculated standardized incidence ratios (SIRs) for the 2-year period after AIDS onset. We used Poisson regression to compare SIRs for AIDS onset in 1990-1995 (90-95) vs. 1996-2002 (96-02, HAART era) and examine linear SIR trends over time.

Results: Among 296,546 PWAs in 1990-2002, 1405 developed non-AIDS cancers (SIR 1.8 in 90-96, 1.7 in 96-02). Risk was high for several cancers, notably Hodgkin lymphoma (HL, n=149, SIR=10.1), and cancers of anus (n=96, SIR=20.2), liver (n=47, SIR=3.6), lung (n=344, SIR=3.1), and kidney (n=40, SIR=1.5), most of which were renal cell carcinomas (RCC, n=30, SIR=1.5). Only 3 non-AIDS cancers showed changes over time. SIR for HL was higher in 96-02 than 90-95 (13.6 vs. 8.1), due to an increase over 1990-2002 (relative risk [RR] 1.09, 95%CI 1.04-1.13 per yr). The SIR for RCC increased (1.9 in 96-02 vs. 1.3 in 90-95), because of a steep rise within 96-02 (RR 1.54, 1.09-2.18 per yr). Lung cancer risk decreased from 90-95 to 96-02 (SIR 3.3 vs. 2.6), due to a steady decline over 1990-2002 (RR 0.95, 0.92-0.99 per yr).

Conclusions: For most non-AIDS cancers, we observed no change in risk attributable to the introduction of HAART. Risk was high for lung cancer, the most common malignancy, but appeared to decline over time relative to the general population. The rising risks for HL and RCC are unexplained, but for HL could reflect the effects of partial immune restoration from antiretroviral therapy.

S8

REPROGRAMMING OF CELL GENE EXPRESSION BY KSHV LANA

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The KSHV latency associated nuclear antigen LANA is expressed in the tumor cells of Kaposi's sarcoma, primary effusion lymphoma and Castleman's disease. LANA is a multifunctional protein being essential for maintenance of KSHV episomal genomes during latency and affecting cell growth and gene expression in ways that are likely to contribute to the genesis of KSHV associated malignancies. LANA induces expression of -catenin target genes by binding to the serine/threonine kinase GSK-3and causing nuclear accumulation of GSK-3. We find that LANA is also a substrate for GSK-3 and that phosphorylation of LANA regulates the LANA-GSK-3 interaction. However, LANA bound GSK-3 appears to be sequestered. Thus LANA may further modify transcriptional activity by reducing phosphorylation of the normal nuclear targets of GSK-3. LANA also mediates transcriptional repression. We provide evidence that LANA acts in part by recruiting the DNA methyltransferase Dnmt3A to chromatin to facilitate repression of growth regulatory genes.

S9

GENETIC AND GENOMIC ANALYSIS OF KSHV INFECTION AND PATHOGENESIS

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Kaposi's sarcoma-associated herpesvirus (KSHV) infection is necessary for the development of Kaposi's sarcoma, an angiogenic vascular spindle cell tumor of endothelial cells commonly found in patients with AIDS. Although many KSHV genes have been examined extensively in vitro, their functions in KSHV infection and replication remain unclear. The lack of an efficient infection system and a genetic manipulation system has tremendously impeded the advancements of the KSHV field in the last decade. We have recently developed an efficient bacteria-mammalian shuttle system for KSHV genetic manipulation by cloning the full-length KSHV genome as a bacteria artificial chromosome (BAC). Simultaneously, we have established a highly efficient KSHV infection system of primary human umbilical vein endothelial cells (HUVEC). The developments of these systems have enabled us to employ comprehensive genetic, genomic, molecular, cellular, and biochemical approaches to define the molecular basis of the sophisticated interplays of virus-cell interactions. To this end, we have shown that efficient KSHV infection of HUVEC is productive at the early stage of infection but the virus switches to latency at the later stage of infection. Unsurprisingly, KSHV infection modulates multiple cellular pathways, including MAPK pathways, to facilitate its initial entry and infection of cells. Significantly, KSHV infection suppresses the overall host transcriptional program but selectively activates cellular genes to promote cell growth and cell cycle progression, enhance cell adhesion and invasiveness, and induce inflammatory and angiogenic cytokines. Importantly, we have shown that KSHV infection induces chromosome instability, which could predispose the infected cells to transformation. Using the genetic approach, we have identified viral genes that are essential for KSHV infection and replication. Together, these results have contributed to the delineation of viral genes and cellular pathways that are essential for KSHV infection and pathogenesis.

S10

THE ROLE OF HPV DNA METHYLATION IN CERVICAL AND ANAL NEOPLASIA

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Human papillomavirus-16 (HPV-16) and related viruses infect mucosal epithelia of the male and female anogenital tract and are the primary cause of malignancies such as carcinoma of the cervix. The lifetime incidence rate of HPV infection is likely close to 100%, while the incidence rate of developing HPV associated anogenital cancer is by about two orders of magnitude lower, suggesting additional cofactors and mechanisms that determine the etiological outcome of an HPV infection. A declining immunity due to human immunodeficiency virus (HIV) infection increases the risk to develop intra-anal malignancies, pointing toward a successful immune response against HPVs in healthy individuals. However, long-term persistence of HPVs suggests yet other mechanisms that determine the clinical outcome.

DNA methylation at CpG dinucleotides is known to be part of an epigenetic mechanism to repress transcription. We have found that HPVs are efficiently targeted by DNA methylation, apparently as consequence of two independent mechanisms. HPV DNA is methylated after infection of the basal layers of epithelial cells, possibly as a cellular defense mechanism, and the escape from this methylated state is a prerequisite for oncogene expression and cellular transformation. This mechanism may underlie long-term subclinical infection with HPVs.

In the course of malignant progression, HPV DNA normally recombines with cellular DNA, a well-known trigger for methylation of any DNA. While this mechanism can lead to methylation and therefore repression of the majority of the HPV genomes in a transformed cell, one or some few genome copies must escape this mechanism to maintain the tumorigenic state. An example is the cervical cancer cell line CaSki, which expresses the HPV oncogenes from one of 500 HPV genomes, all other genomes being heavily methylated. Therefore, and seeming in contradiction to the role of methylation as a repressive mechanism, HPV DNA methylation may serve as a diagnostic criterion to identify progressing cells.

Ongoing research addresses the mechanism of the initial DNA methylation, which may lead to long-term subclinical persistence of the viruses, and the power of using the secondary methylation as prognostic biomarker.

S11

REGULATION OF THE IMMUNE RESPONSE IN HODGKIN'S LYMPHOMA

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Classical HL is characterized by the presence of RS cells, which are transformed post germinal center B cells destined for apoptosis since they have not undergone successful immunoglobulin gene rearrangement. Several mechanisms, including latent infection by EB virus allow these cells to survive. A key role is played by the presence of constitutive nuclear NF-κB, which is induced by LMP1, as well as by CD30, CD40, TNFα and Notch1 interactions, and results in the upregulation of at least 45 genes including chemokines, cytokines, receptors, apoptotic regulators, intracellular signalling molecules and transcription factors. The other characteristic of classical HL is the presence of an extensive inflammatory infiltrate. Key features of this infiltrate are that it comprises Th2 and T regulatory cells and generally lacks Th1 cells, CD8 cytotoxic T cells and natural killer cells. The RS cells appear to induce this infiltrate by the secretion of Th2 type chemokines like TARC and MDC. The RS cells also produce cytokines that inhibit Th1 responses, like IL-10 and TGFβand express CD95 ligand that induces apoptosis of activated Th1 and CD8 T cells. Other important mechanisms that allow the RS cells to escape an effective anti EB virus immune response include the downregulation of HLA class I in EBV negative cases, or the presence of a polymorphism in HLA class I in EBV positive cases that allow escape from CD8 mediated cytotoxicity. On the other hand, expression of HLA G allows the escape from natural killer cells which would normally recognize the HLA class I negative RS cells. Overall, the cellular infiltrate in HL appears to play a decisive role in allowing the RS cells to survive by creating an environment that suppresses cytotoxic immune responses and by providing cellular interactions and cytokines that support the growth and survival of RS cells. Future therapeutical strategies could focus directly on the NF-kB activation, on various receptors to ligand interactions, on the chemokine and cytokine network or on the induction of effective anti EBV latent protein immune responses.

S12

THE INFLUENCE OF HLA CLASS I ON HIV-1 DISEASE PROGRESSION

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HLA class I molecules present antigenic epitopes to T lymphocytes, thereby initiating a specific immune response. The genes encoding HLA class I molecules are highly polymorphic and this polymorphism is thought to have arisen through natural selection by infectious diseases. The clinical course and outcome of HIV-1 infection are highly variable among individuals, depending on a combination of viral, host, and environmental determinants. Genetic resistance to HIV-1 involves a complex array of host genetic effects involving variants that have subtle, but significant consequences on gene expression or protein function. Of the multiple genetic effects that appear to influence HIV-1 disease progression, among the strongest are the HLA class I loci. The effects of HLA on outcome to HIV-1 exposure have been studied more thoroughly than that pertaining to any other infectious disease. Specific alleles at the HLA-B locus and zygosity at the three classical class I loci have been shown to associate significantly with altered rates of disease progression. Using a linear mixed effect model we have recently also demonstrated the relative effects of HLA-A, -B and –C on disease progression, increase in viral load over time and CD4 decline over time. In addition, we show that three distinct *HLA* alleles known to alter the overall rate of AIDS progression act during distinct intervals following HIV-1 infection. The discrete timing of *HLA* allele influence suggests alternative functional mechanisms in immune defense against this dynamic and chronic immunosuppressive disease.

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S13

INTERFERON REGULATORY FACTORS, ONCOGENESIS AND IMMUNE EVASION

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Interferon Regulatory Factors (IRFs) mediate the effects of interferons (IFNs) in regulating innate and adaptive immune responses as well as cell growth, differentiation and survival. IRF3 and 7 are essential for transcription of IFNα/β. IRF7 is the master regulator of Type I IFN responses. Its expression is induced by IFNα/β, LPS, TNFα/β, and virus infection. Toll-like receptor signaling, DNA-damaging agents and the EBV oncoprotein LMP1 induce and activate IRF7. IRF7 also regulates EBV latency. With IRF2 it represses transcription of the EBNA1 gene in Type III latency and favors long term persistence of infection. IRF7 is activated by phosphorylation by the IKK-related kinases, IKKε & TBK1. Recently the roles of RIP and Lys-63-linked ubiquitination in the activation of IRF7 by LMP-1 have been discovered. IRF-7 may contribute to EBV oncogenesis through complex regulatory transcriptional circuits whereby the factor augments expression of LMP-1. Biologically these interactions are mirrored by the potentiating effects of IRF7, which itself has oncogenic properties, on the oncogenic activity of LMP-1 in model systems. Activated IRF-7 is consistently expressed in EBV-positive CNS lymphomas. Finally LMP-1 activates the Tap-2 promoter via IRF-7 during latent EBV infection. In cytolytic infection the activity of IRF-7 in immune responses is inhibited by the EBV BZLF-1 immediate-early gene product, which may account for the absence of T-helper and mucosal Langerhans cell responses in hairy leukoplakia of the tongue. Thus IRF-7 is a multifunctional protein whose central role in IFN responses, LMP-1 signalling and oncogenesis and in immune responses to viral infection and other stimuli are continuing to emerge.

S14
HEPATITIS C VIRUS IN THE HIV INFECTED PATIENT

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In the United States, approximately 25% of all HIV infected patients are also infected with hepatitis C virus, and the prevalence of HCV in HIV infected patients can be nearly 90% in those who have acquired HIV via injection drug use. The impact of HCV infection on the course of HIV remains controversial, but HIV so adversely affects those infected with HCV that HCV has been labeled an opportunistic infection by the US Public Health Service. HIV infection has been shown to increase the proportion of HCV infected persons who develop liver failure, shorten the interval of time over which cirrhosis occurs, and increase the risk of death from liver disease. In some centers, HCV-related liver disease has become the leading cause of death among HIV-infected persons.

Hepatitis C virus (HCV) is the major cause of hepatocellular carcinoma (HCC) in the United States, Europe, Japan, Australia, and many other parts of the world. Estimates from the year 2000 indicate that liver cancer remains the fifth most common malignancy in men and the eighth in women worldwide, and it is becoming more common. The incidence of HCC has doubled over the last two decades in the United States. Between 1992-2002 in the US, HCC was second only to thyroid cancer for the largest increase in incidence and was responsible for the largest increase in cancer death rates. The increase in rates of HCC has been attributed to earlier increases in HCV infection rates and the HIV pandemic has contributed to the increase in deaths from liver cancer. HCV prevention and therapy are therefore important mechanisms of HCC prevention in HIV infected patients.

The diversity of HCV presents a barrier to development of an effective HCV vaccine. A virus capable of genetic variation and of causing chronic infection will evolve to optimize its fitness in each host, a process that is the net sum of immune recognition and functional constraints on replication. We recently defined an HCV consensus sequence, for which each amino acid is that most commonly found at that position, and demonstrated that many immune responses generated against the consensus sequence are cross-reactive with the HCV strains circulating in HCV infected patients. Because the consensus sequence is composed of the amino acid most commonly observed at each position, it likely represents the most fit state of the virus. Thus, effective evasion of the immune response by selection of a sequence divergent from consensus may result in a less fit virus from a replicative standpoint. We have recently demonstrated that immune pressure drives mutation of the virus away from the consensus sequence and toward a potentially less fit strain, suggesting that immune responses to consensus sequences (rather than a product based on a single host's sequence) will establish the highest barrier to viral escape and consequently the most effective protection against chronic infection.

S15

INHIBITION OF HUMAN PAPILLOMAVIRUS DNA REPLICATION BY SMALL MOLECULE ANTAGONISTS OF THE E1-E2 PROTEIN INTERACTION.

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Human papillomavirus (HPV) DNA replication is initiated by the assembly of the viral E1 and E2 proteins at the viral origin of replication. E1 is the replicative helicase of HPV. E2 is a DNA binding protein that binds with high affinity to specific sequences within the origin and also interacts with E1 to recruit it specifically to the origin. Small molecule inhibitors of the E1-E2 protein-protein interaction were identified by high-throughput screening of a large compound collection with an assay measuring the cooperative binding of E1 and E2 to origin DNA. Isothermal titration calorimetry and changes in protein fluorescence showed that the inhibitors bind to the transactivation domain (TAD) of E2, the region that interacts with E1. Crystal structures of both the HPV11 TAD and of a complex between this domain and an inhibitor were obtained at 2.5- and 2.4 Å resolution, respectively. Inhibitor binding caused no significant alteration of the protein backbone, but movement of several amino acid side chains at the binding site, in particular those of Tyr-19, His-32, Leu-94, and Glu-100, resulted in the formation of a deep hydrophobic pocket that accommodates the indandione moiety of the inhibitor. Further mutational analysis of E2 provided functional evidence for specific interactions between Tyr-19 and E1 and between His-32 and the inhibitor. This class of inhibitors was found to antagonize specifically the E1-E2 interaction in vivo and to inhibit HPV DNA replication in transiently transfected cells. These results highlight the potential of the E1-E2 interaction as a small molecule antiviral target for the treatment of HPV infections.

S16

INHIBITORS OF EBNA-1 OF EBV

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A chemical inhibitor of EBNA1 dependent transcription inhibits EBNA1 binding to cognate DNA. Myung-Soo Kang, Hyun Jin Kwun, and Elliott Kieff Departments of Medicine and Microbiology and Molecular Genetics, Channing Laboratory, Brigham and Women's Hospital and Harvard University, 181 Longwood Avenue, Boston MA, 02115

Epstein Barr Virus (EBV) infection is prevalent in all human populations and is an important cause of cancers, particularly in immune compromised individuals. EBV DNA persistence in latently infected dividing cells requires EBV encoded EBNA1, which dimerizes and binds to tandem EBNA1 binding sites in the EBV genome. A high throughput assay with cells that have both EBNA1 dependent and independent reporters was used to screen $>4X10^4$ compounds for specific inhibition of the EBNA1 dependent reporter. Ten compounds that specifically inhibited EBNA1 reporter at 10uM levels and had no significant affect on the EBNA1 independent reporter at 100uM were identified. Cpd H inhibited an EBNA1 gel shift with cognate DNA, at 5-10uM level. Cpd H also inhibited EBNA1 dependent expression from a plasmid transfected into EBNA1 expressing cells. Further, **C**hromatin **I**mmune **P**recipitation revealed that Cpd H treatment of cells inhibited EBNA1 association with cognate DNA. Also, Cpd H stopped EBV episome dependent infected lymphoblastoid cell line (LCL) growth, but had no effect on an LCL with integrated EBV DNA.

S17

CYTOKINES AND PRIMARY EFFUSION LYMPHOMA

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Primary Effusion Lymphoma (PEL) is a rare form of anaplastic or large-cell lymphoma characterized by liquid growth in the body cavities, often without a solid mass. The malignant cells are of B-cell lineage, as they express clonal Ig gene rearrangements, but have predominantly a non-B and non-T cell phenotype. The cells are believed to originate from post-germinal center cells with a plasma cell-like phenotype based upon expression of CD138 and IRF4, and display a gene expression profile linked to inflammation and invasion. PEL cells are latently infected with KSHV, often in association with EBV, and occur predominantly in HIV-1 infected individuals. In vitro, PEL cell growth is dependent, in part, upon autocrine growth factors, including cellulatr IL-10 and viral (v)IL-6. Culture supernatants of PEL cells contain cellular IL-6 and VEGF-A, but PEL cells are not dependent upon these these growth factors for growth and survival. PEL effusions from AIDS patients often contain measurable levels of IL-10, IL-6, vIL-6 and VEGF-A. In a murine model of PEL, in which human PEL cells grow progressively in the peritoneal cavity of most immnunodeficient mice, we found that neutralizing antibodies directed at human VEGF-A consistently prevented the development of ascites in 100% of animals, and prevented the dissemination of PEL cells in most animals, whereas control antibodies were ineffective. PEL cells express a constitutively active form of STAT3, at least in part attributable to autocrine growth stimulation by IL-10 and vIL-6. Transduction of dominant-negative STAT3 and pharmacologicat STAT3 inhibition caused caspase-dependent cell death, and induced transcriptional repression of the inhibitor of apoptosis survivin. Forced over-expression of survivin in PEL cells rescued them from apoptosis induced by STAT3 inhibition. These results suggest that activated STAT3 signaling directly contributes to malignant progression of PEL by preventing apoptosis, acting through the pro-survival protein survivin. Targeting STAT3 activation and survivin may provide a new therapeutic approach for the treatment of PEL.

S18

CYTOKINES AND DEVELOPMENT OF AIDS RELATED LYMPHOMA

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The aim of this study was to determine if elevated serum levels of B cell-stimulatory cytokines and/or molecules associated with B cell activation precede AIDS-related lymphoma (ARL). In a nested case-control study, we assessed pre-ARL serum levels of several molecules that are associated with B cell activation (IL6, IL10, soluble CD23 [sCD23], sCD27, sCD30, C'-reactive protein [CRP], and total IgE), utilizing samples from participants in the Multicenter AIDS Cohort Study (MACS). The expression of these molecules preceding ARL was determined in 181 HIV+ cases and in an equal number of lymphoma-free HIV+ controls, matched to cases on the actual or putative length of infection with HIV. Serum levels of these molecules were assessed in longitudinal samples collected at three time points prior to lymphoma diagnosis (3-5 years, 1-3 years, and <1 year pre-ARL diagnosis) in cases and equivalent time points in controls. Significantly elevated mean serum levels of sCD27, sCD30, IL6 and CRP were seen at all three time points preceding ARL. When assessed as the paired difference between cases and matched controls at each time point, levels of these markers also were significantly elevated in those who developed ARL. IgE was not seen to be significantly elevated in cases. A greater proportion of those who developed ARL had detectable, and therefore, elevated serum IL10 pre-diagnosis, when compared to controls, although this was seen only at the most ARL diagnosis-proximal time point. In contrast, significantly elevated mean serum sCD23 was elevated in those who developed ARL, but only at the study visits 3-5 years and 1-3 years pre-ARL diagnosis. These results indicate that significantly elevated levels of several B cell activation-associated molecules precede the diagnosis of ARL, in some cases by several years.

R1

THE ROLE OF RITUXIMAB IN HIV-ASSOCIATED LYMPHOMA: THE AIDS MALIGNANCIES CONSORTIUM EXPERIENCE

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The addition of rituximab to cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy results in significant improvement in clinical outcome for individuals with non-HIV-associated aggressive B-cell lymphoma. In order to assess the potential risks and benefits of this agent administered with concurrent chemotherapy in HIV-infected individuals with aggressive B-cell lymphoma (HIV-NHL) 150 patients receiving CHOP chemotherapy for HIV-NHL were randomized (2:1) to receive 375mg/m^2 rituximab with each chemotherapy cycle (n=99) or no immunotherapy (n=50) in a multicenter phase III trial. The primary endpoint was complete response rate. Secondary endpoints included time to progression, progression-free survival (PFS), overall survival (OS) and treatment-related toxicity. The complete response rate (CR + CRu) was 57.6% for R-CHOP and 47% for CHOP (p=.147). With a median followup of 137 weeks, time to progression, progression-free and overall survival times were 125, 45, and 139 weeks respectively for R-CHOP and 85, 38 and 110 weeks respectively for CHOP (p=ns, all comparisons). Treatment-related infectious deaths occurred in 14% of patients receiving R-CHOP compared with 2% in the chemotherapy-alone group (p=.035). Of these deaths, 60% were in patients with baseline CD4 < 50/mm^3. PFS was significantly influenced by CD4+ count (p< .001) and IPI score (p=022), but not bcl-2 status. The addition of rituximab to CHOP chemotherapy in patients with AIDS lymphoma may be associated with improved tumor responses. However, these benefits may be offset by an increase in infectious deaths, particularly in those individuals with CD4+ lymphocyte counts < 50/mm^3.

R2

RITUXIMAB PLUS CYCLOPHOSPHAMIDE, DOXORUBICIN AND ETOPOSIDE (R-CDE) IN HIV-NHL: POOLED RESULTS FROM THREE PHASE II TRIALS

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Evidence suggests that infusional therapy is a more effective means for administering cytotoxic therapy than intravenous bolus therapy for lymphoma, and offers greater potential for therapeutic synergy with rituximab, which has a long half-life.

We pooled the results of three prospective phase II trials evaluating rituximab in combination with a 96 hour infusion of cyclophosphamide (187.5-200 mg/m^2/day), doxorubicin (12.5 mg/m^2/day) and etoposide (60 mg/m^2/day) (called "R-CDE") plus granulocyte colony-stimulating factor (G-CSF) in 74 patients with human immunodeficiency (HIV)-associated B-cell non-Hodgkin's lymphoma, of whom 56 patients (76%) received concurrent highly active antiretroviral therapy (HAART).

The complete remission rate was 70% (95% confidence intervals [CI] 59%-81%), and the estimated 2-year failure-free survival and overall survival were 59% (95% CI 47%-71%) and 64% (95% CI 52%-76%), respectively. Ten patients (14%) had an opportunistic infection during or within three months from the end of R-CDE, and 17 (23%) developed non-opportunistic infections. There were six deaths related to infection (8%), including two from bacterial sepsis during R-CDE (3%), and four opportunistic infections that occurred between 2-8 months after completion of R-CDE (5%).

R-CDE produced a 70% CR rate and 59% 2-year failure free survival in patients with HIV-associated lymphoma. Consistent with other reports, addition of rituximab to cytotoxic therapy in this population may increase the risk of life-threatening infection. Further studies evaluating rituximab in combination with infusional chemotherapy are warranted, but need to proceed cautiously.

R3

USE OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) AND CHEMOTHERAPY (CT) FOR AIDS-RELATED LYMPHOMA (ARL): CONCOMITANT OR SEQUENTIAL?"

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ARL prognosis has improved substantially in the HAART era. Pre-HAART, death from opportunistic infections diminished the role of lymphoma prognostic features recognized in the non-AIDS setting. In the HAART era, evidence suggests event-free survival (EFS) is more related to lymphoma-specific factors. It follows that lymphoma subtype and treatment approach may now be more important to outcome. Standard-dose conventional and dose-adjusted infusional CT approaches are now replacing low-dose approaches in ARL. No randomized trials specifically address the question of concomitant or sequential HAART. Clinical studies support the feasibility of both approaches. Observations in favor of combined therapy include that fewer CT dose reductions appear necessary; and that stable HAART adherence with low HIV load is feasible during CT and predictive of longer EFS. Favorable outcome can also be seen if HAART is delayed until completion of CT. (Sparano JA. Curr Opin Oncol 2003). Concerns against combined therapy include potential overlapping toxicity and untoward pharmacokinetic interactions. These have not been substantiated in most clinical trials, though rigorous evaluation is problematic (Scadden DT. Blood 2003). Also, CT-induced CD4 depletion may negate HAART immune protection. Pre-HAART, ARL prognosis was correlated with distinct CD4-related clinicopathologic disease patterns. While HAART may have an influence on this during lymphomagenesis, it is unlikely to have a significant effect on subsequent tumor biology (Yarchoan et al. Nat Clin Pract Onc 2005). There is no compelling data to suggest an advantage to stopping a stable non-myelotoxic HAART regimen at CT initiation. Likewise, delaying HAART until completion of CT appears feasible and may be rational for some patients, especially those not already on HAART.

R4

AIDS-LYMPHOMA: PROGNOSTIC FACTORS FOR SURVIVAL IN THE ERA OF HAART

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In the era prior to the availability of HAART, treatment of patients with AIDS lymphoma resulted in overall survival times of only 6 to 8 months. Prognostic factors for survival included age >35 years, poor performance status, stage III/IV disease, elevated LDH, CD4 cells <100, history of clinical AIDS, and injection drug use. Pathologic type was not a significant factor, and pts with Burkitt (BL) or diffuse large cell (DLCL) fared similarly. Survival is significantly longer for ARL patients in the era of HAART, and prognostic factors for survival may have changed. We evaluated 363 pts with ARL, including 262 in the pre-HAART and 101 in the HAART eras, all of whom were treated with curative intent. After receipt of standard or low dose CHOP or mBACOD, significant improvement in OS was seen for patients with DLCL in the HAART era (8.3 mos versus 43.2 mos, p=0.0003), while no difference was found for pts with BL (6.4 vs 5.7 mos) [JCO 23:4430-38,2005]. In analyzing prognostic factors for survival in pre-HAART and HAART eras among 192 pts with DLCL, both HIV related (CD4 <100) and NHL related factors (No attainment of CR, and increasing IPI score) were statistically related to survival in the pre-HAART era on multivariate analysis. However, in the HAART era, only lymphoma related factors were statistically predictive of survival (No CR=Hazard Ratio 7.6 (3.1-18.5), p<0.001; and increasing IPI score= HR 1.6 (1.1-2.3, p=0.007), while CD4 count was no longer significant. We conclude: (1) Prognostic factors for survival have changed with the advent of HAART; (2) Pts with BL do poorly with standard CHOP or mBACOD in the HAART era and should receive alternative therapy; (3) In pts with DLCL treated with HAART, prognostic factors for survival are similar to those in de novo DLCL.

1

IMMUNESUPPRESSION AND CANCER RISKS: AN EPIDEMIOLOGICAL STUDY IN HIV-POSITIVES AND IN TRANSPLANT PERSONS IN ITALY AND FRANCE

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Introduction: It is well known that individuals with immune deficiencies area at higher risk of cancer than age- and sex-matched persons in the general population, particularly of virus-related cancers. The comparison of cancers that occur in excess in population groups like HIV-infected and transplanted persons may help to better elucidate the relationship between immune surveillance, viral infections and cancer occurrence. **Methods:** A multi-cohort longitudinal study was conducted in Italy and France, including 2002 HIV-infected persons from Italy with known dates of seroconversion, 6072 HIV-infected persons from France and 2755 Italian recipients of solid organ transplants (1844 kidney transplants, 702 heart transplants, 159 liver transplants and 50 lung transplants). Sex- and age-standardized incidence ratios (SIR) and 95% confidence intervals (CI) were computed to quantify the risk of cancer of these persons, as compared to the general population of France and Italy. Among HIV-infected individuals, the risk of cancer was also assessed according to treatment with highly active antiretroviral therapies (HAART). **Results:** The SIRs for all cancers were 9.5 (95% CI:8.7-10.2) for HIV-infected persons and 2.2 (95% CI: 1.9-2.5) for transplants. In both groups, virus-related cancers accounted for the vast increase in cancer risk. In both groups, most of the excess risk was attributable to virus-related cancers, like KS (SIR=451 in HIV-positives, 128 in transplants), NHL (SIR= 62.1 and 10.5, respectively), liver cancer (SIR= 9.4 and 4.3, respectively). Significantly increased SIRs for anal and cervical cancers and Hodgkin's lymphoma (HL) were found only among HIV-positive individuals. In HIV-infected persons, HAART treatment drastically reduced SIRs for Kaposi's sarcoma (KS) (SIR=548 in never treated and SIR=120 in ever treated), non-Hodgkin's lymphoma (NHL) (SIR=72 in never treated and SIR=35 in ever treated), but it did not affect SIRs for anal, liver and cervical cancers and HL (SIR=11.1 in never treated and SIR=9.4 in ever treated). Significantly increased SIRs were observed for lung cancer in non-HAART treated HIV-positive patients (2.4, 95% CI: 1.0-5.0) and in transplants (SIR=1.6, 95% CI: 1.1-2.3). **Conclusions:** HIV-infected persons and in recipients of organ transplant have a similar pattern of cancer risk, which is largely due to virus-related cancers. Interestingly, only for some of these cancers HAART treatment seems to play a protective effect and, in these cases, HAART treated patients have excess risks similar to transplant patients.

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HIV IS NOT A RISK FACTOR FOR LUNG CANCER IN WOMEN: DATA FROM THE WOMEN'S INTERAGENCY HIV STUDY (WIHS)

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Background: An increased incidence of lung cancer (LC) has been reported in HIV-infected individuals. Nonetheless, prior studies have not always included control groups, nor considered other risk factors, such as smoking. In an attempt to ascertain the effect of HIV infection, and of HAART on the incidence of LC, we studied 3,378 women (2521 HIV-infected and 857 HIV-uninfected at risk) from the Women's Interagency HIV Study (WIHS), followed for up to 9 years. **Methods:** Lung cancers were identified by matching to state cancer registries and by verifying self reports through medical record abstraction. LC incidence rates (IR) were calculated from the number of incident LCs in HIV positive and negative women, divided by the number of person years (PYs) of follow-up. Standardized incidence ratios (SIRs) were calculated to compare the observed LC incidence among the WIHS participants to that among women in the USA using the population based SEER cancer registry. Lastly, we compared the prevalence of selected behavioral characteristics in WIHS versus women in the USA by adjusting the population based cross sectional survey data from NHANES III to the age and race distributions of the WIHS. **Results:** Nine cases of incident lung cancer were diagnosed in HIV-positive women, versus 2 in the HIV negative comparators; rates did not differ between HIV positive and negative women (Rate ratio=0.8 [95% CI 0.1, 3.6]; p=0.75). Likewise, among HIV positive women we observed similar LC incidence rates in the pre-HAART (68.4/100,000 PYs) and HAART eras (68.8/100,000 PYs) (RR=1.0, 95% CI= 0.02-7.4) Overall, the lung cancer SIR was not different in HIV-positive versus negative women (16.5 [95% CI=7.6, 29.0] versus 15.2, [95% CI=1.8, 42.4]; exact p=1.0). All WIHS women with lung cancer had a history of smoking, and compared to US women (NHANES III), WIHS women were statistically more likely to have smoked ($p<0.0001$). Further, the risk of lung cancer in the WIHS increased by smoking history, in a dose dependent manner (p=0.02 for trend). **Conclusion:** We conclude that HIV infection, per se, is not a risk factor for lung cancer; the association appears to be related, instead, to tobacco exposure. Smoking cessation programs are likely to increase survival of these individuals over time.

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SPECTRUM OF CANCERS AMONG HIV-INFECTED PERSONS IN AFRICA: THE UGANDA AIDS-CANCER REGISTRY MATCH STUDY

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Introduction: More than 25 million people in sub-Saharan Africa have human immune deficiency virus (HIV) infection, but little is known of their risk of cancer. We investigated cancer risk among persons with HIV/AIDS in Uganda. Methods: Cancer incidence among 12,607 HIV-infected persons registered by The AIDS Support Organization (TASO), in Kyadondo County from October 1988 through December 2002, was evaluated through record-linkage to the Kampala Cancer Registry. Standardized incidence ratios (SIRs) were calculated to identify increased cancer risks in the early (4-27 months after TASO registration), late (28-60 months), or combined (4-60 months) incidence periods. Results: We linked 378 cancers (181 prevalent, 197 incident) to TASO participants. Of the incident cancers, 137 (70%) were AIDS-defining cancers. Compared to the general population, risks for AIDS-defining cancers were increased in the early-incident period: Kaposi sarcoma (SIR 6.4, 95%CI 4.8-8.4), non-Hodgkin lymphoma (6.7, 1.8-17), and cervical carcinoma (2.4, 1.1-4.4). Risks for these three cancers were also increased in the combined periods. Risks of five non-AIDS-defining cancers were increased in the combined periods: Hodgkin lymphoma (5.7, 1.2-17) and cancers of the conjunctiva (SIR 4.0; 1.5-8.7), kidney (16, 1.8-58), thyroid (5.7, 1.1-16), and uterus (5.5, 1.5-14). Cancers of the breast, nasopharynx, and lung were increased either in the early or late incident periods only. Seven cancers were observed among 407 children, including five cases of Kaposi sarcoma. Conclusions: We confirm the increased risks of AIDS-defining cancers and report increased risks of a few non-AIDS-defining cancers in sub-Saharan Africa. Our study demonstrates that record-linkage studies are feasible in Africa, where the HIV epidemic remains considerable.

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HODGKIN LYMPHOMA AND IMMUNITY IN PERSONS WITH AIDS

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Objective: To examine the impact of immunity on the risk of Hodgkin lymphoma (HL).
Background: HL risk is increased 5-15-fold in persons with AIDS (PWA), and the incidence has increased despite better antiretroviral therapy in recent years. In PWA, the mixed cellular (MC) subtype is more common than the nodular sclerosing/lymphocyte predominance (NS/LP) subtype. We speculated that HL histology would be affected by immunity and change the subtype distribution. Additionally, if HL was not diagnosed as such, incidence would decline with low CD4 counts.

Methods: We examined data from matching nationwide AIDS and cancer registry data through 2002. Incident rates were obtained 4-27 months after AIDS onset (477,368 person-years (py) of prospective follow-up). Standardized incidence ratios (SIR) were age-, sex-, race- and calendar-time-adjusted. Immunity was assessed by CD4 cell counts (from1990-2002) reported within -6 to +3 months of AIDS onset.

Results: 173 HL cases occurred (36.2/105, SIR: 9.5), of which 119 (69%) had CD4 count data. The SIR in 1996-2002 (13.2) was elevated significantly. HL incidence decreased as CD4 count declined (ptrend= 0.008). In PWA with 150-199 and >200 CD4/uL, the incidences were 55.1/105 py and 46.8/105 py, respectively, whereas in PWA with <50 CD4/uL, it was 21.0/105 py. By subgroup, NS/LP incidence declined more rapidly than the MC incidence, with no NS/LP cases observed in the PWA with <50 CD4/uL.

Conclusion: In PWA, HL subtype distribution changed and incidence decreased steadily as CD4 counts decreased, supporting our hypothesis that HL histology is influence by immune response. Severe immunosuppression could prevent the occurrence of HL, but this seems unlikely. Instead, it may be being diagnosed as another condition (probably NHL) or it may be undiagnosed. The rising HL incidence in PWA is attributable to better CD4 counts in recent years.

5

HUMAN HERPESVIRUS 8 IN AMERICAN ADOLESCENTS: EVIDENCE FOR EARLY INFECTION AMONG PERSONS AT RISK FOR HIV ACQUISITION

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Background: Human herpesvirus 8 (HHV-8) infection is common among children and adolescents in areas where Kaposi sarcoma (KS) is endemic. In the United States, HHV-8 is a prevalent sexually-acquired infection among persons at elevated risk for HIV infection, including adult men who have sex with men (MSM) and women with a history of multiple sex partners or intravenous drug use. Infectious HHV-8 virions are frequently shed in saliva. We hypothesized that HHV-8 is acquired shortly after sexual debut, due to its ubiquity in communities at risk for HIV infection and the possibility of transmission through saliva. We therefore investigated the prevalence and predictors of HHV-8 infection among adolescents with or at high-risk for acquiring HIV.

Methods: 537 adolescents from the REACH cohort were followed with detailed questionnaires and laboratory testing. Sera were assessed for the presence of antibodies to HHV-8.

Results: 60 of 537 (11%) adolescents were HHV-8 seropositive, including 20% of MSM, 6% of male heterosexual youths and 9% of females. HHV-8 infection was not significantly more common among HIV-infected adolescents, and no characteristic predicted HHV-8 infection among MSM. Among women, recent infection with gonorrhea (odds ratio (OR) 2.8, 95% confidence interval (95% CI) 1.4-5.7, p=0.002), history of sex with other women (OR 2.4, 95% CI 1.1-5.3, p=0.026), and African American race (OR 3.4, 95% CI 1.1-10.0, p=0.028) were all associated with an elevated risk of HHV-8.

Conclusions: HHV-8 infection is common among American teenagers at risk for HIV infection, but its prevalence is lower than that of similar adults. Gender, race and sexual behavior may influence the risk of infection with HHV-8

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AMC 035: A PHASE I/II TRIAL OF SGN-00101 IN THE TREATMENT OF HIGH-GRADE ANAL INTRAEPITHELIAL NEOPLASIA (AIN) IN HIV-POSITIVE INDIVIDUALS

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Background: Effective treatment of anal intraepithelial neoplasia (AIN) 2-3 may prevent progression to invasive anal cancer but there are few proven treatment options for AIN 2-3. AMC 035 was a recently completed phase I clinical trial of a therapeutic vaccine, SGN-00101 (Hsp-E7), for AIN 2-3 in HIV+ individuals.

Methods: HIV+ patients with biopsy-proven AIN 2-3, on HAART with CD4+ ≥ 200 x 10^6/L and HIV viral load (VL) ≤ 500 copies/ml were sequentially assigned to Cohort 1 (100 mcg SGN-00101), Cohort 2 (500 mcg) or Cohort 3 (1000 mcg). Five subjects in each cohort received 3 doses injected subcutaneously every 4 weeks. Anal cytology, high resolution anoscopy and biopsy were used to monitor changes in AIN status at 8, 12, 24 and 48 weeks. HIV VL and CD4+ level were measured at baseline, 12 and 24 weeks. Complete response (CR) was defined as regression to normal and partial response (PR) as regression to AIN 1.

Results: The mean age of subjects was 47.5 years, 87% were male and 93% were Caucasian. 5/15 subjects had HPV16 infection at baseline and all had at least one oncogenic HPV type. No significant changes in HIV VL or CD4+ counts were detected. CD8+ counts were increased at 24 weeks (p=.02). The most common adverse event was injection site reaction, in all subjects, with one subject having grade 3 toxicity and the others having grade 1-2 toxicity. There were no drug-related serious adverse events. At 48 weeks, subjects showed histologic improvement (PR in 2/5 subjects in Cohorts 1 and 2, CR in 1/5 subjects in Cohort 3).

Conclusions: SGN-00101 was well tolerated in HIV+ individuals with preliminary evidence for histological activity.

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LACK OF CONCORDANCE BETWEEN ORAL AND GENITAL HUMAN PAPILLOMAVIRUS (HPV) IN WOMEN'S INTERAGENCY HIV STUDY (WIHS) COHORT

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Background and Significance: Human Papillomavirus (HPV), a well established cause of anogenital squamous cell cancers, has recently been implicated as an etiologic agent for oropharyngeal cancer. HIV seropositive individuals are at increased risk for oropharyngeal cancer and other HPV associated malignancies. In anticipation of a potential rise in incident oropharyngeal cancers among HIV-infected individuals in the era of HAART, the prevalence, predictors and patterns of oral HPV infection and their relationship with cervical HPV infection were studied in the WIHS cohort. **Methods:** 182 HIV-positive and 88 HIV-negative women participating in WIHS provided questionnaire, cervical vaginal lavage and oral rinse. DNA purification was performed by Puregene (Minneapolis, MN). HPV genomic DNA was detected by PGMY09/11 L1 consensus primer PCR and type specified by reverse line blot hybridization for 38 HPV types and *B*-globin (Roche Molecular Systems, Alameda, CA). Differences in prevalence were tested using a t-test of proportions. To determine the predictors of oral and cervical HPV infection, multivariate logistic regression was used. Concordance was measured by kappa statistic. **Results:** HIV-positive patients were more likely to have an oral HPV infection than HIV-negative patients (23%, [95% CI 16.4-29.5] vs 8.4%, [95% CI 2.3-14.5]), were more likely to be infected by a high-risk type (13.7%, [95% CI 8.3-19.0] vs [3.6%, 95% CI 0-7.7]) and to have multiple concurrent oral HPV infections (8.1%, [95% CI 4.4-13.5] vs 1.2%, [95%CI 0-6.5]). Similar differences were observed with cervical HPV infection, although prevalence at that site was higher: 75.8% (95%CI 69.0-82.6) in HIV-positive vs 44.4% (95% CI 33.4-55.5) in HIV-negative. HPV type frequency distributions were similar at oral and cervical sites. Women with a cervical HPV infection were more likely to have an oral HPV infection (25.2% vs 7.6%, p= 0.01). Risk of oral HPV infection increased with increasing number of cervical infections (P_{trend}<0.0001), and this trend was independent of HIV serostatus and CD4 count. There was poor overall and type-specific concordance between oral and cervical HPV infection, Kappa range (0-0.29). In an exploratory analysis, factors which appear to be associated with increased risk of oral HPV infection in HIV positive subjects are CD4<200, history of sexually transmitted infection, recent male oral sex partner and cervical HPV infection. In contrast, predictors of oral HPV infection in HIV negative patients were recent female sex partners, cervical HPV infection and alcohol use. **Conclusion:** HIV positive patients are at increased risk for oral HPV infection. Type distributions of oral HPV and cervical HPV are similar, yet prevalence of oral infection is lower and poorly concordant with cervical HPV infection.

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PAPILLOMAVIRUS INFECTION REQUIRES FURIN CLEAVAGE OF THE VIRAL CAPSID PROTEIN, L2

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Although papillomaviruses enter cells via an endocytic pathway, many of the later events in the infectious process remain unclear. We have recently determined that uncoating of papillomaviruses occurs in endosomes, and L2 and the viral genome subsequently leave this compartment and traffic into the nucleus. To test the possibility that cell-encoded proteases might participate in this process, we examined the ability of protease inhibitors to interfere with infection by HPV16 and BPV-1 pseudoviruses. Both pseudoviruses were potently inhibited by a specific inhibitor of furin, a proprotein convertase that is present in the trans-Golgi network, at the cell surface, and within endosomes. Furin inhibition did not impair virus adsorption or endocytic trafficking. A requirement for furin was established by determining that the pseudoviruses were unable to infect a Chinese hamster ovary (CHO) cell line in which the furin gene had been disrupted, while a companion CHO line in which furin had been reintroduced was readily infected. Inspection of the amino acid sequence of L1 and L2 revealed that a consensus RXXR furin cleavage site near the N-terminus of L2 is conserved among all papillomaviruses. Mutation of this site abrogated infectivity.
We conclude that papillomavirus infection requires furin cleavage of the L2 protein. Although furin has been implicated in the production of infectious virions for some enveloped viruses, this is the first demonstration, for any virus, that furin has an essential role during the process of infection.

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IN VIVO IMAGING OF PRIMARY EFFUSION LYMPHOMAS IN MICE, AND EFFECTIVE TREATMENT BY INHIBITION OF NF-KB.

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Activated NF-κB is a critical mechanism by which lymphoma cells infected by KSHV are protected from apoptotic stress. Using a mouse xenograft model of PEL, we have demonstrated that Bay 11-7082, a selective pharmacological inhibitor of NF-κB, prevents or delays tumor growth and prolongs disease-free survival. To document NF-κB inhibition in vivo, and to establish a better in vivo model of PEL, we have made a traceable PEL cell line, derived from BC3, that expresses luciferase under the control of NF-kB. These cells have relatively high basal luciferase activity and a dose and time-dependent suppression of luciferase upon treatment with Bay11. BC3-NFkB-Luc cells were injected intraperitoneally into NOD-SCID mice and PEL development was monitored at different time points by in vivo imaging (IVIS). Treatment of these mice with Bay 11 (three injections of 5 mg/kg at days 3, 5 and 7 post-tumor inoculation) resulted in NF-κB inhibition and tumor regression. While most mice receiving vehicle alone had visible tumors from the day of inoculation onwards, and developed ascites within 20 days, all mice treated with Bay 11 showed disappearance of the luciferase signal and were free of tumor for more than 30 days after inoculation. Our results support the use of selective NF-κB inhibitors in the treatment of KSHV-associated lymphomas.

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TARGETED KILLING OF KSHV-INFECTED PEL CELLS WITH GANCICLOVIR AND ZIDOVUDINE VIA HYPOXIC ACTIVATION OF ORF21 AND ORF36

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Primary effusion lymphoma (PEL) is a rare B-cell lymphoma caused by Kaposi's sarcoma-associated herpesvirus (KSHV) that arises in the pleural space and other body cavities. PEL is poorly responsive to cytotoxic chemotherapy and carries a poor prognosis; new therapies are needed. KSHV encodes for two lytic genes that can phosphorylate certain antiviral drugs to toxic moieties: ORF21 (a thymidine kinase) can phosphorylate zidovudine (AZT) or ganciclovir (GCV), while ORF 36 (a phosphotransferase) can phosphorylate GCV. Pleural effusions are a hypoxic environment, and we have shown that hypoxia can activate KSHV. We hypothesized that this process could be used to target PEL. We found that ORF21 and ORF36 are upregulated in PEL cells exposed to hypoxia (1% O_2) as assessed by Northern blot. Using a new RPHPLC/MS method, we found that 293T cells transfected with ORF21 and treated with 25μM AZT or 500μM GCV produced 432 pMoles AZT-triphosphate (AZTTP) /10^6 cells or 27.5 pMoles ganciclovir-triphosphate (GCVTP)/10^6 cells, respectively. Similarly, 293T cells transfected with ORF36 produced 74.7 pMoles of GCVTP, but no AZTTP. In normoxia, PEL cells produced 18.0 pMole/10^6 cells of GCVTP. This increased 2.8 fold to 50 picomoles in hypoxia and 3.6 fold to 65 pMole with TPA. Treatment of PEL cells with 200 μM GCV killed less than 20% of the cells but this was increased to 35% in hypoxia. Although 10 μM AZT killed less than 5% of the cells in hypoxia, the combination of AZT and GCV resulted in >60% cell killing. Under the same conditions, little or no killing of the herpesvirus-negative B cell lines Ramos or CA46 was observed. This data suggests that treatment with GCV and AZT may be able to specifically target PEL cells and therefore be useful in the development of an effective multi-agent therapy.

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ACTIVATION OF LMP1- AND LMP2-SPECIFIC T-CELLS FOR THE IMMUNOTHERAPY OF EBV POSITIVE MALIGNANCIES WITH AN ADENOVIRAL VECTOR ENCODING FULL LENGTH LMP1 AND LMP2

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Background: LMP1 and LMP2 are potential targets for the immunotherapy of EBV-positive malignancies. We have shown in the past that LMP1- and LMP2-specific T-cells can be activated from EBV-seropositive individuals with adenoviral vectors encoding either an inactive form of LMP1 (dLMP1) or LMP2. The aim of this study was to construct and characterize an adenoviral vector containing dLMP1 and LMP2 (Ad_dLMP1-I-LMP2) for the generation of LMP1- and LMP2-specific T cells.

Materials/Methods: Blood samples from 10 HLA-A*2, EBV-seropositive individuals were screened with a new method for the activation of antigen-specific T-cells that takes advantage of the tropism of Ad5F35 adenoviral vectors, which preferentially transduce monocytes after incubation of peripheral blood mononuclear cells (PBMC).

Results: Stimulation of PBMC with Ad-dLMP1-I-LMP2 transduced monocytes resulted in an at least 30 – 100 fold expansion of LMP1- or LMP2-specific T-cells as judged by the presence of HLA-A*2 restricted, tetramer positive T-cells. LMP2-specific responses were detected in all donors where as LMP1-specific responses were found in 7/10 donors. The presence of LMP1- and LMP2-specific T-cells was confirmed in a subset of donors with Elispot assays. In addition, generated CTL killed autologous fibroblasts expressing LMP1 and LMP2 as well as EBV transformed B cells.

Conclusion: LMP1- and LMP2-specific T-cells can be activated in EBV-seropositive individuals with Ad_dLMP1-I-LMP2. The constructed adenoviral vector is a promising candidate for the *ex vivo* and *in vivo* activation of LMP1- and LMP2-specific CTL.

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TYMIC OUTPUT AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION (ASCT) HIV-RELATED LYMPHOMA

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Background: One of the major concern for ASCT in HIV+ patients (pts) is that post-transplant immunosuppression might worsen the immunosuppression and enhance the HIV replication. Therefore we analyzed whether the immune system of HIV+ pts might support an immune recovery similar to the HIV- pts who underwent the same ASCT program.

Methods: The kinetics and the extent of immunereconstitution were assessed by measuring: TCR excision circles (TRECs), CD4, CD8, CD56, CD4, CD45RA, CD19 cells, and HIV pro and viraemia.

Results: 15 HIV+ and 7 HIV- pts with relapsed DBCL underwent ASCT. Before the induction therapy TRECs/10^6 PBMC mean value was 962±2183 and 2948+5485 in HIV+ and HIV- pts respectively. The nadir was reached before ASCT (150±11 vs 927±1842). At one year the mean value of TRECs/10^6 PBMC returned to the baseline in HIV- pts, while in HIV+ pts largely overcame the baseline (2440±2799). Before the induction therapy, HIV+ pts showed a significant lower CD4 (174±110 vs 386±200) CD56 (86±129 vs 144±92) and CD4/CD8 ratio in comparison with HIV- pts. CD4 count nadir was reached during aplastic period in both groups. No differences were present in the dynamics of CD4, CD45RA, CD8 and CD56 recovery between the two groups. At one year CD4 count returned to the baseline value in HIV- pts while in HIV+ pts overcame 70% the baseline value. Before ASCT, all HIV+ pts were on HAART and HIV-viraemia was <50cp/ml in 10/15; 6 pts discontinued HAART, but HIV viraemia and proviraemia did not increase significantly during the overall observational period.

Conclusions: The dynamics of the immunereconstitution was similar in HIV+ and HIV- pts, but the tymic output unexpectedly seemed to be enhanced in HIV+ pts.

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REGULATION OF KSHV LATENT GENE EXPRESSION

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Primary effusion lymphoma (PEL) and Kaposi's sarcoma are closely associated with the lymphotropic gamma herpesvirus Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8). By profiling viral transcription genome-wide we have defined an extended latency locus in KSHV that encompasses essential latent genes, putative oncogenes and the viral microRNAs. The regulation of each of these mRNAs is dependent on a 1200 bp cis regulatory element that exhibits B-cell specificity in transgenic mice. We will present a working model of KSHV latent gene regulation, based upon transcriptional profiling in primary KS lesions, as well as mouse and tissue culture models of KS tumorigenesis.

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KSHV FUSION/ENTRY RECEPTOR: FUNCTIONAL cDNA CLONING OF A SPECIFIC AMINO ACID TRANSPORTER

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Kaposi's sarcoma herpesvirus (KSHV, HHV-8) infection is linked to development of Kaposi's sarcoma (KS) and peripheral effusion lymphomas, particularly in immunocompromised individuals. Whereas mechanisms of KSHV infection remain poorly defined, limited data based on current models of herpesvirus entry indicate a requirement for initial attachment to surface lectins, heparan sulfate, or integrins, followed by specific interactions with a fusion receptor with/out a cofactor. In vitro, efficient KSHV-induced cell fusion with presumably receptor-positive target cells requires KSHV glycoproteins B (gB), gH, gL and the attachment glycoprotein K8.1A, suggesting that fusion-dependent entry of KSHV follows the general paradigm of other human herpesviruses. To identify a host cell receptor responsible the fusogenic activity mediated by KSHV glycoproteins, we performed functional selection of a recombinant vaccinia virus-encoded cDNA library, based on activation of a novel tripartite reporter gene upon cell fusion. We isolated a cDNA (which we designate Mel-10) encoding a known 12 TM amino acid transport protein. When expressed in a non-susceptible target cell background, Mel-10 conferred strong permissiveness for KSHV glycoprotein-mediated cell fusion and virion entry. With naturally permissive target cells, anti-Mel-10 antisera blocked both KSHV glycoprotein-mediated cell fusion and virion entry. Endogenous Mel-10 expression on various target cells correlated with permissiveness for fusion and entry. Mel-10 therefore fulfills the essential criteria of a functional fusion/entry receptor for KSHV, and is presumably involved in KSHV infection and/or dissemination *in vivo*. Moreover, reported changes in Mel-10 regulation under certain physiological states known to be altered during HIV infection provide exciting new frameworks for studying KSHV-associated diseases and their epidemiologic relationships with AIDS.

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LONG-TERM INFECTED TELOMERASE-IMMORTALIZED ENDOTHELIAL CELLS: A TOOL TO STUDY KSHV LATENCY IN VITRO AND IN VIVO

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Kaposi's sarcoma-associated herpesvirus (KSHV) is associated with KS, primary effusion lymphoma (PEL), and multicentric Castleman's disease. Most KS tumor cells are latently infected with KSHV and of endothelial origin. While PEL-derived cell lines maintain KSHV indefinitely, KS tumor-derived cells to date lost viral genomes upon ex vivo cultivation. To study KSHV latency and tumorigenesis in endothelial cells, we generated telomerase-immortalized human umbilical vein endothelial cells, termed TIVE. TIVE cells express all KSHV latent genes 48 hours post infection and productive lytic replication could be induced by Rta/orf50. In accordance with Grundhoff et al. infected TIVE cells gradually lost the viral episomes at later times.

However, we obtained, for the first time, two endothelial cell lines, in which KSHV episomes were maintained indefinitely in the absence of selection. Long-term KSHV maintenance correlated with loss of reactivation and complete oncogenic transformation of TIVE cells (LTC): LTC grew in soft agar and proliferated under low serum conditions. Furthermore, LTC but not parental TIVE cells formed tumors in nude mice. The tumors continued to express high levels of the latency-associated nuclear antigen (LANA). The LTC tumors expressed lymphatic endothelial specific antigens as found in KS (LYVE-1). Furthermore, host genes like IL-6, VEGF, and bFGF known to be highly expressed in KS lesions were also induced in LTC.

KSHV-infected LTCs represent the first and only xenograft model for KS and should be of use to study KS pathogenesis and the effect of anti-tumor drugs in KSHV infected endothelial cells.

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LATENT KSHV INFECTION CONVERTS PRIMARY VASCULAR ENDOTHELIAL CELLS FROM A FUNCTIONAL METABOLIC VESSEL WALL PHENOTYPE INTO AN ANGIOGENIC PHENOTYPE.

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We have previously described the "spindle cell conversion" phenotype as well as altered cellular mRNA gene array profiles associated with LANA1-positive latent state KSHV infection of primary adult dermal microvascular endothelial cells in cell culture. Here we have addressed changes in important endothelial cell (EC) protein levels as examined by IFA and IHC, which suggest that KSHV infection of vascular EC induces a change from contact-inhibited cuboidal metabolic state EC into angiogenic proliferating spindle cells. We found that the uninfected DMVECs and HUVECs express high levels of CD31 (PECAM1), Von Willebrand factor, VE-Cadherin (CD144) and β-Catenin in nearly all cells, and can be induced into an activated state by treatment with TNFα which increases VCAM1 (CD106)-positivity from 1% to 85%. However, KSHV infection leads to a near total loss of expression of CD31, VWF, VE-Cad and β-Catenin within 48 h in all LANA1-positive spindle cells, but without increased VCAM1 expression. This result parallels but is faster than the shutoff of these same characteristic metabolic EC proteins by TPA-treatment and is also mimicked after immortalization by telomerase in TIME and TIVE cells.

To address whether this is also the case in vivo in LANA1-positive KS spindle cells, we have carried out double-label IHC in archival paraffin blocks of nodular KS tumor tissue. The results revealed that although many normal vascular wall EC in KS tissue retain CD31 and VWF positivity, LANA1-positive perivascular fascicular spindle cells, as well as LANA1-positive immature neovascular wall EC, all fail to do so. These same LANA1-positive spindle cells in KS nodular tissue are also nearly all positive for Prox1 indicative of a partial change to lymphatic EC programming. Therefore the cultured KSHV-infected DMVECs closely parallel the properties of the LANA1-positive spindle cells in KS tumor cells.

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A CELL AND ANIMAL MODEL OF KSHV- INDUCED KAPOSI'S SARCOMA

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Kaposi's sarcoma-associated herpesvirus (KSHV) is the etiologic agent of Kaposi's sarcoma (KS), a highly vascularized spindle cell sarcoma associated with AIDS. The mechanisms whereby KSHV causes the KS angioproliferative response are not well defined, and the reproduction of KS by experimental KSHV infection has not yet been achieved. Here we show that bacterial artificial chromosome-mediated KSHV infection into bone marrow endothelial hematopoietic cells (mEC) leads to immortalization, angiogenic activation and KSHV-mediated KS-like tumorigenicity. KSHV-infected mEC (mECK36) formed highly vascularized spindle cell sarcomas in nude mice and multifocal sarcomas in lungs of SCID/NOD mice. Similar to KS cells, mECK36 displayed increased telomerase activity with VEGF secretion in culture and upregulated VEGF-R2 and VEGF-R3 gene expression in tumors. To establish a link between KSHV expression and tumorigenesis, we examined the role of KSHV vGPCR, an early lytic angiogenic gene, in the KS phenotype. We found that vGPCR short hairpin RNAs (shRNAs) efficiently suppressed vGPCR expression leading to reduced VEGF secretion, angiogenicity, and tumorigenicity. These results 1) Identify endothelial hematopoietic cells as possible KS-progenitors 2) Demonstrate the ability of the KSHV infection to induce KS-like tumorigenesis 3) Define a cell and animal model for KSHV-mediated pathogenesis of KS.

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ETIOPATHOGENETIC MECHANISMS OF CONJUNCTIVAL SQUAMOUS CELL CARCINOMA EPIDEMIC IN UGANDA

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Introduction: A conjunctival miniepidemic has beer reported in Uganda since the start of the HIV epidemy. Several etiopathogenetic factors, such as physical (UV radiation) and infectious (human papillomaviruses, HPVs) agents, have been claimed to be involved in the development of conjunctival carcinoma in particular for Ugandan patients. The objective of our study was to determine the presence and the role of mucosal as well as cutaneous (including *Epidermodysplasia Verruciformis*-related [EV]) HPV types and the genetic susceptibility of *TP53* Arg/Arg polymorphism to conjunctival neoplasia, along with the HIV immunodeficiency. **Methods:** The study included 41 invasive conjunctival squamous cell carcinoma (ICSCC), 33 conjunctival intraepithelial neoplasia of grade 3 (CIN3), 33 of moderate grade (CIN1 and CIN2), and 115 controls. Mucosal as well as cutaneous and EV HPV types have been searched with a combination of eight pairs of PCR primers. The *TP53* genotype was determined by codon 72 allele-specific PCR. HIV status was identified by serologic methods. **Results:** Seven EV-HPV types, including a putative new HPV, and 2 mucosal HPV types have been detected in 19.7% of cases and rarely (0-1.6%) in control subjects with a 33% peak of infection in CIN2 which decreased to 10% in ICSCC. The analysis of *TP53* alleles showed that codon 72 Arg/Arg genotype, found in 21.9% of ICSCC and in 18.2% of CIN3 but only in 6% of CIN1-2 and in 5.2% of controls ($P<0.05$), was associated with an increased risk of ICSCC (odds ratio (OR) = 6.2, 95% confidence interval (CI): 1.6-24.6). There was no statistically significant difference in the distribution of genotypes among ICSCC and CIN3 subjects according to their HIV serological status. Nearly 21% of ICSCC and 20% of CIN3 seropositive subjects were homozygous for the arginine allele compared with 22% and 17% of the seronegative ICSCC and CIN3 subjects, respectively. **Discussion:** The absence of high risk HPVs and the low detection frequency of EV-HPV types in ICSCC do not support a role for such viruses in the development of conjunctival neoplasia. The higher frequency of *TP53* Arg homozygosity in more advanced tumor stages suggests that *TP53* polymorphism is a relevant risk factor for invasive squamous cell carcinoma of the conjunctiva and for CIN3 in the Ugandan population. Although HIV-related immunodeficiency is associated with incidence increase of both conjunctival neoplasia and HPV detection, HIV-infection does not alter ICSCC etiopathogenetic mechanisms nor does it modify *TP53*-associated susceptibility.

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CERVICAL STEM CELLS: ISOLATION, CHARACTERIZATION, AND POTENTIAL ROLE IN HUMAN PAPILLOMAVIRUS (HPV)-INDUCED CERVICAL CARCINOGENESIS

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Cervical cancer is the 2nd most common cancer in women worldwide. HIV infected women have a higher prevalence of infection with HPV and are more likely to develop persistent infection, resulting in a higher incidence of, and more rapid progression to cervical cancer. HPV persistence is a necessary state for the emergence of cervical cancer, but the crucial factors determining persistence are still largely unknown. It has been proposed that viral persistence only occurs upon targeted infection of specific cervical cells, potentially with stem cell properties. As specific markers have yet to be identified, cervical stem cells remain poorly characterized. Using fluorescence activated cell sorting, we successfully isolated a distinct minor subpopulation of putative cervical stem cells with undetectable transferrin receptor and very high integrin α6 cell surface expression. These cells possess many phenotypic characteristics and functional properties expected for epithelial stem cells, such as small cell size with a high nuclear:cytoplasmatic ratio, marker expression of undifferenciated epithelial cells ($K14^+$, $K1^-$), quiescence (as determined by cell cycle analysis and Ki-67 expression), and increased colony formation efficiency in vitro. All other cervical epithelial cell types differed significantly in phenotype and biological behavior. Interestingly, transit amplifying cells expressed a 2-fold higher telomerase activity than putative stem cells. Most importantly, we demonstrate that virtually all of these putative stem cells have a 10-fold higher binding capacity for papillomavirus-like particles than any other cervical epithelial subpopulation, suggesting that HPVs might preferentially bind and infect these cells in vivo. Our results represent an important step towards identification of cervical epithelial stem cells and provide the basis for further investigations to determine if persistent infection and progression to pre-malignant and malignant cervical lesions requires initial infection of these cells.

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HIV ASSOCIATED KAPOSI'S SARCOMA, A PARADIGM FOR ANTI-ANGIOGENESIS RESEARCH

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An aggressive form of Kaposi's sarcoma (KS) associated with HIV is one of the leading cancers in many areas of sub-Saharan Africa, where the HIV epidemic overlaps with high incidences of endogenous HHV8. KS is characterized by an abnormal growth of blood vessels and a prominent inflammatory infiltrate; in fact the initial phases KS resembles granulation tissue. We and others have contributed to the elucidation of KS cell nature and the possible involvement of extracellular HIV Tat. Tat is directly angiogenic, being able to bind and activate VEGFR2 both on KS and endothelial cells. Tat is also indirectly angiogenic, as it is able to bind and activate chemokine receptors on monocytes and granulocytes causing a pro-angiogenic inflammatory state. Evaluation of the effects of extracellular Tat on KS cells by microarray analysis after 24 h of incubation shows an interesting clustering of gene products involved in signal transduction, especially GTP-ase, Kinase and cAMP activity, confirming that Tat acts extracellularly by ways that are probably unrelated to its nuclear transactivation activity. The same analysis revealed the activity of two G-protein coupled receptors, which could turn out to be potential Tat receptors. KS occurrence is reduced by HAART, however it remains a prevalent disease in Africa where HAART has not yet become a viable therapeutic option and parasitic infections push immune responses towards Th2 profiles, favorable to viruses. To find suitable drugs for these KS patients, we have tested several molecules and gene therapy approaches targeting angiogenesis in KS models *in vivo*.

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ARE DIFFERENT MEASUREMENTS OF CD4 CELLS ASSOCIATED WITH THE RISK OF KAPOSI'S SARCOMA IN HIV-POSITIVE HOMOSEXUAL MEN?

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Introduction: To assess and to compare the association between different types of measurements of CD4 cells and kaposi's sarcoma (KS) risk.

Methods: Follow-up data on 519 homosexual men enrolled in the Italian Seroconversion Study (ISS) were analyzed. Time to KS with respect of three modalities of CD4 cell count (i.e., current, nadir and the speed of CD4 cells decline) was investigated using the Cox model. Hazard ratios (HR) and 95% confidence intervals (CI) were computed.

Results: The model with the current count of CD4 cells showed the best statistical fit. As compared to a CD4 cell count $\geq$350, the HR for KS ranged from 2.9 (95% CI:1.0-8.2) for CD4 cell count between 200 and 349 cells/mm^3 to 31.2 (95% CI: 12.6-77.2) for CD4 cell count $\leq$99 cells/mm^3. Whereas the inclusion of the nadir CD4 cell count did not improve the fitting of the model with the current CD4 cell count (p=0.637), the rate of CD4 cell decrease seemed to improve such fit (HR=1.3, 95% CI 1.0-1.6).

Conclusions: When adjusted for the current count, the nadir CD4 cell count is not an independent indicator of KS risk. Moreover, the slope of CD4 cell decline could significantly help to identify HIV-positive homosexual men at higher risk of developing KS.

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CHANLLENGES OF MANAGING AIDS RELATED KAPOSI'S SARCOMA IN RESOURCE LIMITED SETTING

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Background: Sub-Saharan Africa has been most severely affected by the HIV/AIDS pandemic which appears to de augmenting the cancer burden in this region. Prior to the first HIV/AIDS report in Nigeria in 1986, Kaposi Sarcoma was a relatively indolent tumor which principally affects men. Cutaneous lesions affecting the limbs were predominant and the disease was reported to be unrelated to HIV infection. With individual average income of less than 1US dollar per day, majority of patients in Nigeria are unable to afford the cost of antiretovirals and cytotoxic drugs. Radiotherapy is not available in this center. Treatment is therefore mostly suboptimal and many patients decline or default treatment. The paucity of information regarding management of AIDS- related KS in Nigerian patients necessitated this study. **Patients and Methods:** Between January 2000 and December 2004, 44 patients with histologically diagnosed cutaneous KS were prospectively evaluated for HIV infection at Aminu Kano Teaching Hospital using Enzyme Linked Immunosorbent assay (ELISA) and CD4 cell count. This followed ethical clearance from the hospital and informed consent of the participating patients. **Results:** Of the 44 patients, 34(77.3%) were males and 10 (22.7%) were females with age range 19-72 years and mean ±SD of 36.7± 11.3 years. Thirty four (77.3%) tested HIV positive while 10(22.7%) were HIV negative out of which 2 where renal transplant recipients on immunosuppressants. Out of 23 (52.3%) patients that were able to commence ARVT and/or vincristine, or radiotherapy (done at another hospital), 18 (78.3%) were lost to follow-up and 5(21.7%) succumbed to their disease due to progression. Survival ranged from 2 to 12 months, median survival of 4 months. None of the patients could afford a protease inhibitor which is believed to be active against this KS. Of 21(47.7%) that couldn't afford treatment 18(85.7%) were due to poverty and 3(14.3%) had the old traditional belief that cancer aggravated by injections. **Conclusion:** Kaposi's sarcoma in Nigerian patients is strongly associated with HIV infection, and runs an aggressive course in the face of profound immunosuppression. Treatment is often unrewarding due to unaffordable potent therapeutic options. There is urgent need for strengthening and subsidy of diagnostic and therapeutic facilities for the management of HI/AIDS and its associated malignancies in Nigeria. The introduction of the President's Emergency Relief Package for HIV/AIDs (PEPFAR) holds great promise for the future decline in morbidity and mortality. It however requires additional support from other government and non-governmental agencies for the control and treatment of cancer.

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PATTERN OF AIDS RELATED KAPOSI SARCOMA (AKS) IN JOS, NIGERIA

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Introduction: AKS is the most important neoplasm in HIV/AIDS. This report describes the pattern of AKS in patients attending an antiretroviral (ARV) clinic in Jos, Nigeria.

Methods: Forty-six HIV-1 positive adults presenting with AKS had their demographics measured and lesions characterized. CD4-T lymphocyte cells was measured using cyflow and viral load was assayed using the Roche-Ampiclor kit (undetectable level<400copies/ml). Kaplan-Meier statistic was used to compare survival in patients with localized and disseminated disease.

Results: The prevalence of AKS was 1%. 43.5% were males and 56.5% females. The mean age was 37.0 ± 8.7years. Twenty three point nine percent had plaques, 30.4% had nodules and 45.7% had a combination of both. The disease was localized in 21.7% and disseminated in 78.3%. The mean CD4 cell count was 92.9 ± 78.1 cells. Viral load was undetectable in 7 patients while those with detectable levels had a mean value of 196,981 ± 304,013 (n = 39). Fifty-six percent of the patients had tuberculosis. Only 1/5th of the patients had accessed ARV's at presentation and 2 had received a course of cytotoxics. Eleven (23%) had died at one year and mortality was similar in patients with localized and disseminated disease (Wilcoxon test = 2.23, p=0.13).

Conclusion: Disseminated disease is the commonest form of AKS in our centre. The priority for us as a resource limited nation is to make highly active antiretroviral therapy (HAART) more accessible by scaling up our national ARV programme as this has been shown to decrease the incidence of AKS in other countries.

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TRENDS OF KAPOSI'S SARCOMA IN CAMEROUN (CENTRAL AFRICA): FROM THE PAST TO THE FUTURE

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Introduction: The pandemic of AIDS and the discovery of Human Herpès Virus type 8 in 1995 have changed the concept of an old disease, Kaposi's sarcoma. In Cameroon, a developing country of 15 millions people and 12 % AIDS prevalence, 257 cases of Kaposi's sarcoma were reported between 1968 and 1975 by Ravisse and al. We carried out this study the aim of which is to show the trend of Kaposi's sarcoma in Cameroon, from the past to the future.

Materials and Methods: We carried out a descriptive study over a period of 17 years (from January 1987 to December 2004). We trieved the cases of Kaposi's sarcoma observed in the main pathological laboratories of the country. We noted: -the total number of cancers histologically diagnosed over the period of the study; -the number of Kaposi's sarcoma over the same period; -the incidence of Kaposi's sarcoma; -the clinicopathological aspects; -the HIV status.

Results: 555 cases of Kaposi's sarcoma were recruited during the study, representing 5% of the total number of cancers diagnosed within the same period in the country. There is 35 new cases per year. The mean age of the patients is 44 years. 60% of these patients are less than 50 years old. Most of them are males (sex ratio=3males/1female). 18% of these patients are HIV positive and 22% are HIV negative. In 60% of the cases, the HIV status is unknown. One patient of this series is an Indian while others are Cameroonians. There were 31 children aged less than 15 years and four of them were HIV positive. One case was observed in a newborn of five months, HIV positive, while the mother, also HIV positive, didn't have any clinical sign of Kaposi's sarcoma at this time. The tumour was mostly localised on the skin. The other sites of involvement were lymph node, mosltly in children, ENT region, digestive tract or lung in HIV positive patients. The pathological aspect was mixed form in 80% of the cases and monomorphic form in 19.5% of the cases. In few cases, the diagnosis was made after immuno-histochemistry or polymerase chain reaction to identify HHV8 in tumour cells.

Conclusion: The epidemiology of Kaposi's sarcoma is changing in Cameroon as elsewhere, but many AIDS patients do not undergo biopsy. Then, our cases represent only 10% of the normal amount of Kaposi's sarcoma expected. In the other hand, the recently introduced Highly Active Antiretroviral Therapy would change this picture.

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A LIMITATION OF THE SIR AS A MEASURE OF THE EXCESS INCIDENCE OF KAPOSI'S SARCOMA IN AN HIV+ COHORT VERSUS A REFERENCE POPULATION.

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Background: The standardized incidence ratio (SIR) is often used to compare cancer incidence in an HIV+ cohort to that in a reference population such as SEER (Surveillance, Epidemiology, and End Results program). Using simple algebra, we show that the SIR may be solely dependent on the prevalence of HIV in the reference population.

Illustration: The SIR is defined as the observed (O) divided by expected (E) number of incident cancers, where E is based on the cancer-specific incidence rate (R_R) in the reference population, e.g., SEER. While E may account for important cofactors (e.g., age, race, sex), we assume for simplicity that adjustment is unnecessary in this illustration. Thus, $E=T_O*R_R$ where T_O is the sum of the follow-up time accrued by the observed HIV+ cohort. For KS, R_R differs by HIV status, so R_R must be stratified into R_R^+ and R_R^- such that $E=T_O*(\alpha R_R^+ + (1-\alpha) R_R^-)$ where α is the proportion of the SEER population that is HIV+. Since $O=T_O*R_O^+$ in the observed HIV+ cohort,

$$\mathbf{SIR = O/E = R_O^+ / (\alpha R_R^+ + (1-\alpha) R_R^-).}$$

Assuming that the incidence of KS among HIV+ persons is the same in the observed and reference cohorts ($R_O^+=R_R^+$) and that HIV status is not associated with the incidence of KS ($R_R^+=R_R^-=R$), then SIR = R/R = 1, as expected. However, when $R_R^+ \neq R_R^-$, as with KS, then the SIR depends on α. Interestingly, if $R_R^+>0$ and $R_R^-\approx 0$, then SIR $\approx \mathbf{R_O^+ / \alpha R_R^+} = 1/\alpha \geq 1$. Thus, if the risk of KS among HIV- persons in SEER is nearly 0, then the SIR will be approximately equal to the reciprocal of the prevalence of HIV in SEER. It follows that comparisons to different reference populations with different HIV prevalence rates will yield different SIR estimates.

Conclusion: For KS, which is strongly associated with HIV, the SIR must be interpreted with caution since this statistic may be biased above unity by an amount that is inversely proportional to the prevalence of HIV in the reference population.

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KAPOSI'S SARCOMA IN TRANSPLANT AND IN HIV-INFECTED PERSONS: AN EPIDEMIOLOGICAL STUDY IN ITALY AND FRANCE

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Introduction: A follow-up study was conducted in Italy and France to compare the epidemiology of Kaposi's sarcoma (KS) in persons infected with the human immunodeficiency virus (HIV) and in transplant recipients. **Methods:** 8074 HIV-positive persons (6072 from France and 2002 HIV-seroconverters from Italy) and 2705 Italian transplants (1844 kidney transplants, 702 heart transplants and 159 liver transplants) were followed up between 1970 and 2004. Standardized incidence ratios (SIR) and 95% confidence intervals (CI) were computed to estimate the risk of KS, as compared to sex- and age-matched populations of Italy and France. Incidence rate ratios (IRR) were used to identify risk factors for KS.
Results: A 451-fold higher SIR for KS was recorded in HIV-infected persons and a 128-fold higher SIR was seen in transplants. Significantly increased KS risks were observed in HIV-infected homosexual men (incidence rate ratio-IRR=9.7, in France; IRR=6.7 in Italy), as compared to intravenous drug users, and in Italian transplants born in the South (IRR= 5.2, 95% confidence intervals: 1.8-15.2), as compared to those born in the North. Increasing CD4+ cell counts were associated with reduced KS risks in HIV-infected patients. In relation with the duration of immunesuppression, KS occurred earlier in transplants than in HIV-seroconverters. **Conclusions:** As compared to age- and sex-matched general population, HIV-infected persons were at higher risk of KS than transplant persons, while a high level of immunosuppression reached in a short time interval was a main determinant of KS occurrence in transplants.

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RECREATIONAL DRUG USE AND KAPOSI'S SARCOMA, NON-HODGKIN'S LYMPHOMA AND TOTAL CANCER RISK IN HIV POSITIVE HOMOSEXUAL MEN IN THE MULTICENTER AIDS COHORT STUDY

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Objective: Determine the associations between marijuana, cocaine and poppers use and risks of Kaposi's sarcoma (KS), non-Hodgkin's lymphoma (NHL), or any cancer in HIV positive homosexual men. **Methods:** This analysis was performed using data collected from seropositive men participating in the Multicenter AIDS Cohort Study (MACS), an ongoing longitudinal cohort study of gay men in the United States. Follow-up time was restricted to the time from baseline for the 2,234 seroprevalent men and from the first seropositive visit for the 525 men who seroconverted after study entry to 1996 (the pre-HAART era). Cox proportional hazards models were used to study the association between recreational drug use and risks of developing KS, NHL, or any cancer in HIV seropositive MACS men. Multivariate analyses were conducted to estimate the effect of marijuana, cocaine, and poppers use on these outcomes adjusting for age, race, education, CD4 cell count, tobacco smoking and alcohol use. Recreational drug, tobacco and alcohol use as well as CD4 cell count were examined as fixed covariates using the data from baseline or the first seropositive visit (baseline model) as well as time-varying covariates (time-dependent model). **Results:** In the baseline model, marijuana use was significantly associated with the risk of developing KS (HR=1.36, 95% CI: (1.01, 1.81)). No evidence of association between cocaine/poppers use and KS was found in this analysis. Tobacco smoking at baseline was associated with a reduced risk for KS (HR=0.80 (0.65, 0.98)); similar results for tobacco use were obtained in the time-dependent model. Baseline cocaine use, but not time-dependent cocaine use, was found to be associated with a reduced risk for NHL (HR=0.53 (0.30, 0.91)). No association was observed for recreational drug use and risk of total cancer. Higher baseline CD4 count as well as time varying CD4 count were protective for the outcomes examined. **Conclusions:** Marijuana and cocaine use may modify the risk of developing malignancy outcome in HIV positive men. Larger studies in other cohorts should be conducted to confirm these findings.

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WHO IS STILL DEVELOPING KAPOSI'S SARCOMA IN THE HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) ERA?

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Background: Although HAART has dramatically reduced KS incidence, KS remains the most common HIV-associated malignancy in most areas where HAART is readily available. Despite this, little is known about why patients are still developing KS in the HAART era or about their survival after diagnosis.

Methods: We selected all HIV-infected patients who were receiving their primary care at any UCSF-affiliated clinic (including a university-based private practice, county-based municipal practice, and Veteran's Affairs Medical Center) and who were diagnosed with KS from 1999 to 2002. A standardized instrument was used to obtain demographic and clinical data at the time of diagnosis and for 18 months post-diagnosis. The National Death Index was used to determine vital status as of 12/31/2003.

Results: A total of 68 patients with newly diagnosed KS were identified; incidence was stable over 4 years. The median age was 40 years, 97% were men, at least 28% were homeless sometime in the prior year, and 50% had visceral disease at diagnosis. Overall, 61% of patients developed KS when their CD4+ T cell count was < 200 cells/mm^3 and while not on HAART; 9% had a CD4 count of 200 to 350 and not on HAART; and only 6% had a CD4 count > 350 and not on HAART. Mortality was 35% at 18 months post-diagnosis, only somewhat lower than the 55% estimated for those with KS in the San Francisco Men's Health Study (a natural history study in 1984-90) and was related to HAART use post-diagnosis.

Conclusions: Most KS in the HAART era occurs in patients with indications for but who are not receiving HAART. Similarly, mortality after KS diagnosis is strongly linked to HAART use. Improvements in health care utilization and perhaps screening for human herpesvirus 8 infection to identify those at risk for KS are needed if KS incidence and mortality are to be further reduced.

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EXPRESSION OF THE PREGNANCY-RELATED HORMONE RECEPTORS PROGESTERONE AND HUMAN CHORIONIC GONADOTROPIN IN KAPOSI'S SARCOMA

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Introduction: Kaposi's sarcoma (KS) has undergone spontaneous remission during pregnancy. This observation resulted in investigations of the pregnancy-related hormone human chorionic gonadotropin (hCG) to treat KS. However, it is unclear why clinical trials using hCG preparations showed a variable therapeutic effect. The aim of this study was to determine if progesterone (PR) and hCG receptors are expressed in KS lesions.

Methods: Archival cases (n=15) of formalin-fixed, paraffin-embedded KS lesions, including 10 AIDS-related and 5 classic KS specimens, were studied. Cutaneous samples (9 from extremities, 3 head and neck, and 1 trunk) at different histological stages (3 patch, 4 plaque, 6 nodular) and 2 lymph node-based KS tumors were included. Immunohistochemistry with antibodies directed to the latent nuclear antigen-1 (LNA-1) of Human Herpesvirus-8 (HHV8), PR and hCG receptor was performed. For all stains appropriate positive and negative controls were evaluated.

Results: Patients were of median age 54 years (range 32-92 years), including 11 males and 4 females. PR immunostaining was absent in all cases. KS tumor cells in all cases demonstrated immunoreactivity for LNA-1 and hCG receptor.

Conclusions: HHV8-positive Kaposi's sarcoma lesions in both men and women, of various epidemiological and histological types, express hCG receptors. This suggests that the inhibitory action of hCG on KS lesions likely occurs through its interaction with the hCG receptor. The lack of progesterone receptor expression in KS lesions implies that progesterone is unlikely to be directly responsible for KS regression observed during pregnancy.

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CELL CYCLE PROTEIN EXPRESSION IN AIDS-RELATED AND CLASSICAL KAPOSI'S SARCOMA

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Kaposi's sarcoma (KS) is one of the most important malignancies in patients with acquired immunodeficiency syndrome (AIDS). The current literature indicates that KS is initiated by the human herpes virus 8 (HHV8) as a reactive polyclonal process but with deregulation of oncogene and tumor suppressor genes, it can progress to a true malignancy with monoclonality. Clinically, classical KS often presents as an indolent disease affecting mainly the lower extremities whereas AIDS-related KS has no site predilection and can progress rapidly with systemic involvement. Histologically, classical and AIDS-related KS are indistinguishable and this suggests that AIDS-related KS and classical KS might be initiated by a common etiology but given their different clinical courses, they may progress through different mechanisms. In view of the importance of the cell cycle proteins in the development and progression of many human malignancies, this study aims to examine the role of these proteins in the progression of the two main clinical subtypes of KS.

The cell cycle protein expressions in a cohort of 45 patients with KS with well documented clinical and histological features were studied. Using a monoclonal antibody against the latent nuclear antigen-1 molecule of HHV8, HHV8 was detected in 72% of the cases. The more advanced nodular lesions were found to have a higher level of proliferative activity as measured by the proliferation marker, Ki-67. The role of the Rb/cyclin D1/p16 pathway was examined.

Of the mitotic cyclins examined, cyclin A expression was correlated with the advanced tumor stage. The rate of p34cdc2 expression was high in the lesions and there was no correlation with histological stage. This suggests that p34cdc2 is important in the early development of the tumour but not necessarily in its progression. Along the p53-apoptotic pathway, mutant p53 expression was significantly more common in the nodular stage. The cyclin G1 (a protooncogene, one of the target genes of p53) expression also paralleled that of mutant p53 with the majority of the KS lesions showing cyclin G1 expression and significant correlation between advanced histological stage and increasing rate of cyclin G1 expression. These findings suggest that progression along the p53 pathway may be important in the advanced stage development of KS. On the other hand, expression of the CDK inhibitor, p27, a protein that normally negatively regulates cyclin G1, was reduced in nodular KS. These findings suggest that some KS lesions may progress through a deregulated or abnormal p53 pathway.There were correlations between cyclin D1, cyclin A, cyclin G1, mutant p53 and negative HIV status. Rb protein was the only cell cycle protein whose rate of expression correlated significantly with HHV8 status in KS. The majority of HHV8 positive lesions were also positive for Rb protein, unlike HHV8 negative lesions.

The majority of the KS lesions examined in this study showed HHV8 infection. The Rb/cyclin D1/p16 pathway appears to be important in the progression of the different stages of KS and expression of the proteins involved in the p53 pathway were found to be important in the advanced stages of the development of KS. There were differential expressions of cell cycle proteins between AIDS-related and classical KS, and between HHV8 positive and HHV8 negative lesions. The findings also provided some clues to the possible mechanisms of development in KS lesions that were not initiated by HHV8.

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TRENDS IN KAPOSI'S SARCOMA AND NON-HODGKIN'S LYMPHOMA INCIDENCE IN CALI.

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Background: Non-Hodgkin lymphoma (NHL) and Kaposi sarcoma (KS) are well-recognized tumours with a higher prevalence in HIV patients. Their incidence, in the general population, has markedly increased since the onset of the AIDS epidemic in 1981. We described the general population incidence trends of KS and NHL in Cali, Colombia.

Methods: Age-standardized incidence rates (ASR) for KS and NHL from 1962 through 2001 were obtained from the population-based Cali Cancer Registry (about 2,000,000 inhabitants). Joinpoints (points in time where trend significantly changes from linearity) were found, and estimated annual percentage changes (EAPC) used to summarize tendencies.

Results: During the period, 206 new cases of KS and 2698 of NLH were registered; 15% of the lymphomas were extranodal. Temporal trend for incident cases for KS showed a statistically significant increase of incidence rates in males (EAPC = +7.9%; 95%CI, 5.5, 10.4). Significant changes in linear trends were detected with joinpoint analysis. Male rates accelerated in the late 1980s to early 1990s. The EAPC among females was 0.4%; C.I95%, -0.9, 1.8.
Overall incidence was significantly increasing in NHL; however the increase was higher in extranodal NHL. The EAPC for extranodal NHL was 7.6 % (95%CI, 5.8, and 9.4) among males and 7.7% (95%CI, 6.1, and 9.5) among females. While the increase of NHL began in the seventies (right before the HIV/AIDS epidemic), 85% of KS were diagnosed after 1990.

Conclusion: In contrast with developed countries, the incidence rate of NHL and KS is likely to continue to rise in the coming years. As elsewhere, factors in addition to AIDS are involved in the increasing incidence of NHL. Because diagnostic and classification changes probably do not explain the entire increase unrelated to AIDS, epidemiologic studies are needed to assess the risk factors that might account for the increase in NHL.

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CHILDHOOD CANCERS WITHIN THE ONGOING AIDS EPIDEMIC IN CAMEROON (CENTRAL AFRICA)

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Background: Cameroon is an underdeveloped country with 15000000 (fifteen million) inhabitants located in Central Africa. It is a blank area of world cancer map because structures for the cancers registration are scarce or inexistent. Only 10% of cancers are diagnosed histologically. However, among the 12000 cases of cancers diagnosed in Cameroon per year, 10% occur in children, mostly Burkitt's lymphoa, Wilm's tumour, acute leukemia and lymph node Kaposi's sarcoma. Before 1997, there was no reported cases of cancers in Cameroonian children with HIV infection. AIM: The aim of this study is to show the clinicopathological aspects of Childhood cancers in Cameroon within the ongoing AIDS epidemic.

Materials and Methods: We tried the cases of cancers diagnosed in HIV positive children aged less than 15 years.

Results: Since the last 10 years, 8 histologically diagnosed cases of cancers have been reported in HIV positive children. There was 4 cases of Kaposi's sarcoma, 3 cases of BURKITT's lymphoma and one case of embryonnal rhabdomyosarcoma of the eye. All these children were aged less than 11 years, the youngest aged 5 months and the oldest 10 years. They were HIV-1 infected as well as their motheirs, but their fathers refused blood test for serodiagnosis. For the Kaposi's sarcoma cases, there was one disseminated case with lymph node involvement. The others were only disseminated with ear, skin, eyelid, and genitalia involvement. The histological aspect was the same as in the case of classical or endemic Kaposi's sarcoma. Concerning the BURKITT's lymphoma, there was one maxillo-facial involvement and two disseminated cases. The rhabdo-myosarcoma was diagnosed in a 3 years old child with eye involvement whose mother was also HIV-1 positive. Two children with Kaposi's sarcoma and the one with rhabdomyosarcoma died before two years of follow-up. Two children of this series, one with Kaposi's sarcoma and one other with Burkitt's lymphomas lost their sight and the other children were treated using different chemotherapy protocol.

Conclusion: The pandemic of AIDS will probably have an influence on childhood malignancies in Cameroon; but the situation is still different from what is observed in East and South Africa.

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MANAGEMENT OF HIV/AIDS RELATED CANCERS IN A NIGERIAN TERTIARY HOSPITAL

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The study population consisted of patients with HIV/AIDS related cancers referred to the Radiotherapy and Oncology Centre, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria from January 2000 to December 2004, using proforma, supplementary information obtained from the hospital based cancer registry and the medical records. Twenty patients with HIV/AIDS related cancers were treated during the period. All 20 patients were seropositive for human immunodeficiency virus with confirmed depressed CD4 counts. Four (20%) had Kaposi's sarcoma, 8 (40%) had invasive carcinoma of the cervix uteri, 2 (10%) with non-Hodgkin's lymphoma, 2 (10%) cases of anal cancers, 2 (10%) with basal cell carcinoma of the skin, 1 (5%) case of Squamous cell carcinoma of the eye and 1 (5%) case of Hodgkin,s disease. There were 11 males (55%) and 9 females (45%). Their ages ranged from 20-50 years. The mean age is 40 years and a predominance of metastatic cases. Scarcity of data may be responsible for non-identification of HIV associated neoplasia in children. These patients were treated similarly to patients without HIV infection, but the HIV – induced immunodeficiency causes difficulties in tolerating antineoplastic treatment as they develop intercurrent illnesses. Thirteen (65%) of the patients are already dead.

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HIV-ASSOCIATED MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE: A RETROSPECTIVE ANALYSIS

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Introduction: MGUS is rarely seen in the general population before the age of 60. Various reports indicate a much higher incidence of MGUS in HIV+ patients and a much younger age at diagnosis. We describe the clinical course of 15 HIV+ patients with MGUS seen in an urban HIV outpatient practice, and their association with overt plasma cell disorders, other malignancies and infection.

Methods: We abstracted medical records of all patients with HIV-MGUS identified through the VMMC Hem-Onc clinic between 1996-2005. We reviewed demographic data including age, gender, race, clinical course, and whether they had other malignancies or infections. Lab data included CD4+ counts, HIV VL, quantitative immunoglobulin, SPEP, UPEP, serum viscosity index, skeletal survey and bone marrow biopsy as clinically indicated.

Results: 15 of 500 patients (3%) were diagnosed with MGUS. All were male, their median age was 42 (range, 24-51). The median CD4+count was 350 (range, 48-875) and the mean HIV VL was 24,636 (range, <75 to 100,000). 10 of 15 patients (67%) were HIV viremic despite HAART. One patient had multiple myeloma, 1 marginal zone NHL, 2 KS, and 5 HCV (33%). No patient had renal failure or hypercalcemia and 5 were anemic.

Conclusions: Patients were characterized by a higher incidence of MGUS at a younger age and the presence of other viral infections (HCV and KSHV). CD4+ counts were relatively well preserved and HIV viremia well controlled. HAART did not seem to influence the monoclonal band amplitude. Long-term follow up is needed to better define the natural history of this disorder and the link to other possible contributing factors.

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HIV/AIDS AND CANCER IN INDIA: AUDIT FROM A TERTIARY REFERRAL CANCER CENTER

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Background: Malignancies are a significant cause of illness and death in the population infected with HIV. With the increasing incidence of HIV in India, improved management of opportunistic infections and availability of HAART, there will be an increased incidence AIDS- related and non-AIDS –related cancers. There is limited data from India on the association of cancer with HIV/AIDS. The management of cancer with HIV poses several unique challenges.

Aims: To evaluate the prevalence of different types of malignancies associated with HIV and to assess the presence of associated infections like tuberculosis, hepatitis B and C. Materials and methods: Records of cancer patients who were found to be HIV positive during the period 2000-2002 were studied. A data base was created to examine the relationship between HIV and the cancer types, cancer sites, association with Tuberculosis, HCV and Hepatitis B. Retrospective analysis was done using SPSS software.

Results: There were 163 patients including 100 males. Age ranged between 3-82 years with a median of 40 years. Non–AIDS defining cancers constituted 69.4 % of all cancers. These included head and neck cancers (25.1%), GI cancers (3.6 %), Genito-urinary cancers (7.3%), Breast (6.1%), Hodgkin's disease (4.2%), lung (2.4%) and others (20.7%). The AIDS defining cancers included lymphomas (20.7%), and cancer of cervix (9.2%). Two cancers of the anal canal were seen. Overall 44.3% were squamous carcinoma. 30.1% patients had received chemotherapy, 29.4% received radiotherapy and 19.6% underwent surgery. Only 53.7% received some modality of treatment for their cancer. Only 12 of the above patients received anti retroviral therapy in this cohort. Hepatitis B positivity was seen in 4.3%, Hepatitis C in 0.6%, and tuberculosis in 6.7%. Tobacco usage amongst this cohort was 33.1%.

Conclusions: Indian patients with HIV are relatively young. Kaposi's sarcoma is not commonly seen, while lymphoma and cervix cancers are common. Majority of patients have non-AIDS defining cancers. The prevalence of hepatitis B and C was comparable to that in general population. Approximately one in sixteen patients with cancer and HIV have tuberculosis. Only half the patients with HIV and cancer receive cancer directed therapy and majority of patients do not receive concurrent HAART.

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CERVICAL CANCER SCREENING IN HIV-INFECTED WOMEN IN ZAMBIA

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Introduction: HIV-infected women in resource-limited settings are in serious need of cervical cancer screening as they are living longer due to antiretroviral therapy, but nonetheless have a high risk of cervical neoplasia. To evaluate the usefulness of various screening modalities for HPV-induced cervical cancer in HIV-infected women, we conducted a pilot cross-sectional study in Lusaka, Zambia.

Methods: We screened 146 Zambian HIV-infected women with both liquid-based cytology and Visual Inspection with Acetic-acid (VIA). VIA was performed by nurses who underwent a two-week long competency based training using standardized educational material. All participants were examined by colposcopy and histological confirmation was obtained when indicated. In our preliminary analysis, pending results of histology, we correlated the screening test results of VIA against cytology.

Results: The median age of study participants was 36 years (range 23-49) and their mean CD4+ count was 209/µl. 113/146 (77.4%) women had evidence of any (low or high grade) squamous intraepithelial lesions (SIL) on cytology, while 78/146 (53.4%) women had evidence of high grade SIL. 67/146 (45.9%) women were found to have acetowhite lesions on VIA. The level of concordance between test results of VIA and cytology (high grade SIL threshold) was 0.36 (95% CI: 0.21 to 0.51, $p < 0.001$).

Conclusion: Pending the results of histology, which will allow direct comparison between the accuracies of VIA and cytology, it appears that VIA holds promise as a cost-effective alternative or adjunct to cytology for screening HIV-infected women in resource-limited settings. Immediate treatment without loss to follow-up is an advantage with VIA, should screening efficiency prove to be acceptable.

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CLINICAL FEATURES AND OUTCOME OF COLORECTAL CANCER (CRC) IN HIV-INFECTED PEOPLE: A GICAT EXPERIENCE

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Purpose: To describe the characteristics of CRC in HIV/AIDS patients (pts) and to compare its epidemiological and clinical aspects in HIV-negative pts.

Methods: From September 1983 to November 2003, 17 pts, with HIV infection and CRC, were treated within the GICAT (Gruppo Italiano Cooperativo AIDS e Tumori); their epidemiologic and clinical features were compared with those of 70 HIV-negative CRC pts.

Results: Out of 17 HIV-positive CRC pts, 14 (82%) were males and 3 (18%9 females. Median WHO performance status (PS) at a diagnosis was 2. A primary tumor was localized in the colon in 13 (76%) and in the rectum in 4 (24%). Thirteen (76%) pts presented with stage D disease. Liver was the primary site of metastases. Six (34%) and 11 (66%) HIV-pts underwent palliative and curative surgery, respectively. Eleven /65%) were treated with cytotoxic chemotherapy, one underwent adjuvant radio-chemotherapy and 5 (30%) had only supportive care. In the HAART era we obtained a complete and a partial remission in 1 and 3 pts, respectively.

Conclusions: Malignant neoplasms are a leading cause of death among HIV-infected patients in western countries in the HAART era. Data on CRC in HIV-positive pts are limited and contradictory. They seem to develop the disease at an earlier age and at a much more advanced stage than in the general population. In our experience HIV-positive CRC pts have a worse prognosis than those HIV-negative. It is mandatory to implement early diagnosis screenings in this group of pts.

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AMC 019: A PILOT STUDY OF ANTIVIRAL AND IMMUNOMODULATORY TREATMENT FOR HIV-ASSOCIATED PRIMARY CNS LYMPHOMA (PCNSL)

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Background: A consistent association with EBV distinguishes HIV-PCNSL from that occurring in the general population. Recent descriptions of long-term remissions in patients with post-transplant EBV-associated PCNSL who received EBV-specific therapy, suggests some anti-tumor effect is anti-EBV mediated.

Patients and Methods: 4 patients were accrued to this study, and 2 were treated in a similar fashion off protocol. All 6 had < 50 CD4+ cells/µL and HIV VL ranged from 2170-360,000 copies/ml. Treatment consisted of zidovudine (1.5 gm IV bid), ganciclovir (5 mg/km IV qd), and IL-2 ($2x10^6$ units IV qd). After 2 weeks of therapy, patients were switched to oral ganciclovir (1 gm tid), HAART, and subcutaneous IL-2 ($2x10^6$ units 3x/weekly).

Results: 1 of 4 protocol-enrolled patients remains in complete remission (CR) with > 3 years follow-up. The other 3 patients died from complications of progressive PCNSL. Two patients treated off protocol achieved a favorable response; 1 remains in CR at > 18 months follow-up. Grade 3-4 myelosuppression was uniformly noted, but there were no hemorrhagic or infectious complications.

Comment: For AIDS patients with PCNSL, treatments with dual efficacy against HIV and EBV merit further investigation. Our experience provides a platform for future studies.

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LIPOSOMAL DOXORUBICIN, CYCLOPHOSPHAMIDE, ETOPOSIDE (LACE) AND ANTIRETROVIRAL THERAPY (ART) FOR PATIENTS WITH AIDS-RELATED LYMPHOMA (ARL): A PILOT STUDY

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Purpose: To evaluate the safety and efficacy of LACE in conjunction with ART in patients with ARL and Hodgkin's disease (HD). The impact of HIV viral control on therapy and survival was also assessed.

Patients and Methods: Between 1994 and 2005, 50 patients were diagnosed with ARL (n = 40) and HD (n = 10) at VMMC. 15 of these patients received liposomal doxorubicin (30 mg/m^2 IV d1), cyclophosphamide (700 mg/m^2 IV d1) and etoposide (60 mg/m^2 IV d1 and 120 mg/m^2 po d2-3) q 28 days. All patients received CNS chemoprophylaxis, ART and G-CSF (d 3-12).

Results: The median patient CD4+ count was 181 cells/μL (range, 20 - 853 cells/μL) and the median HIV VL was 34,011 copies/mL (range, <50 – 500,000 copies/mL). 8 patients (53%) had an International Prognostic Index score of 3 or 4. Six patients (40%) were ART-naïve, 7 patients were viremic despite ART and 2 had an undetectable HIV VL. 11 patients (73%) achieved complete response (CR). 3 patients died from relapsed/refractory ARL and 2 patients achieved CR with salvage therapy. 1 CR patient died from complications of PCP and another CR patient died from uncertain causes. Grade 3 or 4 neutropenia occurred in 33 of 78 (42%) chemo cycles. Hospitalization was required after 4% of treatment cycles due to neutropenic fever.

Conclusion: LACE is an effective and tolerable treatment for ARL. HIV viral control could be maintained in the majority of patients during and after completion of LACE. At VMMC, patients who decline enrollment in AMC-034 (dose-adjusted EPOCH + Rituximab) are now offered LACE in conjunction with rituximab.

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HIV-RELATED BURKITT'S NHL (BL) VS DIFFUSE LARGE-CELL NHL (DLCL) IN THE PRE- AND HAART ERAS: SIGNIFICANT DIFFERENCES IN SURVIVAL WITH STANDARD CHEMOTHERAPY

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Background: The survival of patients with HIV-related NHL has significantly improved by the widespread use of HAART. However, the survival of patients with HIV-BL treated with standard chemotherapy regimens remains poor.

Methods: In order to evaluate the outcome of patients with HIV-BL in comparison to that of patients with HIV-DLCL, we reviewed our series of 253 HIV-NHL diagnosed and treated at the National Cancer Institute of Aviano, Italy from 1984 to 2003, including 125 cases in the pre-HAART era (77 HIV-DLCL and 48 HIV-BL) and in the HAART era (93 HIV-DLCL and 35 HIV-BL).

Results: All patients both with HIV-DLCL and HIV-BL were treated with the same doxorubicin containing chemotherapy regimens. The median OS of all patients was significant longer in the HAART era in comparison to that of the pre-HAART era (15 vs 8 months, $p \leq .0001$). In addition, in the pre-HAART era, the median OS was similar in patients with HIV-BL vs patients with HIV-DLCL (7 vs 10 months, $p = .11$) whereas in the HAART era the median OS of patients with HIV-BL was significantly shorter than that of patients with HIV-DLCL (8 vs 22 months).

Conclusions: Our data confirm that the significant improvement of the outcome of patients with HIV-NHL is related to the positive impact of HAART on the survival of patients with HIV-DLCL whereas the prognosis of HIV-BL remains poor despite the use of HAART. Taking into consideration that in the HAART era patients with HIV infection are likely to tolerate intensive chemotherapy regimens, a more intensive approach similar to that employed in the general population should be employed also in patients with HIV-BL.

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THE CLINICAL AND IMMUNOLOGICAL PROFILE OF AIDS-RELATED LYMPHOMA (ARL) IN THE ERA OF HAART

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Background: The morbidity and mortality of people living with HIV has lessened with the advent of HAART. However, the incidence of ARL has not shown as significant of a decline in the HAART era. We compared the epidemiologic, immunologic, and clinical characteristics of patients diagnosed with ARL in the pre-HAART and HAART eras.

Methods: We used the Adult/Adolescent Spectrum of HIV-related Diseases database of Public Health-Seattle and King County to determine incidences and trends among patients diagnosed with ARL in King County, WA.

Results: We noted a significant decrease in the incidence density rate of ARL (1.63 vs. 0.44, $p<0.01$) in the HAART era. There was a significant increase in the percentage of females (2% vs.14%, $p<0.01$), minorities (Blacks 9% vs. 29%, $p<0.01$; Hispanics 6% vs. 21%, $p<0.01$; Native Americans 0 vs. 14%, $p<0.01$), and individuals originating from a country other than the USA (10% vs. 29%, $p<0.01$) in the HAART era. There was also a significant increase in the percentage of ARL patients with a CD4+ ≥200 cells/µl (4% vs. 21%, $p<0.01$) and at HIV VL ≤100,000 copies/ml (10% vs. 29%, $p<0.01$). HBV (0.06 vs. 0.00, $p=0.17$) and HCV (0.03 vs. 0.00, $p=0.33$) co-infection were uncommon events in both eras. Alcohol (0.05 vs. 0.03, $p=0.75$) and injection drug abuse (0.08 vs. 0.03, $p=0.41$) did not differ significantly between eras.

Conclusion: In the HAART era, there is an increased proportion of women and people of color developing ARL in King County, although the overall ARL rate continues to decline. The demographics and immunological profile of patients with ARL are changing, but selected co-morbid conditions have remained constant.

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IMPROVED SURVIVAL WITH CHEMOTHERAPY IN PETIENTS WITH NON HODGKIN'S LYMPHOMA WITH HIV – AN EXPERIENCE FROM EASTERN INDIA

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Background: Usually the patients of Non Hodgkin's Lymphoma with HIV survive 4 to 6 months. Now a days when patients treated both with highly active anti-viral therapy (HAART) & chemotherapy the survival rate often exceeds 2 years. Usually researchers have learnt that two standard chemotheraputic combination for NHL – CHOP (Cyclo Phosphamide, Adrimycin, Vincristin and Prednisolone) and mBACOD (Methotrexate, Bleomycin, Adrimycin, Cyclo Phosphamide, Vincristin and Dexamethasone) can be administered at lower dosage to achieve response rates that are similar to these associated with standard dosages, often with fewer side effects.

Aim: The aim of our study was to see the survival benefit of NHL patients with HIV with regular chemotherapy with reduced dose. Our intention was to see the tolerability of the dosage also.

Material & Method: During the period from July 2003 – June 2005 we selected consecutive 8 patients of Non Hodgkin's Lymphoma with HIV. They all were on HAART. All patients were given two third of CHOP dose and maximum 6 cycles were given.

Result: Out of 8 patients 7 patients could complete 6 cycles of CHOP chemotherapy (1 patient died after 3rd cycle). With median follow up of 8 months (range 3-18 months) the disease free survival rate was 83.33%. All patients tolerated two third dose of CHOP chemotherapy well except 3 (9.09%) episodes of grade IV febrile neutropenia.

Conclusion: CHOP chemotherapy in HIV patients is safe. They improve the survival of NHL patients with HIV.

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EVOLUTIONARILY DISTINCT FORMS OF HIV IN AIDS LYMPHOMA AND DEMENTIA

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Background: AIDS related lymphoma (ARL) and HIV associated dementia (HAD) are diseases mediated in part by HIV infected macrophages and unlike Kaposi's sarcoma, persist in the post HAART era. "Phylodynamic analysis" was performed to test for disease specific tissue HIV DNA sequence evolution.

Materials and Methods: Frozen tissues from patients with ARL, KS and HAD were obtained from the ACSR for HIV DNA analyses (url: http://acsr.ucsf.edu/.).

Results: All HAD involved brain specimens**,** 10/14 ARL and 1/11 KS specimens had HIV DNA concentrations > 1/2000 genomic equivalents. HAD HIV sequences showed compartmentalization and directional evolution with ancestral HIV in the meninges. Of the ARL's, 4/4 primary CNS lymphomas and 3/3 Burkitt's lymphomas were HIV+. Immunostaining studies confirmed the presence of HIV p24 only in macrophages. ARL related HIV sequences were relatively "monophyletic" and uniquely different from non-ARL HIV, with lower frequency of putative gp120 glycosylation sites and evidence for nef gene mutations. In a case with both HAD and CNS ARL, the ARL-LTR HIV sequences were missing one of two NFK-B sites present in the HAD LTR.

Conclusion/Discussion: In this study, HAD related HIV showed evolutionarily related sequence compartmentalization with most sequences evolving from an ancestral form of HIV present in the meninges. HAD sequences were uniquely different from those in parallel ARL within HAD brains or in systemic ARL. These data suggest that CNS ARL HIV does not evolve directly from HAD HIV and in this preliminary study suggests the existence of ARL specific strains of HIV distinct from those involved in the pathogenesis of HAD HAD and ARL are diseases significantly influenced by HIV infected macrophages, distinctly different from KS.

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MOUSE MODEL OF HUMAN IMMUNODEFICIENCY-ASSOCIATED BURKITT LYMPHOMA t(8;14)(q24;q32) TRANSLOCATION

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Purpose: Human immunodeficiency-associated Burkitt lymphoma (iBL) is an aggressive form of *MYC*-driven post-germinal center non-Hodgkin lymphoma that frequently develops in the context of severely impaired immune function occasioned by HIV infection. Accurate mouse models for iBL are needed to study the events involved in its initiation and progression, and to test novel interventions. We generated a mouse model of the *MYC*-activating chromosomal t(8;14)(q24;q32) translocation that is widely believed to be the initiating event in the pathogenesis of iBL.

Experimental procedure: We inserted a histidine-tagged mouse *Myc* cDNA, Myc^{His}, into the mouse immunoglobulin heavy-chain locus, *Igh*, just 5' of Cμ. The intronic heavy-chain enhancer Eμwas deleted during gene insertion.

Result: The newly developed mouse strain, designated $iMyc^{C\square}$, is the most accurate model of iBL t(8;14) available to date. In untreated mice, the $iMyc^{C\mu}$ transgene is only weakly oncogenic in inducing B cell and plasma cell neoplasms. However, in treated mice undergoing chronic inflammation, $iMyc^{C\mu}$ is a strong oncogene, leading to rapid tumor development with full penetrance (100% incidence).

Conclusion: The important biological feature of the $iMyc^{C\mu}$ gene insertion model is that *Myc* is deregulated in all B cells by the appropriate set of correctly spaced regulatory elements residing in the *Igh* 3' Ca enhancer locus. This represents a significant advance over previously developed *Myc* transgenics, which rely on individual immunoglobulin-gene control elements (e.g., Eμ) to simply overexpress *Myc* in B cells.

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HPV-TYPES IN HIV-INFECTED WOMEN IN ZAMBIA

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Introduction: Identification of genital HPV types in HIV-infected women living in resource limited settings is important for designing accurate and appropriate HPV-based cervical cancer screening tests and preventive and therapeutic HPV vaccines.

Methods: We screened 146 HIV-infected Zambian women with liquid-based cervical cytology. We used the Roche linear array PCR to determine presence of 37 specific HPV types in cervicovaginal samples. We correlated the cytology test results with the total number of HPV types present in each sample.

Results: The median age of study participants was 36 years (range 23-49) and their mean CD4+ count was 209/µl. Although 36 of the 37 detectable HPV types were present, the most common were: HPV types **52** (38%), **61** (26%), **62** (26%), **58** (23.3%), **53** (20%), **84** (18.7%), **35** (18%), **81** (18%), **16** (17.3%) and **45** (17.3%). A comparison of test results of cytology and number of HPV types per sample is shown below:

	Cytology results			
	Normal/ ASCUS	Low grade SIL	≥High grade SIL	
0 HPV type	3	1	0	4 (2.7%)
1 HPV type	5	2	6	13 (8.9%)
2-5 HPV types	21	16	45	82 (56.2%)
≥ 6 HPV types	4	16	27	47 (32.2%)
	33	35	78	146

[Pearson Chi-Square Test of significance: ***p<0.05***]

Conclusion: Infection with multiple high risk HPV types was associated with higher risk of cervical cytological abnormalities. Almost one third of the HIV-infected Zambian women had ≥ 6 HPV types identified by the assay. Additional studies with long term follow-up are necessary to determine significance of these findings.

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CLONING AND IDENTIFICATION OF A MICRO-RNA CLUSTER WITHIN THE LATENCY-ASSOCIATED REGION OF KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS

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MicroRNAs are small non-coding regulatory RNA molecules that bind to 3'UTRs of mRNAs to either prevent their translation or induce their degradation. Previously identified in a variety of organisms ranging from plants to mammals, miRNAs are also now known to be encoded by viruses. The human γ-herpesvirus Epstein-Barr Virus has been shown to encode miRNAs which potentially regulate both viral and cellular genes. To determine whether Kaposi's sarcoma-associated herpesvirus (KSHV) encodes miRNAs, we cloned small RNAs from latently and lytically KSHV infected primary effusion lymphoma derived cells and endothelial cells. Sequence analysis revealed 11 miRNAs of 19 to 23 bases in length that perfectly align to KSHV. Surprisingly, all candidate miRNAs mapped to a single genomic locale within the latency-associated region of KSHV (1). The existence of this miRNA cluster has also been reported by two additional groups while this work was under review (2, 3).

To determine whether these novel viral miRNA genes are evolutionarily conserved we cloned the miRNA containing region from several PEL cell lines, KS tumors and PBMC from subjects with KS or MCD that were previously characterized by K1 and K15 genotyping. Sequence analysis demonstrated that these miRNAs are highly conserved between different virus sub-types and different malignant entities.

By performing RT-PCR-based RNA mapping studies, we detected several transcripts that may express the miRNA cluster as a spliced intron, from a long pre-mRNA that originates upstream of the LANA promoter and extends through the kaposin locus. These data suggests that the KSHV-encoded miRNAs are co-regulated with the expression of ORFs 73, 72, 71, and the kaposin proteins all of which regulate different host cellular signal transduction pathways in latently infected cells.

In summary, these data suggest that viral and/or host cellular gene expression may be regulated by miRNAs during both latent and lytic KSHV replication. The high sequence conservation between different KSHV sub-types further underlines that miRNAs may play an important role in the viral lifecycle.

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DUAL ROLES OF RTA IN ORI-LYT-DEPENDENT DNA REPLICATION OF KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS

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Herpesvirus lytic DNA replication requires both *cis*-acting element, the origin, and *trans*-acting factors including virally encoded origin-binding proteins, DNA replication enzymes and cellular factors. Recently, two almost-identical origins of lytic DNA replication (*ori-Lyt*) in Kaposi's sarcoma-associated herpesvirus (KSHV) have been identified and two virally encoded proteins, namely RTA and K8, have been shown to specifically bind to the origin. The studies reported here have been focused on functional roles of RTA in *ori-Lyt*-dependent DNA replication. When binding to an *ori-Lyt*, RTA initiates an RNA transcription. We demonstrated that the transcription from the *ori-Lyt* was absolutely required for the *ori-Lyt*-dependent DNA replication and premature termination of the transcription abolished the DNA replication. In addition, by using a DNA affinity purification and mass spectrometry, several cellular proteins were found to bind to KSHV *ori-Lyt*, including poly(ADP-ribose) polymerase I (PARP-1), RecQL1, Ku86/70 autoantigens and scaffold attachment factor A (SAF-A). PARP-1, RecQL, Ku antigens, but not SAF-A, appear to accumulate in viral replication compartments and be recruited to *ori-Lyt* by interacting with RTA and K8. The roles of these cellular proteins in *ori-Lyt*-dependent DNA replication are being investigated. It was found that inhibition of PARP-1 by using either siRNA or chemical inhibitors (3-aminobenzamide and nicotinamide) resulted in decreases in *ori-Lyt*-dependent DNA replication, whereas hydroxyurea, which raises PARP-1 activity, caused a increase in the DNA replication, suggesting a positive role of PARP-1 in KSHV lytic DNA replication. Our data demonstrated two distinct functions of RTA in *ori-Lyt*, supporting *ori-Lyt*-associated RNA transcription and recruiting the replication compartment, which is composed of both viral and cellular factors, to *ori-Lyt* in the KSHV genome.

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KSHV: ANALYSIS OF THE VIRAL PROTEIN KINASE, ORF-36

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The viral protein kinase (vPK), encoded by ORF-36 of KSHV/HHV-8, is conserved across all herpesviruses. Because of its low homology to cellular kinases, vPK represents an excellent target for novel antiviral drugs. Previously, our studies demonstrated the ability of vPK to phosphorylate JNK/SAPK in a complex with MKK4 and MKK7 (Hamza et al. J. Biol. Chem. 2004). To obtain a fuller understanding of the function of the ORF-36 gene in the viral life cycle, we have identified additional cellular, as well as viral substrates of vPK, by (i) a proteomics approach, via the pepchip kinase substrate array and (ii) studies of binding partners and substrates in both cell-free systems and in intact cells. These approaches revealed specificity for certain serine/threonine substrates with particular motifs, including cellular substrates that are also known targets of the homologous herpesvirus kinases. The cellular substrates include lamin A/C, the carboxy-terminal domain (CTD) of RNA polymerase-II, and elongation factor 1-alpha. Viral targets include K-bZIP (K8), which is efficiently phosphorylated in vitro and in vivo by vPK. In addition, vPK is associated with K-bZIP in co-transfection in 293 cells and in induced BCBL-1 cells. Interestingly, immunofluorescence studies revealed that vPK and K-bZIP both are co-localized to replication compartments. Although vPK is mostly localized in the nucleus, we demonstrate that vPK also binds ORF-45, a tegument protein located predominantly in the cytoplasm, and phosphorylates ORF-45 on a threonine residue. Stable cell lines (VERO) containing wild-type KSHV BAC, or a KSHV BAC with a transposon insertion mutation in ORF-36, have been created. Initial observations suggest that vPK knock-out is not absolutely necessary for expression of K8.1 and ORF29. The consequences of vPK elimination for viral replication will be discussed.

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INHIBITION OF REPLICATION AND TRANSCRIPTION ACTIVATOR AND LATENCY-ASSOCIATED NUCLEAR ANTIGEN OF KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS BY MORPHOLINO OLIGOMERS

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Kaposi's sarcoma-associated herpesvirus (KSHV) is associated with several human malignancies. In this study, the potential of phosphorodiamidate morpholino oligomers (PMOs) to suppress KSHV gene expression in PEL cells was explored. PMOs are analogs of short DNA oligomers with a modified backbone, resulting in highly specific binding and resistance to nucleases. PMO conjugation to an arginine-rich peptide was used to facilitate delivery into PEL cells. The KSHV replication and transcription activator (RTA) gene and latency-associated nuclear antigen (LANA) gene were targeted because of their key roles in activating KSHV lytic replication and maintaining KSHV latency, respectively. High efficiency PMO uptake by BCBL-1 cells was observed and PMO persistence in the cells lasted for over three days after one application. Treatment of BCBL-1 cells with a PMO against RTA resulted in reduction of RTA expression in a dose-dependent manner. Expression of several KSHV early and late genes, including a viral homologue of interleukin-6 (vIL-6), viral interferon regulatory factor-1 (vIRF-1) and a virion-associated envelope glycoprotein encoded by ORF-K8.1A, was also reduced in a dose-responsive manner, suggesting that KSHV lytic replication was blocked by the PMO. Cell viability assay showed no cytotoxicity by the PMO. Treatment of BCBL-1 cells with PMOs against LANA resulted in reduction of LANA expression. The results suggest that PMOs could specifically block KSHV replication, and warrant further study.

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THE KSHV ONCOPROTEIN VFLIP CONTAINS A TRAF-INTERACTING MOTIF AND REQUIRES TRAF2 AND TRAF3 FOR SIGNALING

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Primary effusion lymphomas (PELs) depend on the expression of the viral FLICE/caspase 8-inhibitory protein (vFLIP) for their survival. This effect is achieved by activation of the transcription factor NF-κB. Tumor necrosis factor (TNF) receptor-associated factors (TRAFs) are direct mediators of NF-κB signaling by TNF family receptors and the EBV viral oncoprotein LMP1, so we assessed the role of TRAFs in signaling by vFLIP. A TRAF-interacting motif was identified in vFLIP that is not present in other FLIP molecules, and single amino acid substitutions in this domain abolish induction of NF-κB. vFLIP coprecipitates with TRAF2 in PEL cells, and mutations in the TRAF-interacting motif of vFLIP abolish binding. TRAF2 was shown to be essential for NF-κB activation by vFLIP using a dominant negative TRAF2 deletion mutant. Elimination of TRAF2 and TRAF3 in PEL cells by RNA interference results in dramatic inhibition of NF-κB activity and JNK phosphorylation, but no effect was seen following suppression of TRAF1, TRAF5 or TRAF6. We found that TRAF2, but not TRAF3, mediates association of vFLIP with the IKK complex. These data indicate that TRAF2 and TRAF3 are essential for vFLIP-mediated signaling, which is critical for KSHV-associated lymphomagenesis.

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LATENCY-ASSOCIATED NUCLEAR ANTIGEN (LANA/ORF73) OF KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS (KSHV/HHV-8) ROLE IN B-CELL DIFFERENTIATION IN TRANSGENIC MICE

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Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) is a human lymphotropic herpesvirus. It is implicated in B cell neoplasia, such as primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD). The latency-associated nuclear antigen (LANA) is consistently expressed in all KSHV-associated tumor cells, and was shown to bind the tumor suppressor proteins, p53 and pRb. We generated transgenic mice expressing LANA under the control of its own promoter, which is B-cell specific. All LANA transgenic mice develop splenic follicular hyperplasia, due to an expansion of IgM^+IgD^+ mature B-cells. Furthermore, we observed post germinal center lymphomas in older animals similar to MCD, implying that LANA can predispose activated B-cells to lymphomagenesis.

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CELL AND PROMOTER-SPECIFIC TRANSACTIVATION BY THE KSHV ORF57/MTA PROTEIN

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We have previously demonstrated that the KSHV ORF57/Mta protein is a transactivator that increases expression of heterologous genes both transcriptionally and post-transcriptionally. In transiently-transfected CV-1 cells, ORF57 had no independent ability to transactivate a series of KSHV promoters, but dramatically synergized with ORF50/Rta in a promoter-specific fashion. We extended these studies in six Human cell lines of different lineages: multiple myeloma, Burkitt's lymphoma, and an epithelial cell line. Among our panel of KSHV promoter/reporter plasmids, the PAN promoter was transactivated by Mta in both Akata-31 and 293 cells, and the ORF57 promoter was transactivated only in Akata-31 cells. We extended the studies by testing deletion mutants of the KSHV promoters, and synthetic promoters containing only multimerized consensus sites for single, specific cellular transcription factors. These data demonstrate that Mta-mediated transactivation is specific for particular upstream promoter elements in both cell lines, but requires only a functional TATA box to strongly activate transcription in Akata-31 cells. Multiple experiments demonstrate that the effects of Mta are indeed transcriptional and not post-transcriptional, including: 1. the magnitude of transactivation is independent of the basal strength of each promoter, and 2. transactivation does not differ when the same promoter is tested with different reporter molecules. We also show that Rta activates transcription in all cell lines tested, but that Mta synergizes with Rta only in Akata-31 and BL-41 cells. Direct interaction of Rta with Mta requires the intact leucine repeat in Rta, but the functional consequences of this interaction are currently unknown. We have used DNA affinity chromatography with a promoter element required for synergy as bait to identify a number of candidate cellular proteins that we are testing for their role in these Mta-mediated functions.

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KSHV ORF57 CONTAINS THREE NUCLEAR LOCALIZATION SIGNALS IMPORTANT FOR ACCUMULATION OF CYTOPLASMIC KSHV ORF59 mRNAs

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Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) is etiologically linked to the development of endothelial Kaposi's sarcoma, primary effusion lymphoma (PEL), and multicentric Castleman's disease. KSHV ORF57 is an early lytic, nuclear protein conserved in all members of the herpesvirus family. Its homologs in other herpesviruses have profound trans-regulatory effects on the expression of other viral genes at the posttranscriptional level and are essential for viral replication. Here we have demonstrated by mapping analysis that the N-terminal half of the ORF57 contains three nuclear localization signal (NLS) motifs, all enriched with basic amino acid residues. Nuclear localization of the ORF57 could be blocked by simultaneous introduction of point mutations into all three NLS motifs. In a heterologous cytoplasmic protein, HPV6 E6, individual NLS motifs from ORF57 also functioned independently to convert the cytoplasmic protein into a nuclear protein. Moreover, the newly identified NLS motifs of ORF57 was found being involved in enhancement of KSHV ORF59 expression in transient co-transfection assays. Despite their proper localization, ORF57 with point mutations in any two of the three NLS motifs conferred little or no enhancement of ORF59 expression due to a significant reduction of ORF57-mediated cytoplasmic accumulation of ORF59 transcripts. Together, the NLS motifs identified in this study, besides promoting nuclear import of ORF57, appear to play an important role at the posttranscriptional level.

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GENE STRUCTURE AND EXPRESSION OF KSHV ORF56, 57, 58, AND 59

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Though the structure of KSHV genome is similar to herpesvirus saimiri and EBV, KSHV genome features much more unique split genes and commonly encodes its genes with signature of bicistronic or polycistronic transcripts. We have recently analyzed in details the gene structure and expression of KSHV ORF56 (primase), 57 (MTA), 58 (BMRF2 homologue), and 59 (DNA polymerase processing factor) in butyrate-activated KSHV-positive JSC-1 cells. We have demonstrated that KSHV ORF56 is expressed at much less abundance than ORF57 as a bicistronic ORF56/57 transcript, which utilizes the identical ORF57 intron for its RNA splicing. For polyadenylation, both ORF56 and ORF57 utilize the same poly A site at nt 83608 in the virus genome and the majority of two transcripts are cleaved at nt 83628 for poly A addition. Similarly, KSHV ORF58 and ORF59 are transcribed from the opposite strand of the virus genome by using different transcription start sites, nt 95990 for ORF58 and nt 96794 for ORF59, but share two overlapped, alternative poly A sites at nt 94492 and 94488. Interestingly, each poly A signal has its own cleavage site for poly A addition at a position +11 or +14, both of which are used with equal efficiency. Thus, low abundant KSHV ORF58 is expressed as a monocistronic transcript with an unusually long 5'-untranslated region (UTR), but an extremely short 3' UTR, whereas KSHV ORF59 is expressed in high abundance as a bicistronic transcript with a short 5' UTR, but a long (>1 kb) 3' UTR. Moreover, expression of ORF56 and ORF59 can be up-regulated significantly by KSHV ORF57 in transient co-transfection assays.

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ROLE OF MULTIMERIZATION OF THE LYTIC SWITCH PROTEIN IN REACTIVATION OF KSHV FROM LATENCY

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We have previously demonstrated that the kshv ORF50/Rta protein is necessary and sufficient to disrupt viral latency and induce the viral lytic cycle, a critical step for kshv pathogenesis. Our preliminary mechanistic studies indicate that homo-multimerization of Rta is critical for this function. We have previously described a truncated mutant of rta (called ORF50ΔSTAD) that specifically inhibits transactivation by wild-type Rta and suppresses the ability of wild-type Rta to reactivate the virus. This dominant negative inhibitor is co-immunoprecipitated in complex with wild type rta in vitro and in transfected cells. Rta's N-terminus contains a heptad repeat of leucine amino acids that is part of a degenerate basic/leucine zipper motif (bZIP); deletion of this domain ablates the function of the cognate Rta protein, eliminates the ability of ORF50ΔSTAD to function in a dominant negative fashion, and prohibits multimer formation. However, ORF50ΔSTAD also contains essential domains required for direct DNA binding by Rta and heteromeric interactions with cellular transcription factors, functions that we are currently attempting to separate genetically from multimerization. Among a number of KSHV promoters with direct binding sites for Rta, deletion of the ZIP domain has little effect on DNA binding by purified Rta to the KbZIP promoter, but results in formation of novel DNA-protein complexes. Using a non-denaturing polyacrylamide gel system, we have found that C-terminal truncation of full-length Rta converts the cognate protein from a dimeric or trimeric form to a higher-order multimer. Moreover, N-terminal truncation mutants of Rta are also capable of forming homo-multimers. Taken together, our data suggest that the N-terminal leucine repeat is the primary mediator of Rta multimerization, but the potential of Rta to form higher-order multimers is limited by the C-terminus of the protein.

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THE CHICKEN OR THE EGG PROBLEM: DOES THE LYTIC SWITCH PROTEIN RTA PROMOTE ESTABLISHMENT OF KSHV LATENCY?

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Kaposi's sarcoma and primary effusion lymphoma are AIDS-associated malignancies caused by Kaposi's sarcoma-associated herpesvirus (KSHV, HHV-8). KSHV exploits two programs of viral gene expression (lytic and latent) and both contribute to tumor growth. In advanced tumors, most cells harbor latent KSHV and are resistant to conventional antiherpetic therapies. Design of effective treatments will require a better understanding of how KSHV switches between the two states. Studies from our laboratory and others have uncovered an unexpected degree of crosstalk between two programs. This helps to explain KSHV's preference for latency and its reluctance to undergo reactivation.

Lytic replication is initiated by the transcription factor RTA and involves many viral genes. This contrasts with latency, where only a few genes are expressed, mainly from a gene cluster that codes for 4 proteins (Kaposin, v-FLIP, v-cyclin and LANA) and 11 microRNAs. All are transcribed from three major promoters - termed LT_c, LT_i and LT_d – as an elaborate network of spliced transcripts specifying several combinations of products. The LT_c and LT_d promoters are constitutive, whereas LT_i is dependent on RTA. Activation of LT_i raises levels of LANA, a multifunctional protein that inhibits activation of lytic promoters. Thus, RTA creates a feedback loop that reinforces latency. Activation of LT_i by RTA requires histone deacetylase (HDAC) activity and is blocked by small-molecule HDAC inhibitors. These same agents are potent inducers of lytic reactivation, and it is likely that selective inhibition of LT_i lowers LANA levels, derepressing other the lytic promoters activated by RTA. The intertwined functions of the lyitc and latent regulators bring to mind a popular conundrum: which comes first the chicken (latent) or the egg (lytic)? The answer, as everyone knows, is that one gives rise to the other!

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IDENTIFICATION AND CHARACTERIZATION OF THE KSHV ORF49 PROTEIN

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Kaposi's sarcoma-associated herpesvirus (KSHV) is the etiological agent of Kaposi's sarcoma (KS), primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD). KS is the most common neoplasm among HIV-positive individuals. Like other herpesviruses, KSHV is able to establish a predominantly latent, life-long infection in its host. The KSHV lytic cycle can be triggered by a number of stimuli that induce the expression of the key lytic switch protein, the replication and transcription activator (RTA) encoded by Orf50. The expression of Rta is necessary and sufficient to trigger the full lytic program resulting in the ordered expression of viral proteins, release of viral progeny and host cell death. We have characterized an unknown open reading frame, Orf49, which lies adjacent and in the opposite orientation to Orf50. Orf49 is expressed during the KSHV lytic cycle and shows early transcription kinetics.

We have mapped the 5' and 3' ends of the unspliced Orf49 transcript which encodes a 30kDa protein that is expressed in both the nucleus and the cytoplasm. Interestingly, we found that Orf49 was able to cooperate with Rta to activate a number of KSHV lytic promoters containing AP-1 sites. The Orf49-encoded protein was also able to induce transcriptional activation through c-Jun but not ATF1, ATF2, or CREB transcription factors. We found that Orf49 could induce phosphorylation and activation of the transcription factor, c-Jun, and the Jun N-terminal kinase (JNK). Our data suggests that Orf49 functions to activate the JNK pathway during the KSHV lytic cycle.

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TRANSCRIPTIONAL REGULATION OF THE KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS (KSHV) K15 GENE

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The K15 gene product of KSHV is a transmembrane protein that is encoded by the last open reading frame of the KSHV genome. The K15 protein has been implicated in the modulation of B-cell signal transduction and the activation of the Ras/mitogen-activated protein kinase (MAPK) and NF-κB signal transduction pathways. Here we report the identification of the transcriptional start site of the full-length K15 gene in KSHV-positive BCBL-1 cells. We have mapped the K15 transcriptional start site to a position 152 nucleotides upstream from the translation start site by rapid amplification of cDNA ends (RACE) and RNase protection assays. We have also characterized the K15 promoter element. To analyze the *cis*-acting elements necessary to regulate K15 gene expression, a series of 5' promoter deletion constructs were generated and subcloned upstream of the luciferase reporter gene. Transcriptional assays with these mutant promoters demonstrated that chemical induction in latently infected KSHV-positive BCBL-1 cells activated K15 transcription. In addition, K15 promoter transactivation was also mediated by the viral immediate-early protein, ORF50/Rta, suggesting that the K15 gene is actively transcribed during lytic replication.

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LONG TERM CELL SURFACE IMMUNOMODULATION FOLLOWING KSHV INFECTION IN CULTURE IS INDEPENDENT OF HORIZONTAL VIRAL SPREAD

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KSHV is the causative agent of the highly vascularized endothelial cell tumor Kaposi's sarcoma, as well as several lymphoproliferative disorders. As is characteristic of all herpesviruses, the lifecycle of KSHV consists of two phases: latency, marked by a highly restricted program of viral gene expression, and lytic replication, which ultimately results in productive release of virions. The recently identified phenomenon of an immediate burst of lytic gene expression following de novo KSHV infection challenges the conceptual rigidity of these two lifecycle phases in in vitro tissue culture systems (Krishnan et al, 2004). Similarly, modulator of immune recognition 2 (MIR2), encoded by viral ORF K5, is classified as a lytic gene, but downregulates immune molecules, such as PE-CAM, ICAM-1, and MHC class I, within the first few days following KSHV infection, as demonstrated by reversal of these effects following knockdown of MIR2 expression with anti-K5 siRNA (Tomescu, Adang, Kedes; in preparation). A related viral gene, ORF K3, which encodes MIR1, does not appear to be expressed during the 'lytic burst' following infection but may contribute to immune evasion during lytic replication or latency. We are actively investigating the roles of MIR1 and MIR2 in the downregulation of immunomodulatory molecules in long term KSHV infected cultures in the presence of phosphonoacetic acid (PAA), which blocks horizontal spread of the virus. This will allow us to elucidate the respective roles of MIR1 and MIR2 expression in long term cultures, independent of subsequent rounds of infection with their accompanied bursts of lytic gene expression. Preliminary results suggest that the pattern of downregulation may vary over time. These observations may reflect temporal variation in immune evasion strategies employed by KSHV to counter the equally dynamic changes in host defenses that it encounters during the different phases of infection.

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AN *IN VIVO* MODEL FOR KSHV GENE EXPRESSION AND RECAPITULATION OF A HUMAN SEROLOGIC RESPONSE

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Background: An inability to observe gene expression longitudinally *in vivo* has hampered development of strategies for the treatment and prevention of KSHV-related pathology.

Methods: We investigated KSHV gene expression following IV inoculation of SCID-hu mice. Using RT-PCR, we measured KSHV DNA levels and RNA transcriptional activity while tracking viral protein expression using immunohistochemical (IHC) assays. Mice inoculated with UV-inactivated KSHV served as controls. Using immunofluorescence assays (IFA), we also investigated KSHV-specific human antibody production.

Results: Over the 3 months following infection, transcriptional activity for both latent and lytic genes increased within 13 of 13 experimental murine spleens, at levels 10,000-fold greater than UV-KSHV controls. Within chimeric tissue, IHC revealed expression of the latency-associated nuclear antigen (LANA) that paralleled its mRNA levels. IFA revealed KSHV-specific human IgG responses within 4/13 mice with a staining pattern reminiscent of LANA reactivity. Finally, short-term preemptive treatment with IP ganciclovir (GCV) reduced KSHV RNA and DNA levels in 9/9 treated animals for up to 3 months.

Conclusion: SCID-hu mice are capable of supporting KSHV latent and lytic gene expression longitudinally, and inclusion of human immune tissue grafts in this model results in the gradual appearance of KSHV-specific human serologic responses. Furthermore, preemptive treatment with IP GCV inhibits latent and lytic gene expression in the chimeric model. Future experiments will include more detailed characterization of infected cell types and human immune responses.

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ROLE OF DC-SIGN AND L-SIGN IN THE TRANSMISSION AND PATHOGENESIS OF KAPOSI SARCOMA HERPES VIRUS (KSHV/HHV-8)

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B cells the latent reservoir for KSHV (Kaposi Sarcoma related Herpesvirus) are not readily infected in cell culture. We therefore hypothesize that KSHV may bind to receptors such as DC-SIGN and L-SIGN present on dendritic cells and endothelial cells in order to enhance the infection or transmission of KSHV in these cells. To perform these studies we used monocyte derived dendritic cells, the BTHP-1 cell line (Raji derivative-B cell line) and 293T cells expressing either L-SIGN or DC-SIGN. The cells were infected with a KSHV viral stock derived from BCBL-1 cells. The results of our study show substantial increases in KSHV viral load in the dendritic cells and BTHP-1 L-SIGN expressing cells compared to the control cells as assessed by a K6 quantitative PCR assay. Additionally, these cells showed a striking change in morphology leading us to hypothesize these cells may be lytically infected. RT-PCR assays were used to follow the progression of infection in the BTHP-1 L-SIGN cells and results show both lytic and latent viral gene expression (ORF 50, vGPCR, K8.1 and LANA). Additionally, KSHV infection was greatly inhibited when these cells were preincubated with SIGN specific antibodies or mannan prior to KSHV infection. The viral load in the dendritic cells was decreased when the viral stock was treated with the KSHV specific glycoprotein B antibody prior to infection, leading us to believe gB may bind to DC-SIGN. These data indicate a potential role for DC-SIGN and L-SIGN as receptors for the infection and pathogenesis of these cells by KSHV.

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NOVEL USES OF THE PROTO-ONCOGENIC NOTCH SIGNALING PATHWAY BY KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS

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We have previously demonstrated that the KSHV lytic switch protein, ORF50/Rta, functions as a ligand-independent inducer of RBP-Jk (Recombination Signal Binding Protein-Jk; aka. CBF-1, CSL), the target of the Notch proto-oncogenic signal transduction pathway. However, our data suggest that Rta regulates RBP-Jk activity in a fundamentally different fashion than the well-characterized cellular Notch and EBV EBNA-2 proteins. Although binding of RBP-Jk to the KSHV ORF57/Mta and TK promoters is required for transactivation by Rta, purified Rta makes direct contacts with DNA on both sides of RBP-Jk to form a trimeric complex in a promoter specific fashion. Moreover, neither constitutively-active RBP-Jk (RBP-Jk/VP16) nor Notch 1 (NICD) alone can transactivate the ORF57 promoter in transient transfections; however, co-transfection of an Rta mutant lacking its transactivation domain (ORF50ΔSTAD) robustly restores activation, an effect that requires both the intact Rta and RBP-Jk binding sites on the promoter. This suggests that instead of converting DNA-bound RBP-Jk from a repressor to an activator of transcription, Rta promotes DNA binding by RBP-Jk to use it as a scaffold for its own promoter association and transactivation. We have further tested this hypothesis by immuno-precipitating chromatin from PEL cells using an RBP-Jk-specific antibody; among a series of viral and cellular promoters containing RBP-Jk elements, we have found that RBP-Jk is enriched on the Mta promoter only following TPA-mediated viral reactivation. Finally, we have found that ORF50ΔSTAD can potently synergize with NICD in activating the ORF57 promoter, suggesting that Rta and NICD can contact DNA bound RBP-Jk simultaneously, and further supporting a novel mechanism for Rta's interaction with the Notch pathway.

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SIGNAL TRANSDUCTION BY HUMAN AND HHV-8 IL-6 ARE QUALITATIVELY DISTINCT AND THAT BY VIRAL IL-6 IS INFLUENCED BY THE NON-SIGNALLING gp80 RECEPTOR SUBUNIT

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Human herpesvirus-8 (HHV-8) specifies a functional homologue of interleukin-6, viral IL-6 (vIL-6), that has been implicated as a contributor to HHV-8 associated neoplasia. Like its mammalian counterparts, vIL-6 mediates signal transduction through the gp130 receptor, but in contrast to cellular IL-6 proteins, vIL-6 can effect dimerization of gp130 to initiate receptor tyrosine phosphorylation and signal transduction in the absence of the non-signalling gp80 α-subunit of the IL-6 receptor. STAT1, STAT3 and MAPK signalling by vIL-6 have been reported to mirror that triggered by human IL-6 (hIL-6). By comparing the amplitude and duration of vIL-6 and hIL-6 induced STAT1, STAT3 and SHP2 activation and gp130 tyrosine phosphorylation, we have found that these cytokines have clearly distinguishable signalling activities. Further, utilization in our experiments of a gp80-refractory vIL-6 variant, vIL-6.25 (R189L) revealed that the non-signalling gp80 receptor can have a marked influence on gp130 phosphorylation and downstream signal transduction. Unlike hIL-6 and vIL-6.25, vIL-6 was able to induce sustained hyperphosphorylation of gp130, prolonged superactivation of STAT1, and increased activation of SHP2. Five of the six cytoplasmic tyrosine residues of gp130 were phosphorylated efficiently by vIL-6, with vIL-6.25 and hIL-6 leading to relatively weak gp130 phosphorylation and preferential targeting of two of the tyrosines. Our data provide evidence that ligand- and coreceptor-induced conformational differences in gp130 signalling complexes can have profound quantitative and qualitative effects on downstream signalling events, and in the presence of gp80 vIL-6 is an unusually potent signalling ligand that may be resistant to negative feedback regulation. Such prolonged and high-level signalling may be relevant to the development of HHV-8 associated malignancies.

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STRUCTURAL REQUIREMENTS FOR gp80-INDEPENDENCE AND INTRACELLULAR RETENTION OF HHV-8 IL-6

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Human herpesvirus-8 (HHV-8) interleukin-6 (vIL-6) displays 25% amino acid identity with human IL-6 (hIL-6) and shares overall four-helix-bundle structure and gp130-mediated STAT/MAPK signalling with its cellular counterpart. However, vIL-6 is distinct in that it can signal through gp130 alone, in the absence of the non-signalling gp80 α-subunit of the IL-6 receptor, and that a large fraction of the protein is retained intracellularly, rather than being efficiently secreted. To investigate the structural requirements for gp80-independent signal transduction by vIL-6 and for its intracellular localization, we constructed a series of expression vectors encoding vIL-6/hIL-6 "domain-swap" and site-mutated IL-6 proteins. These constructions were utilized in transient transfection assays to measure activities through gp130 versus gp130/gp80 and secretion versus intracellular localization. Our data indicate: (1) Specific gp130-interacting residues and regions of vIL-6 cannot confer gp80 independence to hIL-6; (2) While the N- and C-terminal regions of hIL-6 can substitute for those of vIL-6 to retain gp80 independence, each helix of vIL-6, including receptor non-interacting helix-B, is indispensable for gp80 independence; (3) Residues within vIL-6 helix-C are necessary for intracellular concentration of the viral cytokine, but cannot confer this property to hIL-6; (4) There is no correlation between intracellular localization of vIL-6 and gp80 independence; (5) Both gp80 dependent and gp80 independent signalling by vIL-6 can occur intracellularly, as well as extracellularly. We conclude that the gp80 independence of vIL-6 is a consequence of its overall primary sequence and tertiary structure, not conferred by discrete residues and regions, and that vIL-6 may interact, in part via C-helical residues, with an intracellular partner to prevent secretion of the viral cytokine. We are currently investigating the signalling consequences of intracellular retention to help elucidate the biological implications of our findings.

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SUBNANOMETER-RESOLUTION STRUCTURE OF KSHV CAPSID FROM ELECTRON CRYOMICROSCOPY

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Kaposi's sarcoma-associated herpesvirus (KSHV) and Epstein-Barr virus are the two known human pathogens of the gammaherpesvirus subfamily of the *Herpesviridae*. Structural studies of these two DNA tumor viruses have been limited by sample preparation. We have recently purified sufficient amounts of KSHV capsids using velocity gradient centrifugation from the media of BCBL-1 cells to determine the structure of the KSHV capsid to a resolution of 9-Å. We used a 200kV electron cryomicroscope with a charge-coupled device to image over 2000 micrographs, from which 4097 particle images were obtained for 3D reconstruction. This reconstruction has allowed structural comparisons with other human herpesviruses at a secondary structure (alpha helices) level. The KSHV capsid shares the organization and icosahedral shape typical of herpesviruses, with pentons, hexons and connecting triplexes arranged on a T=16 icosahedral lattice. The KSHV triplex monomer protein, TRI-1 (ORF62), has a less extended and significantly smaller structure than its structural counterpart in herpes simplex virus type 1 (HSV-1), an alphaherpesvirus, consistent with their different amino acid sequence lengths. The crystal structure of the upper domain of the major capsid protein (MCP) from HSV-1 was fit into the KSHV MCP density. Although internal alpha helices match well between MCP upper domains of KSHV and HSV-1, many unique structure features or rearrangement are apparent in the KSHV MCP, particularly near the highest radial regions. These observed structurally unique features in both MCP and TRI-1 correlate well with the different patterns of tegument interactions between gammaherpesviruses and alphaherpesviruses.

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IN VIVO ANALYSIS OF A GAMMAHERPESVIRUS VIRAL CYCLIN

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Gammaherpesviruses establish a chronic infection that is often associated with disease in immunocompromised patients. Although these viruses encode multiple oncogenes, the contribution of these oncogenes to chronic infection and disease are poorly understood. We have focused our analysis on the viral cyclin, a known oncogene in KSHV that is evolutionarily conserved with many other gammaherpesviruses. While much is known about the capacity of the KSHV viral cyclin in vitro, and in tumor cell lines, its role in virus infection is difficult to address. We have analyzed the in vivo role of the viral cyclin through analysis of murine gammaherpesvirus-68, a closely related small animal gammaherpesvirus. In vivo analysis of viral cyclin-deficient viruses has demonstrated that while the viral cyclin is dispensible for lytic replication and the establishment of latent infection, the viral cyclin is critical for reactivation from latent infection. Significantly, viral cyclin-deficient viruses are dramatically attenuated in chronic disease, including vascular disease and long-term mortality. Based on these initial studies, we have further identified a key role of the viral cyclin in vivo: the viral cyclin is required very specifically to oppose the activity of a cellular cyclin-dependent kinase inhibitor and tumor suppressor, p18Ink4c. As a result, the requirement for the viral cyclin is almost completely removed upon infection of mice that lack p18Ink4c. These studies highlight the importance of the viral cyclin in chronic infection, and suggest that targeted therapeutics to the viral cyclin (or therapeutics that may enhance the activity of p18Ink4c) may attenuate KSHV-associated diseases.

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GENETICS OF TUMOR SUPPRESSION IN THE AVIAN MAREK'S DISEASE MODEL

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Marek's disease virus (MDV) is one of the most oncogenic herpesviruses in nature. T-cell lymphomas caused by MDV infections in chickens provide a model for studying how genetic contributions of the host influence the incidence of herpesvirus-induced tumors. In chickens genetic composition, especially at the major histocompatibility complex (MHC), has a strong role in determining whether MDV infection progresses to tumor formation. We have used MHC recombinant haplotypes to test the hypothesis that allelic differences at antigen presenting MHCI loci are the basis for the strong MHC influence in Marek's disease. We found marked differences in tumor formation in two fully congenic lines carrying double recombinant haplotypes (*BR2* and *BR4*). This difference in tumor incidence between *BR2* (18%) and *BR4* (47%) animals is similar to differences previously observed between other resistant and susceptible haplotypes. Interestingly, rather than *MHCI*, the crossover breakpoints in *BR2* and *BR4* define *BG1* to be the locus contributing to the difference in tumor incidence. *BG1* likely serves in signaling from the cell surface, indicating that differential cell activation, rather than antigen presentation, may be the basis for the MHC-related genetic differences observed in avian MDV tumor formation.
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RHESUS MONKEY RHADINOVIRUS PROTEINS REVEALED: 33 AND COUNTING

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Rhesus monkey rhadinovirus (RRV) is one of the closest phylogenetic relatives of KSHV. Unlike KSHV, RRV replicates to high titer in cell culture and, thus, serves as an effective model for studying primate gammaherpesvirus structure and virion proteomics. Our goal is to develop a better understanding of the repertoire of proteins that comprise intact gammaherpesviruses since at least some of these proteins are likely to have critical functions not only in viral structure and assembly but also in the early stages of infection, including evasion of the cell's rapidly deployed anti-viral defense. We have analyzed the protein content of purified RRV virions using two complementary mass spectrometric approaches and have found the particles contain at least 33 distinct virally-encoded proteins. We have tentatively assigned seven of the virally encoded proteins to the capsid, 17 to the tegument and nine to the envelope. Five of the putative tegument proteins are gammaherpesvirus-specific of which four have no known function. Additionally, we found three novel proteins not previously reported as virion-associated, including two putative tegument proteins and one envelope protein. Seven of the 33 proteins are novel gamma-2-herpes virion-associated proteins. In parallel experiments, we have extracted RRV virions with nonionic detergent and subjected the resultant particles to similar analyses. In addition to the expected capsid structural proteins, MS/MS of these particles consistently detects peptide fragments from six distinct tegument proteins. These data suggest that this subset of tegument proteins may interact more directly with and with higher affinity for the underlying capsid components and, in turn, may play a role in assembly or transport of viral or subviral particles during entry or egress. Experiments to explore these possibilities are underway.

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EVIDENCE FOR RETROPERITONEAL FIBROMATOSIS HERPESVIRUS (RFHV) INVOLVEMENT IN THE ETIOLOGY OF KAPOSI'S SARCOMA-LIKE TUMORS OF MACAQUES WITH SAIDS

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Old World primates are hosts to two distinct lineages of rhadinoviruses related to Kaposi sarcoma (KS)-associated herpesvirus (KSHV), the causative agent of KS. In macaques, the RV1 rhadinovirus lineage is represented by retroperitoneal fibromatosis (RF) herpesvirus (RFHV) which was discovered in RF, a KS-like tumor of macaques with SAIDS. The RV2 lineage is represented by rhesus rhadinovirus (RRV), a more distantly related rhadinovirus, which has, as yet, no human counterpart. In order to determine the roles of the RV1 and RV2 rhadinoviruses in the development of RF, we have cloned and sequenced the majority of the RFHV genome and have developed assays and reagents to differentially detect and quantitate RV1 and RV2 rhadinoviruses. Strong similarities were detected between the RFHV and KSHV genomes, in gene content and sequence. Comparison of the RFHV ORF 73 homolog of the major tumor-associated latency antigen (LANA) of KSHV, which is believed to play an important role in tumorigenesis, revealed a conservation of important structural and functional features which were not found in RV2 rhadinoviruses. We show that a monoclonal antibody to KSHV LANA reacts with RFHV LANA which is detected in the nucleus of the spindle-shaped tumor cells characteristic of RF lesions. Rhadinovirus lineage-specific quantitative PCR assays revealed a strong association between the RV1 rhadinovirus, RFHV, and RF tumors in SIV- and SRV-2 associated SAIDS-RF which was not seen with macaque RV2 rhadinoviruses. Our studies implicate RFHV in the etiology of RF tumors in macaques and support the development of SAIDS-RF as a relevant animal model of AIDS-KS.

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DISCOVERY OF SEVEN NEW POTENTIAL TUMOR MARKERS

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Introduction:

In our previous works using the approach of computational differential display we identified a considerable amount of human tumor-specific EST clusters. The next step was to test their tumor-specificity in experiments *in vitro*.

Material and methods:

To experimentally verify the results obtained *in silico* we conducted a series of PCR experiments with specific primers on the commercial (Clonthech®, Biochain®) and hand-made cDNA panels from different normal and tumor tissues.

RNA from surgery material was extracted using GuSCN/CsCl method. cDNA was then synthesized using Revert Aid® First Strand cDNA Synthesis Kit (Fermentas).

Results:

We studied more than 40 clusters described in silico as tumor-specific and found 7 cDNA sequences among them which were indeed tumor-specific in experiment in vitro. Corresponding RNAs appeared to be expressed in various tumors (such as tumors of breast, lung, ovary, testis, cervix etc.), but at the same time were expressed in a limited number of normal tissues or were not expressed in normal tissues at all.

Conclusions:

Taken together, our computational and experimental data confirm that in silico search may be useful approach for discovery of new tumor-specific sequences.

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BIAS IN STANDARDIZED INCIDENCE RATIOS FOR AIDS-RELATED CANCERS IN REGISTRY STUDIES

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Background: Standardized incidence ratios (SIRs) are commonly used in registry studies to estimate the relative risk (RR) of cancer in people with AIDS (PWA), by comparing incidence in PWA to contemporaneous rates in the general population. The SIR may be a poor approximation of RR when the prevalence of AIDS and/or the risk of cancer in PWA is relatively high. To explore this issue for AIDS-related malignancies (specifically Kaposi's sarcoma [KS] in males), we investigated: a) the extent of underestimation of RR by SIR; b) the magnitude of bias in inter-SIR comparisons; and c) the bias in SIRs when the general population includes more than one exposed group (such as PWA and transplant recipients).

Methods: We used published SIRs from studies conducted in the US, Uganda, and Italy. RRs were calculated using three methods 1) using incidence rates from pre-AIDS era (RR_{pre}); 2) back-calculation (RR_{bc}) from the prevalence of AIDS (P_A), with the expression RR_{bc}= SIR* (1-P_A)/ [1-(P_A*SIR)]; and 3) excluding cases known to be in PWA from general population rates (RR_{ex}).

Results: In both Uganda and US studies, SIRs for KS substantially underestimated RR (Uganda: SIR=5.3 vs. RR_{pre}=73.0 and RR_{bc}= 7.3; US: SIR=116.7 vs. RR_{pre}= 19,887 and RR_{ex}= 656). In the US, trends in SIRs for KS across calendar year of AIDS diagnosis were significantly different from trends in RR. In Italy, our approximation of the SIR for KS in transplant recipients differed greatly from the RR comparing transplant recipients with non-AIDS and non-transplant subjects (72 vs. 376, respectively).

Conclusions: Our results indicate that for certain AIDS-related malignancies such as KS, SIRs are biased estimators of RR, and trends in SIRs must be interpreted cautiously. Results also reveal considerable uncertainty in estimating the underlying RR, as evidenced by diversity of RR using the three methods. In settings where the general population is composed of multiple exposure groups, SIRs may be biased approximations of RR.

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EFFECT OF WHEAT GRASS JUICE FOR SUPPORTIVE CARE THERAPY IN SOLID ORGAN CANCER PATIENTS WITH HIV – EXPERIENCE FROM INDIA

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Background: The wheat grass is often referred to as "the blood of plant life" and has almost the same chemical structure as haemoglobin. The difference between the two is that in human blood the metallic element of the haemoglobin is Iron, while in Chlorophyll this atom is Magnesium, Chlorophyll improves the supply of Oxygen to the circulatory system. The wheat grass is also a complete protein with 30 enzymes, vitamins and minerals. It is also a complete protein with 30 enzymes, vitamins & minerals. It has been seen that it improves the immunity status of cancer patients. No study has been documented on use of wheat grass juice in HIV patients with cancer.

Aim: The aim of our study was to see the effect of wheat grass juice on improvement of immunity and quality of life in cancer patients with HIV.

Material & Methods: During period from July 2003 – June 2005 we selected 16 HIV positive terminally ill solid organ cancer patients for wheat grass juice therapy in the palliative care unit of Netaji Subhash Chandra Bose Cancer Research Institute, Kolkata. We cultivated wheat grass in our campus, when the grasses are 5 days old we took the fresh leaves including roots and made fresh juice out of that and had given 2-table spoon of juice of our 16 HIV positive patients with solid organ cancer for continuous 6 months. The haemoglobin, white blood cell count, serum total protein, IgG level and performance status were assessed in all patients before and after giving wheat grass juice.

Result: Fourteen patients were evaluable. The mean values of haemoglobin, total count, total protein and IgG level were 8.2g/dl, 3100/cum, 5.4gm% and 500mg/dl respectively before wheat grass juice therapy. The mean values of haemoglobin, total count, total protein and IgG level increase significantly (p value <.005) and were observed mean of 9.8gm%, 6.8gm% and 780mg/dl respectively. No significant increase in white cell count were seen. The performance status was improved from 50% to 70% (Karnofsky) after wheat grass treatment.

Conclusion: We concluded that wheat grass juice is an effective theraptic supportive treatment in HIV positive patients with solid organ cancer. Its use in terminally ill HIV positive patients with solid organ cancer is encouraging.

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FATAL HEMOPHAGOCYTIC SYNDROME RELATED TO ACTIVE HHV8 INFECTION IN IMMUNOCOMPETENT SUBJECTS

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HHV-8, which is involved in the etiopathogenesis of KS and rare lymphoproliferative disorders mainly in HIV infected people, has been also implicated in the development of hemophagocytic syndrome in transplant recipients or HIV positive patients with KS, in occasional cases. We report the occurrence of a fatal hemophagocytic syndrome in two immunocompetent patients with active HHV-8 infection, without evidence of any other known HHV-8 related disease. **Patient 1**: a 63-year old woman was admitted with fever, peripheral cytopenia and splenomegaly. One month before she had been started on corticosteroid treatment for autoimmune hemolytic anemia (AHA) and ten days before she had received nefrectomy for renal carcinoma. There was no evidence of metastatic disease and of bacterial or viral infection. She received eritropoietin, G-CSF and high dose intravenous immune globulin with no benefit and died with a rapidly evolving multiorgan failure. **Patient 2**: a 69-year old man was admitted for AHA and splenomegaly. He initially responded to corticosteroid treatment, but rapidly developed pancytopenia and fever. He received high dose intravenous immune globulin, acyclovir and broad spectrum antibiotics but died with rapidly progressive multiorgan failure. A bone marrow aspirate or biopsy showed in both patients a normo/hypercellular marrow with myelodysplastic features associated with signs of hemophagocytosis, without evidence of lymphomatous infiltration. Biochemical data were also concordant with HS. HHV-8 DNA was detected either in the peripheral blood or in the serum from both patients, by PCR for three different viral genes (orf-K1, ORF 73 and orf 26). The molecular analysis also allowed us to determine the HHV-8 subtype which was variant A in patient 1 and variant C in patient 2. The occurrence of different viral genotypes in the two cases was also confirmed by the analysis of ORF 73 polymorphisms. The HHV-8 viral load was determined by real time PCR for orf 26, showing the presence of an extremely high number of viral copies in both cases, indicative of an active viral infection. In patient 1 immunohistochemical analysis in the bone marrow biopsy showed the presence of HHV-8 LNA positive cells. Pancytopenia with HS, myelodysplastic features and AHA occurring in immunocompetent adults should be added to the spectrum of clinical pathologic manifestations associated with HHV-8 infection. Its frequency may be underestimated and it should be always considered in the differential diagnosis of unexplained peripheral cytopenia. Its prompt recognition and early effective antiviral treatment might be the only way to avoid its otherwise rapidly fatal evolution.

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PREVALENCE OF HUMAN HERPESVIRUS 8 AND FACTORS ASSOCIATED WITH HHV-8 INFECTION AMONG MEN WHO HAVE SEX WITH MEN IN PERU

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Background: HHV-8, a virus associated with Kaposi's sarcoma (KS) and other malignancies, is a common infection in some populations. In North America and Europe the highest HHV-8 seroprevalence is found among HIV-positive MSM. No epidemiological study of HHV-8 prevalence and risk factors has been conducted in Peru or the Andean region.

Methods: We performed a cross-sectional study to assess HHV-8 seroprevalence among 497 MSM recruited for the 2002 Peruvian HIV sentinel surveillance program using a whole viral lysate HHV-8 EIA and IFA for equivocal EIAs. We used logistic regression to estimate Odds Ratios (OR) and their 95% confidence intervals (CI) to measure the association between selected covariates and HHV-8 infection.

Results: A total of 497 samples were obtained for the analysis, 483 of which had complete demographic and laboratory data available. The HHV-8 seroprevalence was 66.5% among HIV-positive MSM (95% CI 63.1%-69.9%) and 26.7% among HIV-negative MSM (95% CI 24.4%-29.0%). Factors independently associated with HHV-8 infection were education < 12 years (OR 1.86, 95% CI 1.20-2.88), describing one's sexual role as 'passive' or anal receptive (OR 1.95, 95% CI 1.19-3.18), reporting STI symptoms during the last year (OR: 1.90, 95% CI 1.22-3.00), and HIV infection (OR 4.38, 95% CI 2.88-6.67). Also, being chronically infected with HIV-1 (based on the results of the 'less sensitive' HIV EIA or STARHS assay) was significantly associated with HHV-8 infection compared to those recently HIV-1 infected (OR: 3.98, 95% CI 1.63-9.70).

Conclusions: HHV-8 infection is a common among MSM in Lima, Peru. The factors associated with HHV-8 seropositivity (receptive role in anal sex, recent STI symptoms, and HIV infection) indicate that HHV-8 is associated with higher risk sexual behaviors. Among HIV-positive MSM, HHV-8 is more common among those with chronic rather than recent HIV infection.

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NO EVIDENCE FOR VERTICAL TRANSMISSION OF HUMAN HERPESVIRUS-8 IN SOUTH AFRICA

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In settings of high seroprevalence, human herpesvirus (HHV)-8 infection is common in children, but the rate of vertical transmission is uncertain. We conducted a study of 293 HIV-1 infected women and their infants in Soweto, South Africa in order to assess the prevalence of HHV-8 infection in pregnant women and the rate of vertical transmission of HHV-8.

Blood was drawn from mothers shortly before delivery and from infants at 15-18 months. Plasma was tested for HHV-8 using an immunofluorescence assay to detect antibodies to the latency associated nuclear antigen (LANA) and an enzyme linked immunosorbent assay that detects antibodies to the lytic antigen K8.1. Subjects were considered infected with HHV-8 if they were positive by either assay.

8.3% of infants had evidence of HHV-8 infection by 15-18 months, exactly half the adult prevalence rate found among the HIV-1 infected mothers (16.6%). Quantitative maternal K8.1 antibody optical density (OD) was not correlated with K8.1 antibody OD in the infants ($p = 0.52$). 12.5% of infants born to HHV-8 positive mothers had evidence of infection, compared to 7.3% of infants of HHV-8 negative mothers ($p = 0.25$).

Although our findings do not preclude a low rate of vertical transmission, our data demonstrate that vertical transmission of HHV-8 is uncommon and that horizontal transmission begins to occur very early in life.

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HHV8 VIRAL LOAD IS AN INDEPENDENT PROGNOSTIC FACTOR IN HHV8 RELATED LYMPHOPROLIFERATIVE DISORDERS ASSOCIATED WITH HIV INFECTION

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Background: HHV8 is associated with Multicentric Castleman's Disease (MCD), Primary Effusion Lymphoma (PEL) and some nodal lymphomas with anaplastic morphological characteristics (HHV8-Solid Lymphoma, HHV8-SL).

Patients and methods: Our study describes clinical features, outcome, immunological, virological characteristics and prognostic factors of HIV-infected patients (pts) affected by MCD and HHV8-related lymphomas diagnosed and treated in our center between April 1987 and June 2004.

Results: Nine MCD, 16 HHV-8 related lymphomas (13 PEL and 3 HHV8-SL), were diagnosed and treated out of 327 Non-Hodgkin's Lymphomas (NHL). Four MCD pts were treated only with antiretroviral therapy and highly active antiretroviral therapy (HAART), since it was available, and 5 pts with HAART plus oral etoposide. Three PEL pts were treated with CHOP-like regimen, 7 with CHOP-like regimen plus HAART and 1 with HAART alone. All 3 HHV8-SL pts were treated with CHOP-like regimen. MCD showed lower median values of HHV8 viral load and longer overall survival (OS) in comparison with HHV8-related lymphomas. When OS was stratified according to HHV8 viral load, pts with a value >40000 cp/ml had a significant shorter OS; moreover, the multivariate analysis identified HHV8 viral load >40000 cp/ml and HHV8-related lymphoma diagnosis as independent factors significantly associated with an increased risk of death.

Conclusions: High levels of plasma HHV8 DNA are linked to a bad prognosis in HHV8-related lymphoproliferative disorders in HIV setting, and HHV8 viral load can be an important prognostic marker.

Supported by ISS grants.

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ISOPENTENYLPYROPHOSPHATE AND DAUDI TUMOR CELLS STIMULATE COMMON AND DISCRETE SUBSETS OF Vγ2Vδ2 T CELLS

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Gammadelta (γδ) T cells account for 1-10% of $CD3^+$ lymphocytes in the peripheral blood and mostly express a heterodimeric T cell receptor (TCR) with $V\gamma2^+$ and $V\delta2^+$ chains. Although the Vγ2Vδ2 subset is defined by the shared expression of common TCR gene segments, these TCRs are highly diverse due to characteristic N nucleotide insertion and deletion at the complementarity-determining region 3 (CDR3) of both γ- and δ-chains. Vγ2Vδ2 T cells recognize alkylphosphates that are ubiquitous intermediates in isoprenoid biosynthesis and tumor cells derived from hematopoietic malignancies in a non MHC-restricted, TCR-dependent manner. Previous work from our lab demonstrated that a model alkylphosphate, isopentenylpyrophosphate (IPP), specifically selects $J\gamma1.2^+$ chains and selectively skews the Vγ2 repertoire toward longer chain lengths. We assumed that Vγ2Vδ2 recognition of alkylphosphates and tumor cells was common and hypothesized that Daudi B cells, the model tumor target for Vγ2Vδ2 T cells, would similarly promote the outgrowth of Vγ2Vδ2 lymphocytes with longer, $J\gamma1.2^+$ Vγ2 TCRs. Peripheral blood mononuclear cells (PBMC) from 6 donors were stimulated *in vitro* with interleukin-2 (IL2) alone, IL2 and IPP, or IL2 and irradiated (120 Gy) Daudi tumor cells. Comparison of Vγ2 CDR3 sequences from three donors before and after stimulation revealed specific and general responses to IPP and Daudi antigens. Importantly, the major responses to either antigen were overlapping, thus explaining the observation that IPP stimulated expansion leads to increased cytotoxicity for Daudi.

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ACCURATE PREDICTION OF THE BINDING AFFINITY OF NNRTI WITH HIV-1 REVERSE TRANSCRIPTASE

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Quantitative prediction of the binding affinity of drug candidates to their target, be it protein or DNA, relies on the accurate description of protein/DNA-ligand interactions. We have studied the binding of non-nucleoside HIV-1 reverse transcriptase (RT) inhibitors (NNRTI) with RT in order to facilitate the lead optimization of NNRTI. NNRTI bind in a mainly hydrophobic pocket that is separate from the polymerase active site in RT. We used the Linear-Response method (LRM) with the Liaison software (Schrödinger Inc) for a series of 2,3-dihydrothiasolo[2,3-a]isoindol-5(9bH)-ones. In the LRM method, the binding energy is correlated with the van der Waals and electrostatic interactions between the inhibitor and protein as well as the energy required to create the cavity in the binding pocket. Using the hybrid Monte Carlo sampling method in Liaison, the correlation between predicted and experimental binding energy was good for compounds that are closely related. These compounds differ by only small structural modifications such as having one or two nitrogen atoms in the benzene motif, the location of the nitrogen, and presence of a methyl group. The relationship levels off when more diverse compounds were included. We explore the limitations in the practical use of LRM calculations in real-world drug development, especially for systems that involve mainly hydrophobic interactions.

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MACROPHAGES WITH HIV-1-INTEGRATION INVOLVED IN MALIGNANT PATHOGENESIS

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Background: HIV-1 integration, which typically occurs randomly, is important in establishing viral replication and expression. Recent reports identifying non-random HIV-1 integration suggest that disease pathogenesis may result from disruption or enhancement of gene regulation in selective situations. We recently described high HIV-1 proviral DNA (HIV DNA) in circulating macrophages (CD14/CD16 phenotype). We were interested in determining if $CD14^+/CD16^+$ monocytes/macrophages (M/MΦ) with high HIV DNA had specific HIV-1 integration sites potentially important in the pathogenesis of HIV-1-associated malignancies (HAM).

Methods: Isolated M/MΦ from HIV-1-uninfected donors were infected with HIV-1 strain, p89.6. HIV DNA was assessed using a quantitative real-time PCR assay. Using a linker-primer PCR technique, HIV-1 integration sites were then mapped. Inverse-PCR was used to analyze DNA from specimens obtained from HAM cases as well as assessed for presence of CD14 and CD16 in tissue.

Results: HIV DNA levels were found to be relatively high in the infected M/MΦ compared to controls. In those cultures with high HIV DNA, viral integration was found within toll-like receptor 1, and near toll-like receptors 6 and 10 on chromosome 4. In addition, integration was found 300kb upstream or downstream of genes of hypothetical proteins, i.e. LOC92689 and FLJ23235. In 1 (out of 11 tumors) case of a large cell lymphoma, HIV-1 integration was found in chromosome 22q13.2 in EST cluster Hs.99330 near HSCBCIP1, a CAP-binding protein complex interacting homologue. HAM specimens also stained positive for $CD14^+/CD16^+$ M/MΦ.

Conclusions: These preliminary results demonstrate that HIV-1-infected M/MΦ with high HIV DNA have specific sites of viral integration. In HAM tissue, $CD14^+/CD16^+$ M/MΦ may be important players in oncogenesis as targets of viral integration.
(Supported by G12RR03061, U01A134853)

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ANTIGEN PRESENTATION BY T CELLS INDUCES CD4+CD25+FOXP3+ REGULATORY T CELLS

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Introduction: Antigen (Ag) presentation by T cells induce tolerance among responding T cells and may be a powerful immune evasion strategy in diseases such as HIV/AIDS or T cell malignancies. Therefore, we examined the immune response to T Ag presenting cells (TAPC) and found TAPC induce anergy, apoptosis and CD4+CD25+FoxP3+ regulatory T cells (Treg).

Methods: TAPC were generated by anti-CD3/CD28 stimulation and transduced with retrovirus containing either CMVpp65 or EBV LMP2. Irradiated TAPC were then used weekly to stimulate autologous PBMC. Exogenous IL-2 was added on day 14. Cultures were phenotyped by flow cytometry using MHC/class I pentamers, CD4, CD8, CD25, CTLA4, LAG3, GITR and annexin-V. FoxP3 analysis was performed on sorted cell populations using real-time PCR. To evaluate suppressive function, CD4+CD25+ T cells were sorted and used in proliferation assays.

Results: TAPC express low levels of CD80 and CD86, were efficiently transduced with retrovirus (>90%) and were capable of inducing both MHC class I and II responses. Stimulation by TAPC induced limited antigen-specific CD8+ T cell proliferation followed by anergy/apoptosis, and induced CD4+CD25+ T cells that expressed FoxP3, CTLA4, GITR and LAG-3. These CD4+CD25+ T cells suppressed proliferation of PBMC upon CD3 activation and prevented expansion of Ag-specific CD8+ T cells in a contact-dependent manner.

Conclusions: Ag presentation by activated T cells promotes tolerance through apoptosis and anergy as well as inducing CD4+CD25+ T cells that further suppress T cell proliferation. These mechanisms may be important immune evasion strategies for HIV and T cell malignancies.

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IL-6 SENSITIVITY OF MCL1 EXPRESSION IN CELLS FROM TRANSGENIC MICE PROVIDES A PATHWAY FOR THE REGULATION OF CELL VIABILITY

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Mice that are transgenic for the pro-viability BCL2 family member MCL1 demonstrate increased B and T cell survival, and develop lymphoma at high frequency but with long latency. In a variety of human cancer cells, particularly those of hematopoietic origin elevated levels of MCL1 are present. Apoptosis can be induced using an MCL1 antisense approach thus suggesting that targeting MCL1 in hematopoietic cancers would be beneficial. We are therefore examining the possibility that MCL1 transgenic mice provide a model for studying human cancers associated with increased survival of immune cells, such as multiple myeloma. To begin to probe for parallels, we tested cells from the spleen and bone marrow of MCL1 transgenic mice to determine if IL-6 played a role in MCL1 expression. Our results show that IL-6 resulted in an increase in expression of both the message and protein of MCL1 in transgene cells, and this occurred along with the activation of STAT3. Next we wanted to determine potential therapeutic targets to decrease MCL1 expression and thus decrease cell viability allowing cells to undergo apoptosis. We used a series of approaches such as an IL-6 neutralizing antibody, RNAI, and Pharmacological inhibitors. Our findings showed that either inhibition of IL-6 (e.g., with a neutralizing antibody), or of MCL1 (e.g., with RNAI), resulted in enhanced cell death. Pharmacologic inhibitors of STAT3 or other signaling pathways resulted in non-specific effects. These findings indicate that targeting the cytokines that control the expression of MCL1 may provide a means comparable to targeting MCL1 itself for inducing cell death.

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PROGRAMABLE ENDONUCLEASES AS ANTI-VIRAL AND ANTI-CANCER AGENTS

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We are developing custom HIV-specific endonucleases for the purpose of creating HIV-resistant hematopoietic stem cells, and possibly attacking the reservoirs of latently infected cells. This approach differs from current vial suppression strategies in that the HIV would be permanently inactivated. The DNA form of the viral genome represents a novel target for therapeutic intervention, and its structural features make it ideally suited for endonuclease-mediated inactivation. In particular, the genome lies between two direct-oriented Long Terminal Repeats (LTRs). Targeting a double-strand break between direct repeats should stimulate homologous recombination, resulting in deletion of the viral genome. Towards this aim, we have produced chimeric endonucleases consisting of the cleavage domain of FokI endonuclease and the DNA-binding domain of engineered zinc finger proteins. These endonucleases have been shown to function in human cell lines, inducing targeted chromosomal recombination frequencies as high as 1%. Custom zinc finger proteins can now be designed to bind virtually any desired DNA sequence with high specificity and affinity. Anti-HIV zinc finger proteins have also been constructed and are currently being evaluated for their ability to specifically cleave target sites in vitro and in cells.

A similar approach could be applied as an anti-cancer strategy, since many cancers are associated with the presence of viral genomes. These viruses often contain genomic regions flanked by direct repeats, making them similarly susceptible to endonuclease-mediated inactivation.

This work supported by NIH grant CA103651

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SELECTIVE SUPPRESSION BY SYNTHETIC SIRNA DUPLEXES OF HUMAN PAPILLOMAVIRUS 16 AND 18 E6 AND E7 ONCOGENE EXPRESSION

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RNA interference has been developed as an approach to block viral gene expression by using synthetic siRNA duplexes. Since E6 and E7 of both HPV16 and HPV18 are transcribed from the same promoter and polyadenylated from the same early poly-A site, the two oncogenes in each virus are transcribed as a bicistronic pre-mRNA which undergoes alternative RNA splicing. Our previous study demonstrated that the bicistronic RNA retaining an E6 intron is necessary to encode viral oncoprotein E6, while splicing of the intron from the E6 coding region is essential to produce viral oncoprotein E7. Thus, RNA splicing of E6 and E7 pre-mRNAs was considered when an E6- or E7-specific siRNA was designed for the oncogene knockdown. Two siRNAs were designed to knockdown oncogene E6 and E7 expression of HPV16, and other two siRNAs were designed to block expression of HPV18 oncogene E6 and E7. Those siRNAs were then examined for their inhibitory activities in their corresponding $HPV16^+$ or $HPV18^+$ cervical cancer cell lines. Various approaches demonstrated that those E6 and E7 siRNAs were oncogene-specific and inhibited viral oncogene expression efficiently, accompanied by stabilization of p53 and pRb, respectively, and cell cycle arrest at G1. Data indicate that our siRNA approach is a most efficient way to knockdown HPV oncogene expression in cervical cancer and may extend to treat for other HPV infection or HPV-related cancers.

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KINETICS OF HOST TRANSCRIPTION, EBV AND KSHV IN PRIMARY EFFUSION LYMPHOMA

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Most primary effusion lymphomas (PEL) are co-infected with two gamma-herpesviruses: Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV). The significance of dual infection for the pathogenesis of PEL is unknown, as are most details of the regulatory pathways that ensure latent coexistence of the two viruses within the same cell. This study aims to identify cellular genes which are affected by gamma-herpesvirus reactivation and to examine the kinetics of viral-cellular gene interactions during viral reactivation to better understand the interdependencies in this system. KSHV and EBV were reactivated in the JSC-1 PEL cell line using 0.3mM sodium butyrate in a 72h time course experiment. DNA viral loads were determined using real-time quantitative PCR assays. The Affymetrix™ microarray platform was used to measure cellular gene expression. Real-time quantitative RT-PCR for each KSHV and EBV gene was used to profile viral transcripts. KSHV and EBV transcription commenced in an ordered cascade of early, delayed early and late classes and preceded increases in viral load. KSHV mRNAs clustered together, rather than with their EBV homologs. KSHV genes Rta/orf50 and Mta/orf57, were transcribed 6-12 hours earlier than EBV genes, Rta/BRLF-1, Zta/BZLF-1 and Mta/BMLF-1, suggesting that KSHV controls the cellular signaling pathways that normally reactivate EBV from latency in dually infected PEL. As EBV transcripts accumulated, KSHV mRNA levels declined.

Analysis of cellular transcription profiles showed cellular replication and cell-division genes were coordinately down-regulated by virus reactivation, including a G2/M block. Several genes in the JAK/STAT pathway, implicated in EBV and KSHV tumorigenesis, were up-regulated upon viral reactivation. IL-6 and cytokine signaling typically activate the JAK/STAT pathway and the KSHV encoded vIL-6 was immediately induced upon reactivation in JSC-1 cells.

Viral reactivation from the latent infected state is likely key to the development of the tumors associated with these viruses. The observations from this study provide insight into the interactions of viral and host cell gene transcript regulation and may provide novel targets for anti-viral and anti-lymphoma therapies.

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CHROMATIN REGULATION OF EPSTEIN-BARR VIRUS LATENT ORIGIN OF REPLICATION

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The Epstein-Barr virus (EBV) origin of plasmid replication (*OriP*) uses the cellular licensing machinery to regulate replication during latent infection of human cells. However, additional epigenetic factors regulate the utilization frequency of OriP. We found that the minimal replicator sequence of *OriP*, referred to as the dyad symmetry (DS), is flanked by nucleosomes. These nucleosomes were subject to cell cycle-dependent chromatin remodeling and histone modifications. Restriction enzyme accessibility assay indicated that the DS-bounded nucleosomes were remodeled in late G1. Remarkably, histone H3 acetylation of DS-bounded nucleosomes decreased during late G1, coinciding with nucleosome remodeling and MCM3 loading, and preceding the onset of DNA replication. The ATP-dependent chromatin-remodeling factor SNF2h was also recruited to DS in late G1, and formed a stable complex with HDAC2 at DS. siRNA depletion of SNF2h reduced G1-specific nucleosome remodeling, histone deacetylation, and MCM3 loading at DS. We also show that OriP replicates later in S-phase than several cellular origins. We propose that histone deacetylation delays replication initiation at OriP to a later stage of S-phase. This replication delay may function like a DNA-damage checkpoint that protects the viral genome from replicating during suboptimal cellular conditions. We are presently trying to determine if delayed S-phase replication is important for viral genome stability during latency.

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- Improve the flow of translational research, bedside to bench and bench to bedside. Yes or No
- Provide an opportunity for networking Yes or No
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- Start new research projects Yes or No
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   d) To provide a means by which new investigators may be drawn into the area of research related to AIDS malignancies. 1 2 3 4 5 N/A
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9th International Conference on Malignancies in AIDS and Other Acquired Immunodeficiencies
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