**S1**

## PHASE I STUDY OF INTERLEUKIN-2 AND STEM CELL FACTOR IN PATIENTS WITH AIDS OR HIV AND CANCER

Manisha Shah[1], Andrew Grainger[1], Cheryl Kefauver[1], Patricia Buczak[2], Zale Bernstein[2], Aharon Freud[1], and Michael A. Caligiuri[1]. [1]The Ohio State University Comprehensive Cancer Center, Columbus OH, [2]Roswell Park Cancer Institute, Buffalo, NY.

BACKGROUND: Based on the *in vitro* and *in vivo* animal studies performed in our laboratory, we postulated that r-metHu stem cell factor (SCF), when administered concomitantly with low dose interleukin (IL)-2 in human, will expand natural killer (NK) cells to a significantly greater degree than treatment with IL-2 alone. A phase I study of IL-2 and SCF in patients with acquired immunodeficiency syndrome (AIDS) or human immunodeficiency virus (HIV) and cancer was undertaken to test our hypothesis and to determine the safety and toxicity of this combination cytokine therapy. PATIENTS AND METHODS: Using the standard dose escalation with 3 x 3 design for phase I study, a total of thirteen patients with AIDS or HIV and cancer were treated with subcutaneous (SQ) IL-2 daily, except on Sundays, with or without SCF administered SQ three times a week (Monday-Wednesday-Friday) for consecutive eight weeks. Patients in IL-2 alone (cohort 1, n=3) group received IL-2 at a dose of 900,000 IU/$m^2$/day while patients with IL-2 and SCF were treated with three different dose levels. Patients in cohort 2a (n=4) received IL-2 at a dose of 900,000 IU/$m^2$/day with SCF at 5 mcg/kg/day, cohort 2b (n=3) received IL-2 at a dose of 650,000 IU/$m^2$/day with SCF at 5 mcg/kg/day, and cohort 3 (n=3) received IL-2 at a dose of 650,000 IU/$m^2$/day with SCF at 10 mcg/kg/day. All patients received therapy as an outpatient basis. All had Eastern Cooperative Oncology Group performance status of 0-1. RESULTS: No grade 3 or 4 toxicities were observed in patients in IL-2 alone cohort. As two out of four patients in cohort 2a withdrew from the study due to grade 3 fatigue or grade 2 myalgia, the protocol was amended to de-escalate the dose of IL-2. All three patients in cohort 2b completed therapy without any grade 3 toxicity. Two out of three patients in cohort 3 experienced dose-limiting toxicity that included grade 3 fatigue (n=1), pruritus (n=1) and urticaria (n=1). Thus, cohort 2b dose level [IL-2 administered at the dose of 650,000 IU/$m^2$/day daily (except Sundays) combined with SCF given at the dose of 5 mcg/kg/day, three times a week], was considered a maximum tolerated dose. The other common grade 2 adverse events included myalgia (n=5), fatigue (n=3), sinus congestion (n=2). CONCLUSION: Combination of low-dose IL-2 and SCF can be administered safely as an outpatient basis in patients with AIDS or HIV and cancer. Completion of planned laboratory correlative studies associated with this clinical study will provide insight into the presence and degree of innate immune modulation resulted by combined cytokine therapy.

**S2**

## NIH HIV VACCINE INITIATIVES

Richard A. Koup, M.D., Vaccine Research Center, NIAID, NIH

The Vaccine Research Center (VRC) in the National Institute of Allergy and Infectious Diseases (vrc.nih.gov) is devoted to developing a vaccine for HIV. The program is mission-oriented and can develop vaccine candidates from basic research through analysis of Phase I clinical trials. Expanded clinical evaluation is done in collaboration with extramural clinical trial networks. The VRC development plan for the initial HIV vaccine candidate is based on gene delivery of vaccine antigens using a combination of plasmid DNA for priming and replication incompetent recombinant adenoviral vector (rAd5) for boosting. This combination induces high levels of HIV-specific CD8+ CTL responses in mice and macaques. The initial goal is to ask whether this level of HIV-specific CD4+ or CD8+ T cell induction is sufficient to impact the HIV epidemic by preventing infection, controlling replication, or reducing transmission to others. The vaccine is designed to induce T cell responses against multiple HIV proteins to diminish immune escape, and includes sequences from multiple clades to be relevant for a large portion of the global epidemic.

The initial Phase I study (VRC 004) evaluating a 4-plasmid combination of B clade gag/pol/nef with envelope from clades A, B, and C has been completed in 50 healthy adults in the U.S., with no vaccine-related serious adverse events. The study evaluated doses of 2, 4, and 8 mg delivered by a needless injection device. CD4+ and CD8+ T cell responses were detected in the majority of vaccinees using peptide pools to stimulate PBMCs followed by IFN-g ELISpot and flow cytometric detection of intracellular IL-2 or IFN-g production. HIV-specific antibody response was detected in 12/40 subjects. Responses could be detected after two injections, and the responses to the 4 and 8 mg doses were greater than those to the 2 mg dose. The full immunology and development program for this vaccine will be presented.

**S3**

## INSUFFICIENT PRODUCTION AND TISSUE DELIVERY OF CD4+ EFFECTOR T CELLS IN RAPIDLY PROGRESSIVE SIV INFECTION

LJ Picker*, SI Hagen*, R Lum*, EF Reed-Inderbitzin*, LM Daly*, AW Sylwester*, JM Walker*, JD Lifson[§], VC Maino[‡], and MK Axthelm*.
*Vaccine and Gene Therapy Institute and Oregon National Primate Research Center at Oregon Health & Science University, Beaverton, OR 97006; [§]National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD 21702, and [‡]Becton Dickinson Biosciences, San Jose, CA 95131.

The mechanisms linking replication of HIV-1 to the progressive cellular immunodeficiency of AIDS are controversial, particularly the relative contribution of CD4+ T cell destruction to the disease process. Indeed, in the rhesus macaque (RM)/SIV model, progressive disease can occur in the absence of significant CD4+ lymphopenia, suggesting mechanisms other than direct or indirect CD4+ destruction play a role. Here, new approaches for delineation of systemic CD4+ T cell dynamics were used to determine the extent to which CD4+ T cell insufficiency underlies rapid disease progression in SIV-infected RM (terminal disease within the first 200 days of infection). Of 14 RM infected with wildtype CCR5-tropic SIVmac239, and 4 with CXCR4 tropic SIVmac155T3, 10 of the former and all of the latter were asymptomatic during the first 200 days of infection, whereas 4 of wildtype SIVmac239-infected animals exhibited rapid disease progression. The characteristic CD4+ T cell lymphocytosis of the rapid progressors was found to be composed of naïve phenotype cells, masking a profound systemic depletion of circulating and tissue CD4+ memory T cells. Memory T cell depletion occurred in all CCR5-tropic infections, but in clinically stable RM, was uniformly countered by a dramatic and sustained increase in production of short-lived CD4+ "effector" cells, which rapidly migrated to tissue. In rapid progressors, production of CD4+ effectors initiated but failed by day 42 of infection, and tissue delivery of new CD4+ T cells ceased. Regenerative failure preceded clinical symptoms by many weeks and was the strongest statistical predictor of early disease, suggesting that quantitative CD4+ effector cell insufficiency in tissue may account for the susceptibility to opportunistic infection in rapidly progressive infection, and conversely that the persistently elevated CD4+ memory T cell proliferative activity noted in these infections (a component of infection-associated immune activation) may play a critical role in maintaining immune homeostasis in stable disease.

**S4**

## HPV INFECTION IN THE CONTEXT OF HAART

Joel Palefsky, University of California, San Francisco

The incidence of cervical and anal cancer are increased in HIV-positive men and women compared with the general population. HIV-positive women and men are also at increased risk of anogenital human papillomavirus (HPV) infection, cervical intraepithelial neoplasia (CIN) and anal intraepithelial neoplasia (AIN), compared with age-matched HIV-negative women and men, respectively. The risk of both AIN and CIN clearly increase with lower CD4+ level, in contrast to anal and cervical cancer, whose incidence does not increase at a CD4+ level below 200/$mm^3$. These data suggest that development of CIN/AIN may be more related to immunosuppression than progression of high-grade CIN/AIN to invasive cancer. The advent of HAART has permitted examination of the effect of immune reconstitution on the natural history of anogenital HPV infection. CIN/AIN, and anogenital cancer. To date there have been at least 12 reports addressing this issue. Interpretation of the data on the effect of HAART has been difficult due to the different study methodologies and endpoints used in these reports. Data thus far may be summarized as follows:1) HAART does not appear to result in clearance of cervical or anal HPV infection and the effect of HAART on HPV viral load is not clear; 2) The effect of HAART on CIN is mixed, with some reports showing reduced incidence of CIN and increased regression. The effect however is modest and some reports show no effect at all; 3) HAART has little or no effect on the natural history of AIN; 4) The incidence of cervical and anal cancer have not declined since the introduction of HAART; and 5) These data are consistent with the limited role of immune response in the most advanced stages of anogenital neoplasia. The data also point to the need for continued close monitoring of anogenital HIV-positive men and women whether or not they are on HAART.

**S5**

## DEVELOPMENT OF HPV HUMAN T CELL MEDIATED ASSAYS

Chien-Fu Hung, Ph.D., Johns Hopkins University

Understanding of host immune responses is important for vaccine development. However, data on the cellular immune responses to human papillomavirus (HPV) infections are controversial. Some studies showed cell-mediated immune responses to HPV-16 E7 peptide associated with regression of cervical intraepithelial neoplasia and loss of human papillomavirus infection. Other studies demonstrated that cell mediated immune responses to E6 and/or E7 are associated with persistent HPV-16 infection. The primary difficulty encountered in the study of cellular immune responses against HPV is that HPV infections generate weak immune responses. It may be because of lack of systemic infection, depletion of langerhans cells by HPV mucosal infection, and/or a lack of reliable human immunological assays. In order to circumvent some of these problems, we used HLA-A2 transgenic mice, as HLA-A2 is a common MHC allele in the human population. We generated a potent antigen-specific CD8+ T cell response in HLA-A2-transgenic mice using a DNA vaccine encoding an intracellular targeting molecule linked to an HPV antigen. In order to determine the immunodominant epitope of the encoded HPV antigen, we have used the BIMAS analysis system to identify candidate immunodominant HLA-A2-restricted epitopes. These candidate epitopes were further validated using CD8+ T cells generated by vaccination of HLA-A2 transgenic mice with the potent DNA vaccine. The identification of immunodominant epitopes allows us to develop quantitative human immunological T cell assays, including intracellular cytokine staining, ELISPOT, and tetramer staining. Thus, the vaccination of HLA-A2 transgenic mice with a potent DNA vaccine will allow us to identify immunodominant epitopes, facilitating the development of ideal human immunological assays. The advantages and the potential pitfalls of such a system will be discussed.

**S6**

## OVERVIEW AND CHALLENGES TO ASSESSING T CELL RESPONSES TO HUMAN PAPILLOMA VIRUS

Malcolm John[1], Joel Palefsky[1]. [1]University of California, San Francisco.

The role of peripheral immune responses to HPV persistence or progression is poorly understood. It is believed that Th1 immune responses are important in HPV disease clearance. Yet contradictory findings have been published on the correlation of peripheral T cell responses to anogenital dysplasia and cancer.

Several studies show a link between HPV infection clearance and T-helper cell responses to HPV16 E6 and E7 peptides. Others suggest a link between HPV16 E7 responses and viral persistence and disease progression. Peripheral HPV16 E6 and E7 specific cytotoxic T-lymphocytes (CTLs) have been detected in peripheral blood of HPV16+ women; HPV persistence in women without squamous intraepithelial lesions (SILs) was associated with lack of CTL response to E6. Still others detected E6 and/or E7 responses in ~40% of women with SILs and persistent HPV16. Our clinical data generated using intracellular cytokine staining (ICS) and IFN-$\gamma$ production (by ICS and ELISPOT) and T cell proliferation by carboxy-fluorescein diacetate, succinimidyl ester assays support the presence of weak, waxing and waning peripheral Th1 responses to HPV16 E7 peptides in HPV-infected individuals.

Assessing peripheral T cell responses to HPV remains a challenge primarily due to the low level of such responses. Choice of patient population, test antigens, and assay may significantly impact results. Given theses challenges, efforts to assess local tissue T cell responses will be of importance.

**S7**

## ADVANCES IN THE IMMUNOLOGY OF HPV AND THE CONTINUED NEED FOR A THERAPEUTIC HPV VACCINE

W. Martin Kast, PhD
Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA 90033, USA

Human Papilloma Virus (HPV) infection is linked to the development of cervical carcinoma and some other cancers and pre-cancerous lesions including cervical and anal intraepithelial neoplasia (CIN, AIN). The incidence of HPV associated lesions is increased 6 fold in HIV positive patients indicating a prominent role of the immune system in controlling HPV infection.

Despite major recent progress in the development of a preventive vaccine for HPV, it is clear that such vaccines will have no effect on patients that are already infected with HPV including AIDS patients and will likely incur some hurdles in being introduced in the general population. In addition, the claimed high success rate of surgical procedures for CIN lesions needs to be revisited seen the fact that, when measured over life time, about half of the patients that undergo a successful surgical procedure for CIN will return to the clinic for an additional procedure related to HPV infection as surgery did not eliminate the underlying cause of the lesions. Additionally in AIN patients, surgery is not an option. Combined, these facts underscore the continued need for the development of a therapeutic HPV vaccine that could eradicate HPV after the virus has already caused its damage.

For a successful therapeutic HPV vaccine a thorough knowledge is needed on how the immune system interacts with HPV and how HPV, a human virus that has co-evolved with human beings, is trying to escape from being recognized by the human immune system. New and intriguing evidence will be presented that one of the ways HPV is using to escape immune recognition is the targeting of Langerhans cells and changing them into potentially immunosuppressive cells. The consequences for vaccine strategies will be discussed. In addition a new and promising vector system will be revealed that is based on HPV recombinant Venezuelan Equine Encephalitis Virus Replicons. This vector system has been modified such that the oncogenic potential of two HPV genes is eliminated and is shown to be therapeutic in three HPV induced mouse tumor models including a new HLA-A2 transgenic tumor model. These promising data support the continued development of this vector system for clinical evaluation against HPV-associated disease.

**S8**

## INVESTIGATIONAL HUMAN PAPILLOMAVIRUS (HPV) (TYPES 6, 11, 16, 18) VACCINE

Eliav Barr, Merck Research Labs, West Point, PA

**Background**. ~70% of sexually-active adults will become infected with HPV during their lifetime. Every year, HPV infection causes ~250,000 anogenital cancer deaths and millions of cases of genital warts. HPV 16 and 18 cause 70% of cervical cancers. HPV 6 and 11 cause >90% of genital warts. In animals, systemic vaccination with virus-like particles (VLPs) composed of the L1 capsid protein of non-human papillomaviruses protected against virus challenge and disease by inducing type-specific neutralizing anti-L1 responses. A quadrivalent HPV 6, 11, 16, and 18 L1 VLP vaccine may greatly reduce HPV disease burden in men and women.

**Clinical Program.** Merck & Co. has developed a quadrivalent HPV (Types 6, 11, 16, 18) L1 VLP vaccine expressed in yeast and formulated on aluminum adjuvant. Both the expression system and the adjuvant have an extensive, proven safety record.

In early studies, administration of HPV 11, 16 and 18 HPV L1 VLP vaccines in a 3-dose regimen was generally well-tolerated and induced neutralizing anti-HPV responses. Vaccine-induced anti-HPV responses persisted for 2.5 years following vaccination. Merck then performed a randomized, placebo-controlled study of an HPV 16 L1 VLP vaccine in 2,392 women. The study's primary endpoint was the incidence of persistent HPV 16 infection or related cervical dysplasia in women who were HPV 16-naïve at baseline. In the study's primary analysis, the incidence of this endpoint was 3.8% and 0.0% per 100 subject-years at risk in the placebo and HPV 16 vaccine groups, respectively.

Phase III studies of a quadrivalent HPV (Types 6, 11, 16, 18) L1 VLP vaccine in >25,000 subjects are underway. These studies will provide a definitive evaluation of the impact of prophylactic HPV vaccination on women's risk for genital warts and cervical cancer.

**Conclusion:** HPV infection is a common STD. If proven safe and effective, a vaccine that prevents infection with HPV 6, 11, 16, and 18 will be a major public health advance. Definitive studies to evaluate such a vaccine are underway.

**S9**

## UPDATE ON THE DEVELOPMENT OF AN HPV-16/18 PROPHYLACTIC CERVICAL CANCER VACCINE

Gary Dubin, M.D., Vice President, Global STD Vaccines Clinical Development Programs, GlaxoSmithKline, King of Prussia, PA

HPV-16 and HPV-18 are responsible for the majority of cervical cancers globally. Prevention of infection with these types would likely have significant public health impact. GlaxoSmithKline (GSK) Biologicals has developed a vaccine containing HPV-16 and -18 L1 virus-like particles (VLPs) and AS04, a novel adjuvant with alum and 3-O-deacylated-monophosphoryl lipid A.

In phase I and II trials, the vaccine was shown to be immunogenic and generally well tolerated. Based on these results, GSK initiated a double blind, randomized pilot efficacy trial of the HPV-16/18 VLP-AS04 vaccine in the United States, Canada, and Brazil. 1113 women ages 15-25 years were randomized to receive HPV-16/18 vaccine or placebo on a 0, 1, and 6 month schedule in a double-blind trial conducted in North America and Brazil. HPV infection was evaluated over an 18-27 month period using PCR performed on self-collected cervicovaginal samples and health care provider-collected cervical samples. Primary and secondary endpoints were detection of any incident HPV 16/18 infection and detection of persistent HPV 16/18 infection (2 positive samples over a 6 month interval). 721 women (366 vaccine/355 placebo) without prior HPV 16/18 infection completed the 3-dose immunization schedule. Rates of incident HPV-16/18 infection rates were reduced in the vaccine group by 91% ($p<0.001$) and 74% ($p<0.001$) respectively when considering cervical samples only and all samples combined. Rates of persistent infection were reduced in the vaccine group by 100% ($p<0.001$) and 100% ($p<0.001$) respectively when considering cervical samples only and all samples combined. Vaccination was well tolerated and induced high levels of HPV-16 and HPV-18 specific antibody.

A large phase III efficacy program is now being initiated.

**S10**

## ACTIVATION OF CD21 AND CD23 GENE EXPRESSION BY KSHV RTA

Jae Jung, NEPRC, Harvard Medical School

Expressions of B cell activation maker CD21 and CD23a transmembrane glycoprotein are regulated by Notch-mediated transcriptional factor RBP-Jk/CBF1. Epstein Barr virus (EBV) EBNA2 and Kaposi's sarcoma-associated herpesvirus (KSHV) RTA are recruited to their specific responsive elements by interacting with cellular RBP-Jk. Furthermore, the genetic ablation study has indicated that the interaction of RTA with RBP-Jk is essential for KSHV lytic replication. Despite detailed study of RTA-mediated viral gene expression, its cellular targets have not been characterized. Here, we demonstrate that KSHV RTA dramatically induces CD21 and CD23a gene expression through the RBP-Jk-binding sites in the CD21 first intron and CD23a proximal promoter region, respectively. RTA-induced expression of CD21 protein that is a primary EBV receptor significantly enhanced EBV infection, mimicking the EBV/KSHV coinfection of primary effusion lymphoma cells. In addition, RTA-induced CD23 glycoprotein in B lymphocytes and KSHV-infected primary effusion lymphoma cells underwent proteolysis and gave rise to soluble CD23 molecule, which subsequently induced the activation of primary human B and T lymphocytes at detectable level. These results demonstrate that cellular CD21 and CD23a genes are common targets for B-cell tropic gamma herpesviruses, and that the upregulation of CD21 and CD23 glycoproteins by KSHV RTA facilitates EBV coinfection and lymphocyte activation, which may contribute to the development of virus-associated lymphomas.

**S11**

## DOWNREGULATION OF MHC-I MOLECULES BY VIRALLY-ENCODED E3-UBIQUITIN LIGASES

Laurent Coscoy
University of California, Berkeley

To establish a life-long infection, KSHV has evolved means of subverting normal host defenses. We and others recently showed that KSHV encodes two proteins, MIR1 and MIR2, that prevent antigen presentation by MHC-I molecules. These proteins decrease MHC-I expression on the surface of the infected cell, thus preventing recognition by cytotoxic T lymphocytes (CTLs). The mechanism by which they do so is novel; they act as E3 ubiquin ligases of the ubiquitin pathway. They promote ubiquitination of the MHC-I intracytoplasmic domain, a signal that targets MHC-I molecules for internalization from the cell surface and degradation by the lysosomes. We are characterizing this unusual form of immune evasion to improve our understanding of viral pathogenesis.

We recently discovered the existence of a family of human cellular proteins that are structurally and functionally homologous to the viral MIR proteins. At this point, the putative proteins encoded by these genes remain largely uncharacterized, with no functional activities yet identified. We believe that these novel proteins may participate in the regulation of the trafficking of molecules involved in immune mechanisms. Understanding their function may give us insight into basic immunology and thus help us to understand the mechanisms of defense against pathogens.

**S12**

## IMPROVING SURVIVAL, EXPANSION AND PERSISTENCE OF ADOPTIVELY-TRANSFERRED TUMOR-SPECIFIC CTLS

Rooney CM, Huls MH, Bollard C, Straathof K, Okamura T, Gottschalk SM, Brenner MK and Heslop HE. Center for Cell and Gene Therapy, Baylor College of Medicine, Texas Children's Hospital and The Methodist Hospital, Houston, Texas.

After infusion into recipients of T cell-depleted stem cell transplants (SCT), EBV-specific CTL expand by up to four logs, reconstitute immunity to EBV, reduce high virus load, home to tumor sites and produce complete remission of EBV-associated lymphomas. Gene-marking studies further demonstrated the persistence of the infused cells for up to seven years. By contrast tumor-specific T cell lines or clones infused into immunocompetent tumor bearing individuals have been disappointing, rarely producing anti-tumor responses and persisting only days or weeks. The factors influencing CTL survival and efficacy include the composition of the CTLs, with polyclonal T cell lines recognizing multiple tumor epitopes and containing both CD4+ T helper cells and CD8+ CTL being ideal. Both patient and tumor environment also influence the fate of the infused CTL. In a lymphopenic environment, mature T cells should proliferate by hemostatic lymphoproiliferation, until they fill the T cell compartment, but if the T cell compartment is full, little expansion or even contraction may result. After SCT, the presence of both an antigenic tumor and the presence of normal EBV-infected B cells likely also provide a proliferative boost. Immunogenic tumors that arise in immunocompetent hosts express a range of T cell inhibitory molecules and recruit and induce T regulatory cells. Therefore, for optimal CTL function, either the host or tumor environment must be altered, or the CTL must be made resistant to tumor-mediated inhibition. We will discuss the results of using EBV-specific CTL lines in patients with NPC and Hodgkin, as well as new protocols that use specific antibodies to deplete the T cell compartment before infusion and planned projects for genetic enhancement of CTLs.

**S13**

## SURVEY OF RESEARCH IN AIDS-KAPOSI'S SARCOMA IN ZIMBABWE

M.Z. Borok University of Zimbabwe, Harare

INTRODUCTION: Since the onset of the AIDS epidemic, the incidence and prevalence of Kaposi's sarcoma (KS) has increased dramatically. AIDS-KS is currently the commonest tumour in all age groups in Zimbabwe. The present epidemic of AIDS-KS in sub-Saharan Africa is likely to be the result of overlapping epidemics of HIV and KSHV infection.

EARLY RESEARCH: Prior to the era when antiretroviral therapy (ART) was accessible in Africa, a randomized prospective study was conducted to compare 4 treatment interventions for AIDS-KS to try and determine the most pragmatic approach to the disease. The goal of treatment was palliative and the primary outcome measure was quality of life (QOL). A summary of results will be presented. Independent predictors of survival included clinical stage at presentation, and baseline QOL score. The high mortality and ineffectiveness of conventional treatment emphasized the need to identify markers for disease risk. Rational selection of patients for treatment (or disease prevention) in this resource-poor setting may be feasible.

RECENT RESEARCH: The relationship of KSHV viraemia and KS disease was investigated in 500 subjects in Harare. Cross-sectional analysis of plasma KSHV and PBMC KSHV DNA was performed. The prevalence and magnitude of cell-free KSHV DNA were strongly associated with AIDS-KS and with clinical stage. Plasma and PBMC KSHV DNA levels were linearly related. The data support a connection between untreated HIV-1 infection and KSHV replication. Further studies of the effects on KSHV replication by inhibition of HIV replication with HAART are needed to understand this relationship.

CURRENT RESEARCH: A pilot study of the effects of ART on KS is currently enrolling patients. The study will examine the relationship between clinical response of KS and suppression of KSHV replication.

Preliminary results of a seroprevalence study will be presented.

**S14**

## CERVICAL CANCER PREVENTION – A PARADIGM SHIFT?

Lynette Denny[1], Louise Kuhn[2], Michelle De Sousa[3], Amy Pollack[4] and Thomas C Wright Jr.[2]

[1]University of Cape Town/Groote Schuur Hospital, Cape Town, South Africa, [2]Columbia University, New York, NY, [3]University of Cape Town, [4]EngenderHealth, NY

**Objective:** To determine whether a novel 'screen and treat' approach to cervical cancer prevention, whereby women are screened using either human papillomavirus (HPV) DNA testing or direct visual inspection (DVI) and all test positive women receive cryotherapy, can safely and effectively reduce the prevalence of high-grade cervical cancer precursors in a low-resource setting.

**Methods:** Previously unscreened non-pregnant women, aged 35 to 65 years were screened using HPV DNA testing and DVI and subsequently randomized to one of three study arms: cryotherapy if high-risk HPV positive, treatment with cryotherapy if DVI positive, or delayed treatment for six months. Strict exclusion criteria were utilised to prevent any women with lesions suspicious for cancer from being enrolled in the study. A structured questionnaire was administered to all enrolled women, treated and untreated, at one month post randomisation to evaluate complications of cryotherapy. All women were recalled 6 months later for a further in depth questionnaire, rescreening with all screening tests, colposcopy and histological sampling to evaluate effectiveness of 'screening and treating' women without utilising cytology, colposcopy or histological sampling.

**Results:** Of the 6,555 randomized women, 5652 (86%) completed follow-up. The prevalence of cervical intraepithelial neoplasia – grade 2,3 (CIN 2,3) at six months for both the HPV arm (0.8%) and the DVI arm (2.2%) were significantly lower than the prevalence in the delayed evaluation arm (3.6%). Treatment based on a positive HPV DNA test resulted in a 78% reduction in biopsy confirmed CIN 2,3 ($p<0.01$) compared to the delayed evaluation arm, whereas treatment based on DVI resulted in a 39% reduction ($p=0.015$). Although minor complaints such as discharge and bleeding were common after cryotherapy, only one major complication was reported among the 947 women receiving cryotherapy.

**Conclusions:** "Screen and treat" utilizing HPV DNA testing is a safe and highly effective approach to cervical cancer prevention for low resource settings that produces a 4.5 fold reduction in CIN 2,3 compared to no intervention. Such a program could help eliminate the disparity in cervical cancer rates observed between developed and developing countries.

**S15**

## AUTOLOGOUS STEM CELL TRANSPLANTATION FOR AIDS-RELATED LYMPHOMAS: AIDS MALIGNANCY CONSORTIUM 020

David T. Scadden, Massachusetts General Hospital and Harvard Medical School and the AIDS Malignancy Consortium

AMC 020 is a multicenter trial of autologous stem cell transplantation in the setting of relapsed or refractory Hodgkins or non-Hodgkins lymphoma. Patients had to demonstrate chemosensitivity to a salvage regimen ($\geq$25% tumor response), have a CD4> 50cells/$mm^3$, HIV RNA < 110,000 copies/ml, adequate liver, lung, cardiac and renal function and not have active CNS involvement. Treatment included preconditioning and G-CSF mobilization for stem cell harvesting followed by busulfan (1mg/kg/dose for 14 doses) and cyclophosphamide (60mg/kg). Anti-retroviral therapies were continued or initiated prior to stem cell mobilization and continued as tolerated.

Of the 20 patients enrolled on AMC020, 13 have had a stem cell transplant. One patient was ineligible due to inadequate counts; one patient failed to mobilize and stem cell collection was not completed on one patient. There is inadequate data on the other four patients on which to determine the status of the transplant.

All 13 patients were men: 9 white, one black and 3 Hispanic. The median age was 38 years and ranged from 34 to 55. Median CD4 counts at baseline and post-transplant were 200 and 163.5. The change in CD4 count (Median=39.5) was not significant.

Based on investigator assessment, there are 3 CRs ($23^+$ months, $9^+$ months, $8^+$ months) and 2 PRs that converted to CRs after 3-4 months. There is another CR without documented duration.

Adverse events that were probably or definitely related to treatment were reported in nine patients. The most commonly reported adverse events were neutropenia (46%), thrombocytopenia (46%), anemia (39%), febrile neutropenia (31%) and infection (31%).

There was no treatment-related mortality.

The safety of autologous stem cell transplantation in AIDS-related non-Hodgkins and Hodgkins lymphoma is reasonable with evidence for activity in a multi-center trial.

**S16**

## LONG TERM REMISSION WITH AUTOLOGOUS STEM CELL TRANSPLANTATION IN HIGH RISK HIV ASSOCIATED LYMPHOMA

Amrita Krishnan, A. Molina, J. Zaia, D. Vasquez, D. Smith, N Kogut, J Rosenthal, J Alvarnas, S.J. Forman. Hem/BMT, City of Hope Cancer Center, Duarte, CA, USA

In the HIV negative setting, several randomized studies have shown that high dose therapy with autologous stem cell transplant (ASCT) is the optimal therapy for relapsed HD and NHL. As the procedure related mortality of ASCT has decreased, ongoing studies are exploring its use in high-risk first remission patients. Prior to the advent of highly active antiretroviral therapy this approach was not possible in HIV infected patients with lymphoma. These patients were treated with varied salvage chemotherapy regimens and all had markedly shortened survival. However, now that HIV infected individuals have markedly improved immune and hematologic function, the use of both solid organ and ASCT is being explored in patients with underlying immunodeficiency and concomitant malignancy or organ dysfunction. Herein we report the City of Hope National Medical Center experience on the largest single institution series of patients with HIV associated lymphomas undergoing ASCT. Our initial experience in nine patients demonstrated the feasibility of this approach in terms of stem cell mobilization, engraftment and low regimen related toxicity. The majority of infectious complications were similar to the HIV negative setting with fever and neutropenia predominating. Though opportunistic infections did occur post ASCT most were in patients not on prophylaxis and all were treatable Furthermore, now with long term follow up (median 32 months) in a larger series of twenty patients we demonstrate that ASCT can provide durable remissions for these high-risk patients with a 1 year overall survival of 85%. This suggests that ASCT should be the standard approach for relapsed HIV associated lymphomas akin to the HIV negative setting.

**S17**

## HIGH-DOSE THERAPY PLUS AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR HIV-RELATED LYMPHOMA: RESULTS AND IMPACT ON HIV DISEASE.

Jean Gabarre – Hôpital Pitié-Salpêtrière – Paris - France

**Aim**: To assess the feasibility of high-dose chemotherapy plus autologous hematopoietic stem cell transplantation (HDC/AHSCT) in AIDS-related lymphoma (ARL), and its long-term impact in HIV patients treated with highly active antiretroviral therapy (HAART).

**Patients and methods**: Fourteen patients with relapsed or resistant ARL (8 non Hodgkin's lymphoma and 6 Hodgkin's disease) were treated with HDC/AHSCT while on HAART. HIV-1 proviral DNA load was quantified in 11 grafts.

**Results**: Hematological reconstitution was good. No toxic deaths occurred. Despite the large number of cells harboring HIV-1 proviral DNA ($10^5$ to $10^9$) re-infused with the graft, HAART controlled HIV replication and led to CD4 cell reconstitution in 7 of the 8 patients who were still alive six months after AHSCT. Only two patients had opportunistic infections after AHSCT. There were no significant changes in viral load (VL) or CD4+ cell counts in most patients. One month after AHSCT, 10 patients were in complete remission (CR). Seven patients died from lymphoma between 1 and 10 months after AHSCT, and a further two patients died in CR (one from AIDS at 16 months, one from another tumor at 28 months). Five patients are alive: four are in CR, 14, 19, 32 and 49 months after AHSCT (median CD4+ cell count= 445/μL; undetectable VL in 3 patients), and one is being treated for relapsed lymphoma 36 months after AHSCT.

**Conclusion**: HDC/AHSCT is feasible in AIDS-related lymphoma, in terms of harvesting, engraftement, adverse events and HIV control. It should be proposed to patients with poor-prognosis chemosensitive lymphoma.

**S18**

## ALTERED EBV VIRAL SETPOINT DUE TO HIV-RELATED IMMUNE ACTIVATION

Debbie van Baarle
Dept. of Clinical Viro-Immunology, Sanquin Research at CLB, University of Amsterdam, The Netherlands

In contrast to transplant recipients, in HIV-infected individuals an elevated EBV load is not predictive of EBV-related malignancies. To study whether high EBV load is a normal situation already early in HIV infection, and not related to a decrease in immune function over time, we investigated EBV load and EBV-specific $CD8^+$ T cells over HIV-seroconversion in homosexual men from the Amsterdam cohort. Furthermore, we studied the influence of immune activation on EBV load in healthy Ethiopians and HIV-seronegative homosexual men who both have an activated immune system compared to healthy Dutch heterosexual controls. EBV load significantly increased after HIV seroconversion from a median of 205 to 1002 copies/$10^6$ PBMC ($p<0.001$), whereas no further increase in EBV load was observed between 1 and 5 years after HIV seroconversion (median 1827 to 2478 copies/$10^6$ PBMC, $p=0.530$). Interestingly, the absolute number of EBV lytic epitope RAK-specific $CD8^+$ T cells increased over HIV seroconversion (4.78 to 9.54/μl, p=0,011). Furthermore, the fraction CD27-negative effector RAK-specific $CD8^+$ T cells tended to increase (from 12.2 to 17.31 %$CD27^-$, $p=0.051$), in accordance with antigen-driven differentiation.
In support of a role for immune activation in the increased EBV load, we also found an elevated EBV load in a cross-section of HIV-negative homosexual men ($p<0.01$) and healthy Ethiopian factory workers ($p<0.01$) compared to healthy Dutch controls. Furthermore, the degree of immune activation in the T cells –as measured by HLA-DR and CD38 expression – paralleled the differences in EBV load between the groups. In conclusion, both virological and immunological data support the idea that a new EBV viral setpoint is reached early in HIV infection, probably by EBV reactivation, as suggested by the preferential increase in EBV lytic epitope-specific $CD8^+$ T cells. These data may thus explain the lack of predictive value of EBV load for the occurrence of AIDS-related lymphoma and suggest a role for immune activation in increased EBV load levels.

**S19**

## REAL TIME QUANTITATIVE PCR ASSAYS FOR ACCURATELY MEASURING VIRAL LOAD OF HTLV, KSHV, EBV, HIV, HCV AND HBV: USES AND POTENTIAL PITFALLS

Denise Whitby
Viral Epidemiology Section, AIDS Vaccine Program, SAIC-Frederick, NCI-Frederick, Frederick, MD.

We have developed real time quantitative PCR assays for HTLV-I, KSHV, EBV, HIV, HCV and HBV. Used in conjunction with our cell quantitation ERV-3 assay, these assays provide accurate, sensitive and specific quantification of viruses relevant to AIDS malignancies. Real time PCR assays have a dynamic range of up to 7 logs depending on the efficiency of the primer probe set. Challenges in assay design vary widely depending on the biology of the target virus. For two of our assays, HTLV and EBV, we have compared plasmid based curves with those based on infected cells with known number of viral copies. For HIV, HCV and HBV, virus variability posed a challenge in designing primers that could amplify all known strains with equal efficiency. Conversely, the relative lack of variation between HTLV I and II enabled us to design a primer probe set that would amplify both viruses. For KSHV and EBV the rarity of infected cells in peripheral blood, especially in asymptomatic subjects is problematic both in terms of absolute sensitivity and reproducibility. Virus can be quantified in PBMC, plasma, saliva, other body fluids as well as tissue culture cells and supernatants. We have applied our assays extensively in large scale epidemiological studies, clinical trials and basic virology research. Innovative applications for real time PCR assays in our laboratory include a viral reactivation assay for KSHV.

**S20**

## EBV AND KSHV COPY NUMBER IN HODGKIN'S LYMPHOMA AND AIDS LYMPHOMA

Richard F. Ambinder, Johns Hopkins School of Medicine

EBV copy number in peripheral blood mononuclear cells has been shown to predict risk of development of post-transplant lymphoma in organ transplant recipients. EBV copy number in serum or plasma predicts clinical course in patients with patients with nasopharyngeal carcinoma and nasal lymphoma. Data will be presented showing that viral copy number in PBMC is not different among untreated patients with EBV(+) Hodgkin's lymphoma, EBV(-) Hodgkin's lymphoma and healthy blood donors. However, viral copy number is much higher in the serum or plasma of patients with EBV(+) Hodgkin's lymphoma than in the other groups and disappears with therapy. Viral DNA detected at high levels in this setting appears to be released from tumor cells undergoing apoptosis. In patients with AIDS lymphomas, viral load in PBMC is elevated but not different from viral load in patients with AIDS Kaposi's sarcoma. EBV DNA is commonly detected in plasma and in contrast to the situation with Hodgkin's disease appears to represent virion (i.e. encapsidated DNA). With chemotherapy the viral copy number in PBMC falls in concert with a fall in B cell numbers. This fall in B cells (measured per million PBMC) occurs with or without rituximab therapy, but occurs more rapidly in patients treated with rituximab. KSHV is also detected in a fraction of patients with non-Hodgkin's lymphomas and viral copy number also falls with lymphoma therapy.

**S21**

## RETHINKING THE PATHOGENESIS OF KAPOSI'S SARCOMA

Don Ganem, HHMI/UCSF, San Francisco, CA 94143

KS is an angioproliferative lesion that is linked to infection with the herpesvirus KSHV. Earlier work has shown that, in KS tumors, most spindle cells are latently infected with KSHV. Only a small subpopulation of KS cells displays lytic KSHV infection. Traditional views of herpesviral oncogenesis emphasize the primary role of latent infection in driving tumor formation. However, latent infection of primary endothelial cells is not immortalizing. In addition, under conditions favoring rapid cell proliferation, latently infected cells frequently lose the latent viral episome. These observations raise questions about whether viral latency is sufficient for tumorigenesis, and encourage rethinking the potential roles that lytic infection might play. Such a reconsideration is also supported by clinical studies that show a strong dependence of KS formation on continuous lytic replication.

One model for lytic cycle contributions to KS involves induction of VEGF synthesis by infected cells. However, the ability of this pathway to function in authentic KSHV infection depends upon whether host gene expression is inhibited during lytic growth. By examining host marker gene expression during lytic KSHV growth we have found that there is in fact a global block to host gene expression under these conditions. The block is manifest at the level of mRNA accumulation. A single viral gene, termed SOX (shut off factor and exonuclease; the product of ORF 37) is responsible for this phenotype, which strongly impairs VEGF production by early-mid cycle. Gene expression profiling reveals that a small number of host genes escape this shut-off, chief among which is hIL-6. The implications of this for KS and MCD pathogenesis will be discussed.

We suggest that lytic KSHV infection can play two roles in KS biology. First, it allows recruitment of new cells to KSHV latency to replace those infected cells lost to cell death or segregation of viral DNA. Second, during lytic replication, viral proteins with potent signaling activity are synthesized and secreted. These can trigger angiogenic and proinflammatory programs in surrounding tissue, with the consequent elaboration of cytokines and growth factors that in turn promote spindle cell survival and growth.

**S22**

## THE LATENCY-ASSOCIATED NUCLEAR ANTIGEN OF KSHV MODULATES CELLULAR GENE EXPRESSION AND PROTECTS LYMPHOID CELLS FROM P16INK4A-INDUCED CELL CYCLE ARREST

[1]Feng-Qi An, [1]Nicole Compitello, [1]Edward Horwitz, [2]Michael Sramkoski, [3]Erik S. Knudsen and [1]Rolf Renne

[1]Division of Hematology/Oncology, and Department of Molecular Biology and Microbiology, [2]Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH 44106, USA, [3]Department of Cell Biology, University of Cincinnati, Cincinnati, OH 45229

Kaposi's sarcoma-associated herpesvirus (KSHV) is believed to be the causative agent of several human tumors of endothelial and lymphoid origin. The majority of tumor cells are latently infected and express a small number of viral genes. One of these genes is orf73 encoding the latency-associated nuclear antigen (LANA), a multifunctional protein that functions in viral DNA replication, episome maintenance and acts as transcriptional regulator. LANA has been shown to interact with cellular proteins involved in transcriptional regulation such as the tumor suppressors pRB and p53, and RING3 family members. In addition LANA signals through the APC/wnt pathway by inhibiting GSK-3β.

Although, there are several reports about specific promoters that are regulated by LANA only limited data is available that address the question how LANA expression in KSHV-infected cells affects global cellular gene expression. To directly address this question we generated an EBV-negative Burkitts lymphoma line (BJAB) that expresses LANA from a tetracycline-inducible promoter and performed micro-array-based gene expression profiling.

Expression profiling at different time points post induction revealed that 187 genes were induced or repressed more than 2-fold in the presence of LANA. Of these genes 41 are regulated in the Rb/E2F pathway. To determine whether these observed mRNA expression changes translate into LANA-dependent changes in cell cycle regulation we over expressed p16INK4a, a potent CDK inhibitor that efficiently induces cell cycle arrest in RB positive cells. We show that under these conditions LANA expression can efficiently protect cells from p16INK4a-induced cell cycle arrest. Profiling results from cells that either harbor aberrations in the Rb or the wnt/β-catenin pathway will also be discussed.

**S23**

## TUMORIGENIC MECHANISMS OF KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS

Shane McAllister, Camilo Raggo, Scott Hansen, Rebecca Ruhl, Helen Li, Scott Wong, Klaus Früh and Ashlee Moses. Vaccine & Gene Therapy Institute, Oregon Health & Science University, Beaverton, OR USA.

Kaposi's sarcoma-associated herpesvirus (KSHV/HHV8) is the infectious cause of Kaposi's sarcoma (KS), a cancer characterized by proliferating spindle cells of endothelial origin, and some B cell malignancies. To determine oncogenic pathways that contribute to KS development, we employed a genomic and proteomic expression profiling analysis of endothelial cells (EC) infected with KSHV *in vitro*. These methods revealed a diverse group of cellular proteins with potentially important roles in EC biology and KS pathophysiology. Two of these proteins, the receptor tyrosine kinase c-Kit and the catabolic enzyme heme oxygenase-1 (HO-1), have known oncogenic or angiogenic properties that appear to operate in KSHV-infected EC. Both proteins are expressed in KS tissue *in vivo* and may therefore represent valid therapeutic targets. Additional KSHV-induced proteins include RDC-1, an orphan G-protein coupled receptor, and neuritin, a protein implicated in neurogenesis. The steps taken to validate these genes and their potential roles in KS development will be discussed. We have recently applied a genomics approach to the study of rhesus rhadinovirus (RRV), a simian homolog of KSHV. A comparative analysis revealed both common and unique influences of these related viruses on the cellular transcriptome. Further investigation of KSHV- and RRV-modulated cellular proteins will illuminate viral transformation mechanisms, cellular oncogenic pathways, and novel targets for therapeutic intervention.

**1**

## INCREASED CANCER SURVIVAL TIME FOR SOME BUT NOT ALL CANCERS, AMONG ADULTS WITH AIDS, IN THE ERA OF HAART

Nancy A. Hessol[1] and Susan Scheer[2]. [1]University of California, San Francisco, CA; [2]Department of Public Health, San Francisco, CA.

**Background:** The incidence rate of several cancers is increased in people with HIV when compared to those without HIV. Since the introduction and widespread use of highly active antiretroviral therapy (HAART), HIV-related morbidity and mortality has declined. Less is known about the impact of HAART on cancer survival time.

**Methods:** The San Francisco AIDS surveillance registry was matched with the California Cancer registry to determine cancer incidence among adult AIDS cases. Death information was ascertained through active AIDS and Cancer surveillance and death registry matches. To evaluate the effect of highly active antiretroviral therapy (HAART), cancer survival time (from initial diagnosis to death) during the pre-HAART and HAART eras were compared among persons diagnosed with AIDS between 1990 and 2000. Survival time was evaluated for the four most commonly occurring cancers: Kaposi's sarcoma (KS), non-Hodgkin's lymphoma (NHL), anal and lung.

**Results:** Among the 17,755 AIDS cases diagnosed between 1990 and 2000, there were 3514 cases of KS, 940 cases of NHL (including 173 cases of brain NHL), 136 cases of anal cancer, and 79 cases of lung cancer. In stratified Kaplan-Meier analyses, comparing pre-HAART to HAART eras, median survival time for KS was 19 vs. 93 months ($p<0.0001$); 7 vs. 26 months for NHL (excluding brain) ($p<0.0001$); 3 vs. 7 months for brain NHL ($p=0.005$); 57 months vs. "not yet reached" for anal ($p=0.28$); and 14 vs. 15 months for lung cancer ($p=0.46$), respectively.

**Conclusion:** Cancer survival time in the HAART era significantly increased for NHL and KS but did not change for anal or lung cancer. Further investigation is needed to elucidate why HAART increases survival time for some but not all cancers.

**2**

## CANCER INCIDENCE AMONG ADULT AIDS CASES IN THE HAART ERA

Susan Scheer[1] and Nancy A. Hessol[2]. [1]Department of Public Health, San Francisco, CA; [2]University of California, San Francisco, CA.

**Background:** Highly active antiretroviral therapy (HAART) heralded in a new era of clinical care for the HIV-infected individual. HAART has dramatically reduced the incidence of HIV-related opportunistic diseases but there is more uncertainty about the impact of HAART on AIDS- defining and non-AIDS defining cancers.

**Methods:** To determine cancer incidence among adult AIDS cases in San Francisco the local AIDS surveillance registry was matched with the California Cancer registry. To evaluate the effect of highly active antiretroviral therapy (HAART), cancer incidence during the pre-HAART and HAART eras were compared for the five most commonly occurring cancers.

**Results:** Among the 17,755 AIDS cases diagnosed between 1990 and 2000, there were 5,274 cases of cancer. In age, race, risk group, and sex adjusted Poisson regression analyses, the Kaposi's sarcoma (n=3514) rate ratio (RR) among HIV-infected adults who initiated HAART was 0.55, 95% confidence interval (CI) 0.51-0.59, the RR for non-Hodgkin's lymphoma (n=940) was .49 and 95% CI 0.43-0.57, the RR for anal cancer (n=136) was 3.97 and 95% CI 2.72-5.79, the RR for lung cancer (n=79) was 0.99 and 95% CI 0.63-1.56, and the RR for Hodgkin's disease (n=68) was 1.56 and 95% CI 0.97-2.51.

**Conclusion:** Among the five most frequently occurring cancers in adults with AIDS, only Kaposi's sarcoma and non-Hodgkin's lymphoma showed a significant reduction in incidence among those initiating HAART. Additional factors, such as CD4 cell count and HIV viral load at HAART initiation, should also be considered when interpreting these results.

**3**

## ASSESSMENT OF HUMAN PAPILLOMAVIRUS (HPV) DNA TESTING AS AN ADJUNCT TO PRIMARY CERVICAL CYTOLOGIC SCREENING IN HIV$^+$ WOMEN

TG Harris, JM Palefsky, RD Burk, LS Massad, JY Bang, K Anastos, H Minkoff, C Hall, M Bacon, AM Levine, DH Watts, MJ Silverberg, X Xue, S Melnick, HD Strickler. For the Women's Interagency HIV Study (WIHS).

Objective: Studies suggest that the interval between pap smears can be safely extended in HIV$^-$ cytologically normal women who are HPV$^-$. We studied whether this might be safe in HIV$^+$ women. Methods: The cumulative incidence (cum inc) of squamous intraepithelial lesions (SIL), including high-grade SIL (HSIL) was measured by baseline HPV in HIV$^+$ ($CD4_{<200}$ [n=221], $CD4_{200-500}$ [n=419], $CD4_{>500}$ [n=311]) and HIV$^-$ (n=386) WIHS subjects with normal baseline cytology. Cervical specimens for cytology and HPV testing by PCR were collected every 6 months. Standard life-table methods were used to calculate cum inc, and Cox models were used to conduct multivariate analyses. Results: Data from HIV$^+$/HPV$^-$ women at 3 years are shown in the table.

| HIV status | Year | N | # SIL | SIL Cum Inc (95% CI) | # HSIL | HSIL Cum Inc |
|---|---|---|---|---|---|---|
| $CD4_{<200}$ | 0-1 | 80 | 1 | 2% (0%, 5%) | 0 | 0% |
| | 1-2 | 42 | 3 | 9% (1%, 18%) | 0 | 0% |
| | 2-3 | 35 | 7 | 29% (14%, 44%) | 0 | 0% |
| $CD4_{200-500}$ | 0-1 | 197 | 5 | 3% (1%, 5%) | 0 | 0% |
| | 1-2 | 155 | 9 | 9% (4%, 13%) | 0 | 0% |
| | 2-3 | 137 | 7 | 14% (8%, 19%) | 0 | 0% |
| $CD4_{>500}$ | 0-1 | 179 | 2 | 1% (0%, 3%) | 0 | 0% |
| | 1-2 | 147 | 4 | 4% (1%, 7%) | 0 | 0% |
| | 2-3 | 133 | 3 | 6% (2%, 10%) | 0 | 0% |

After a total of 5 years, oncogenic (hazard ratio [HR]=2.8, 95% CI=2.1, 3.8) and non-oncogenic (HR=1.6, 95% CI=1.2, 2.2) HPV detection were associated with increased SIL risk (adjusted for HIV/CD4+). For HSIL the HR were 10.2 (95% CI=2.4, 43.1) for oncogenic and 1.8 (95% CI=0.4, 9.2) for non-oncogenic HPV. Conclusion: Our data showed that <10% of cytologically normal HIV$^+$/HPV$^-$ women developed SIL within 2 years, with little or no risk of HSIL. Formal trials of HPV testing in primary cervical cancer screening of HIV$^+$ women should be conducted.

**4**

## ARTEMISININ IS CYTOTOXIC FOR HPV-EXPRESSING CERVICAL EPITHELIAL CELLS BUT NOT PRIMARY CERVICAL CELLS

Astrid Baege[1], Gary Disbrow[2], Kate Kierpiec[2] and Richard Schlegel[2].
[1] NCI, NIH and Department of Pathology, Georgetown University Medical Center.

Artemisinin is a sesquiterpene lactone isolated from the plant Artemisia annua and is commonly used for the treatment of otherwise multidrug-resistent malaria in humans. This plant-derived compound contains an endoperoxide bridge that reacts with a ferrous iron atom to form free radicals and cells that express high levels of the transferrin receptor and contain increased levels of iron are particularly sensitive to its cytotoxic effects.

We demonstrate that HPV-immortalized and HPV-transformed cervical cells, similar to some cancer cells, express increased levels of transferrin receptor and are differentially sensitive to artemisinin. Artemisinin at concentrations ranging from 5-50 μM is highly effective in killing cervical cancer cell lines or HPV-immortalized cell lines, but primary cervical cells are very resistant. Furthermore, we demonstrate that artemisinin induces cell death in HPV-expressing cervical cells by triggering apoptosis via caspase 9, 3 activation and PARP cleavage. The presence of DFOM antagonizes the cytotoxic effects of artemisinin by reducing the availability of ferrous iron required for the generation of unstable organic free radicals.

We are currently investigating the cytotoxic effects of artemisinin on cervical carcinomas in a mouse model. Since the pharmacokinetics, dosages and side effects of artemisinin have been established for the treatment of malaria, our results offer promise for the topical and/or systemic treatment of premalignant and malignant cervical lesions due to HPV infection.

**5**

## A NOVEL THERAPY FOR CERVICAL CANCER USING SPLICEOSOME-MEDIATED RNA *TRANS*-SPLICING (SMART)

Michael S. Patrick[1], M. Puttaraju[2], Janet DiPasquale[1], Jesse Quintero[1], Mariano A. Garcia-Blanco[2], Lloyd G. Mitchell[2] & Carl C. Baker[1]. [1]LCO, NCICCR, NIH, Bethesda, MD; [2]Intronn Inc., Gaithersburg, MD.

Cervical cancers universally express human papillomavirus (HPV) E6/E7 pre-mRNAs. Predominantly spliced, HPV pre-mRNAs are ideal targets for an RNA splicing-based therapy (SMaRT™) for cervical cancer. *Cis*-splicing removes introns and ligates exons from a single pre-mRNA. Although rare in mammals, splicing can occur in *trans*- between separate pre-mRNAs. We have achieved efficient and specific *trans*-splicing (TS) by tethering a pre-*trans*-splicing molecule (PTM) to HPV- 16 E6/E7 pre-mRNA through base-pairing. QRT-PCR was used to assess PTM expression and TS efficiency. PTMs are RNAs with an antisense binding domain, a 3' splice site (ss), and a 3' therapeutic exon (TE). Expression of the TE should be restricted to cells expressing target pre-mRNA. Cotransfection of 293 cells with PTM and target expression plasmids converted >80% of the HPV pre-mRNA into chimeric *trans*-spliced mRNA. TS efficiency to a heterologous pre-mRNA was below 0.3%. Efficient TS required high-level PTM expression and the elimination of PTM *cis*-splicing. The maximum TS efficiency obtained by transfecting PTM into a stable cell line expressing HPV-16 pre-mRNA (293/HPV-16 cells) was only 7%. FACS was used to investigate the reason for low TS efficiency to endogenous targets. With the observation that cotransfected plasmids are expressed at similar levels in the same cell, we cotransfected PTM and green fluorescent protein (GFP) plasmids into 293/HPV-16 cells. Transfected cells were sorted into low and high GFP expression populations. PTM expression paralleled GFP expression. TS efficiency in low and high GFP expressers was 4% and 17%, respectively. These results show that TS to endogenous targets can be efficient and suggest that efficient PTM delivery and high expression are important.

**6**

## THE MUTATOR PATHWAY IS A FEATURE OF IMMUNODEFICIENCY-RELATED LYMPHOMAS

Alex Duval[1], C Brennetot[1], O Buhard[1], H Poirel[2], A Martin[3], G Gaidano[4], F Berger[5], M-H Delfau[6], D Cannioni[7], V Leblond[2], R Hamelin[1], M Raphael[2]. [1]INSERM U434, Paris, France. [2]INSERM EMI109, Bicêtre, Paris, France. [3]Hôpital Avicenne, Bobigny, France. [4]Amedeo Avogadro University of Eastern Piemont, Novara Italy. [5]Centre Hospitalier Lyon Sud, Lyon France. [6]Service d'Immunologie biologique, Creteil, France. [7]University Paris V, Hôpital Necker-Enfants Malades, France.

The mutator phenotype caused by defects in the mismatch repair (MMR) system is observed in a subset of solid neoplasms characterized by widespread microsatellite instability (MSI-H). It is known to be very rare in non hodgkin lymphomas (NHL) whereas mutator NHL is the most frequent tumor subtype in MMR deficient mice. By screening a series of 603 human NHL with specific markers of the mutator phenotype, we found here 12 MSI-H cases (12/603, 2%). Of interest, we demonstrated that this phenotype was specifically associated with immunodeficiency-related lymphomas (ID-RL) since it was observed in both post-transplant lymphoproliferative disorders (PTLD) (9/111, 8,1%) and HIV infection-related lymphomas (HIV-RL) (3/128, 2.3%), but not in a large series of NHL arising in the general population (0/364) ($p < 0,0001$). The MSI pathway is known to lead to the production of hundreds of abnormal protein neoantigens that are generated in MSI-H neoplasms by frameshift mutations of a number of genes containing coding microsatellite sequences. As expected, MSI-H ID-RL were found to harbour such genetic alterations in 12 target genes with a putative role in lymphomagenesis. The observation that the MSI-H phenotype was restricted to HIV-RL and PTLD suggests the existence of the highly immunogenic mutator pathway as a novel oncogenic process in lymphomagenesis whose role is favoured when host's immunosurveillance is reduced. Since MSI-H positive cases were found to be either EBV positive or negative, the mutator pathway should act synergistically or not with this other oncogenic factor playing an important role in ID-RL.

**7**

## EXPRESSION OF ACTIVATION INDUCED CYTIDINE DEAMINASE (AID), GERMINAL CENTER B CELL GENE, IS INDUCED BY INFECTION WITH EPSTEIN-BARR VIRUS (EBV)

Marta Epeldegui[1], Elizabeth Crabb Breen[2], Amy McQuay[3], Richard Ambinder[4], and Otoniel Martinez-Maza[1, 2]. Microbiology Immunology and Molecular Genetics[1], David Geffen School of Medicine[2], Molecular Cell and Developmental Biology[3], UCLA; Johns Hopkins School of Medicine[4].

Non-Hodgkin's lymphoma (NHL) is the second most common cancer in HIV+ subjects. There are several subtypes of AIDS-NHL, which differ in the fraction of tumors that are EBV+, as well as in the central molecular lesions thought to result in cancer. Also, many AIDS-NHL express genes typically expressed by germinal center (GC) B cells. The GC is the site of B cell class switch recombination (CSR) and somatic hypermutation (SHM). Activation induced cytidine deaminase (AID) is involved in both CSR and SHM. Errors in CSR and SHM are believed to contribute to the genesis of AIDS-NHL, so aberrant AID expression may contribute to the development of NHL. Typically, AID expression is induced by simultaneous exposure to B cell-stimulatory cytokines (IL-4) and ligation of CD40 on B cells by CD40L on T cells. Since EBV encodes a CD40L homolog (LMP1), EBV infection has the potential to induce AID expression. We have observed that infection of B cells with EBV (B95.8 supernatant) induced AID expression by 10 days post-infection. AID expression was assessed by RT-PCR. Two different size products were noted, one that corresponded to the size expected for AID, as well as a larger product. After sequencing, the larger PCR product was seen to represent a variant form of AID mRNA ($AID_{var}$). We have seen that $AID_{var}$, which appears to represent an RNA splice variant, is expressed commonly in non-activated circulating B cells. LMP1 expression following EBV infection, (confirmed by Western blot) was seen at the same time as AID mRNA. These findings are of great interest as they indicate that EBV can induce AID expression, and may induce subsequent aberrant SHM and CSR, suggesting a direct link between EBV infection and the molecular lesions that lead to the genesis of NHL.

**8**

**EBV INFECTION OF NOD/SCID MICE RECONSTITUTED WITH HUMAN HEMATOPOIETIC CD34$^+$ CELLS.** Miguel Islas-Ohlmayer[1], Angela Padgett-Thomas[1], Rana Domiati-Saad[2], Michael W. Melkus[1], Petra D. Cravens[1], Maria P. Martin[1], Rona S. Scott[3], John W. Sixbey[3], George Netto[2], J. Victor Garcia[1]. [1]Southwestern Medical Center, at Dallas, [2]Baylor Medical Center, [3]Louisiana State University Health Sciences Center.

EBV-induced lymphoproliferative disease is an important complication in the context of immune deficiency. Inadequate T-cell suppression allows the outgrowth of transformed cells, predominantly B-cell lymphomas. The mechanism by which EBV transforms B-cells is not completely understood. Currently there is no in vivo model that can adequately recapitulate EBV infection and its association to B-cell lymphomas. We hypothesized that NOD/SCID mice engrafted with human CD34$^+$ cells and reconstituted mainly with human B-lymphocytes could serve as a good xenograft model to study EBV infection and pathogenesis. To test this hypothesis, we infected reconstituted mice with EBV tagged with the enhanced green fluorescence protein (EGFP). High levels of viral DNA were detected in the peripheral blood of all infected mice. These mice had significant weight loss and were lethargic. Infected mice presented large visible tumors in multiple organs most prominently in the spleen. These tumors had morphologic and immunophenotypic features consistent with monomorphic B-cell PTLD. Interestingly, tumor cells expressed EBNA1, LMP1 and LMP2a, a type II pattern of latent gene expression characteristic of Hodgkin's lymphoma. Culture of cells from the bone marrow and spleen of infected mice resulted in the establishment of EBV$^+$ LCLs (CD79a$^+$, CD19$^+$, CD5$^+$, and CD30$^+$, EBNA1$^+$, EBNA2$^+$, LMP1$^+$ and LMP2a$^+$). These data demonstrate that NOD/SCID mice transplanted with human CD34$^+$ cells are susceptible to infection by EBV and accurately recapitulate important aspects of EBV pathogenesis in vivo in an immunosuppressed host.

**9**

## RRV-ASSOCIATED MALIGNANCIES IN THE SIV-INFECTED RHESUS MACAQUE

Scott W. Wong[1,2], Michael F. Powers[1], Jerald Sprague[1], He Li[1], Lisa I. Strelow[1,2] and Michael K. Axthelm[1,2]
[1]Vaccine and Gene Therapy Institute, Oregon Health & Science University, and [2]Division of Pathobiology and Immunology, Oregon National Primate Research Center

We have previously reported that experimental inoculation of SIV-infected rhesus macaques (RM) with rhesus rhadinovirus strain 17577 (RRV) is associated with B cell hyperplasia and a lympho-proliferative disorder (LPD) resembling multicentric Castleman's disease (MCD). MCD is frequently observed in HIV-infected patients also infected with Kaposi's sarcoma-associated herpesvirus (KSHV), the etiological agent of Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), and some non-Hodgkin lymphoma (NHL). Here we report RRV-associated NHL in two RM with SIV-associated MCD and retroperitoneal fibromatosis (RF) in one RM with SIV-associated MCD. The two RM developing SIV-associated MCD and NHL exhibited persistent RRV infection, with one of the two RM exhibiting B cell hyperplasia in the peripheral blood compartment. In one RM, the lymphoma manifested as multiple subcutaneous lumps, with significant splenic, hepatic and kidney infiltration, while in the second RM the lymphoma developed in the bone marrow. The lymphomas were positive for CD20 and strongly positive for RRV by combined immunohistochemistry and *in situ* hybridization. Persistent RRV infection was also observed throughout the infection study in the RM with advanced LPD and RF. Immunohistochemical staining of the RF tissue revealed that it was negative for von Willebrand's factor, but positive for RRV by *in situ* hybridization and for CD68 by immunohistochemistry. These results strongly suggest that RRV may have a role in abnormal cellular proliferations in the SIV-infected RM. Further analysis is currently underway to determine the exact role of RRV in these malignancies.

**10**

## IMMUNERECONSTITUTION AFTER HIGH DOSE CHEMOTHERAPY (HDC) AND PERIPHERAL BLOOD STEM CELLS TRANSPLANTATION (PBSCT) IN HIV+ PATIENTS (PTS) vs HIV- PTS

Cecilia Simonelli, Mariagrazia Michieli, Michele Spina, Rosamaria Tedeschi, Maria Teresa Bortolin, Maurizio Rupolo, Luciano Abruzzese, Paolo De Paoli, Ivana Sartor, and Umberto Tirelli. National Cancer Institute, Aviano Italy.

In our Institute, refractory and/or relapsed non-Hodgkin (NHL) and Hodgkin (HD) lymphomas in HIV+ pts are treated with HDC followed by PBSCT, as the general population. As of December 2003, we enrolled 17 HIV+ pts (14M/3F; median age 40 yrs; 4 HD/13 NHL; 5 pts completed the treatment and are now in follow-up) and 10 HIV- pts with high grade NHL (6M/4F; median age 62 yrs; 5 completed the treatment and are now in follow-up). Before the induction therapy, mean value of CD4 count/$mm^3$ was 190±123 in HIV+ and 340±301 in HIV- pts, with no significant differences. On the contrary, CD4/CD8 ratio in HIV+ pts was significantly lower than in HIV- pts (0.2 vs 1.2), as well as CD56 count (54 ± 45 vs 140± 96 p=0.01). Before the conditioning treatment, CD4 count was still lower in HIV+ pts, but CD4/CD8 ratio was less than 1 in both groups. CD4 count nadir was reached during aplastic period and was similar in both groups (109± 104 vs 122± 86). CD4/CD8 ratio was 0.2 and 0.5 in both groups, respectively. Three mos after PBSCT, CD4 count returned to baseline in HIV+ pts (174±71), while in HIV- pts it was still 100 cells lower than baseline (240±95). Moreover, CD4/CD8 did not return to baseline values in HIV- pts and remained low as in HIV+ pts. CD56 population returned to baseline values faster than CD4 population in both groups, but it remained significantly lower in HIV+ pts. During follow-up no significant differences were seen in the incidence of opportunistic infections. All HIV+ pts were on HAART and HIV-viraemia was <50cp/ml; 4 pts continued HAART during the whole treatment and their HIV-viraemia remained undetectable. Our observation suggests that, despite some differences in the immunological profiles present at the beginning of the induction, no significant differences exist in the dynamic of immunereconstitution between HIV+ and HIV- pts.

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## HIGH DOSE THERAPY AND AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION AS SALVAGE TREATMENT FOR HIV-ASSOCIATED LYMPHOMA

A. Re [1], C. Cattaneo[1], M. Michieli[2], S Casari[3], M. Spina[2], M. Rupolo[2], GP. Carosi[3], U. Tirelli[2], G. Rossi[1].
[1]Ematologia, [3]Malattie Infettive, Spedali Civili di Brescia; [2]Oncologia Medica A, CRO, Aviano, Italy.

We report the results of a multiinstitutional program of high dose therapy (HDT) and autologous peripheral blood stem cell (PBSC) transplantation in HAART responsive patients (pts) with HIV-Ly refractory or relapsed after standard dose chemotherapy (CT). Twenty-five pts entered the study: 10 HD and 15 NHL. Median age was 38 (28-56); CD4 count 207/cmm (17-506); 24% had detectable HIV-viremia. Pts received debulking therapy with 2-4 courses of standard dose CT. Five were refractory and died. Adequate collection of PBSC was obtained in 17/20 evaluable pts (85%) after cyclophosphamide 4 gr/sqm + G-CSF or G-CSF-supported CT. Two pts had early disease progression and died; one is on treatment and 14 received the BEAM conditioning regimen and PBSC transplantation with prompt engraftment in all. Pts received HAART during the entire program. Treatment-related toxicities (WHO grade 3-4): 3 oral mucositis (3) and 2 hepatic toxicity (3). Infections: 1 Staph. Epidermidis and 1 E. Coli sepsis, 1 Clostridium colitis and 3 episodes of FUO. HIV viremia was undetectable after HDT in all pts except 2 temporary positivity after a brief discontinuance with HAART. CD4 count decreased after treatment, but recovery is seen few mo after transplant. OI were seen in 3 pts: varicella zooster at 5 mo and esophagus candidosis at 9 mo in 1 pt; varicella zooster at 3 mo in a pt and esophagus candidosis at 1 mo in 1 pt. 12/13 evaluable (1 is too early) pts (92%) achieved CR and 1 PR. Relapse occurred in two pts. Eleven are alive and ten disease-free at 9 mo (2-24) after transplant with a projected overall survival (OS) of 64% at 2 ys. The median survival of the entire series (25 pts) from the study entry was 17 mo with a projected OS of 46% at 2 ys. Our data confirm on a multiinstitutional basis that HDT and PBSC transplantation is feasible and active as salvage therapy in HIV-Ly in unselected HAART responding patients.

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## TREATMENT OF SCID MICE WITH A STATIN INHIBITS THE DEVELOPMENT OF EPSTEIN-BARR VIRUS ASSOCIATED LYMPHOMA

Harutaka Katano, Lesley Pesnicak, Jeffrey I. Cohen
Medical Virology Section, Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Immunocompromised persons, such as those with AIDS or organ transplant recipients, may develop Epstein-Barr virus (EBV) associated lymphomas. Therapy for this disease may be ineffective in patients whose immune response is severely impaired. Simvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase and is used to treat patients with elevated serum cholesterol. Simvastatin also binds to leukocyte function antigen-1 (LFA-1) and inhibits the function of LFA-1, including adhesion and costimulation of lymphocytes. EBV transformed lymphoblastoid cell lines (LCLs) express high levels of LFA-1 on their surface and grow in tight clumps. Here we show that simvastatin induces apoptosis of EBV-transformed LCLs and inhibits clump formation. Simvastatin dissociates EBV latent membrane protein 1 from lipid rafts of LCLs resulting in down-regulation of NF-κB activity and induction of apoptosis. In contrast, pravastatin which does not bind to LFA-1, did not induce apoptosis of LCLs and did not dissociate latent membrane protein-1 from lipid rafts or inhibit NF-κB activity when used at concentrations similar to simvastatin. Severe combined immunodeficiency (SCID) mice given simvastatin followed by inoculation with LCLs had delayed development of EBV-lymphomas and prolonged survival. These data suggest that simvastatin may be a promising therapy for the treatment or prevention of EBV-associated lymphomas that occur in immunocompromised persons.

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## ANTIVIRAL TARGETING OF NF-κB AND EBV VIRAL LATENCY IN BURKITT LYMPHOMA

Dirk Dittmer[1], Juan Carlos Ramos[2], Subrata K. Ghosh[2], William J. Harrington, Jr.[2]. [1]The University of Oklahoma Health Sciences Center, [2]University of Miami School of Medicine.

The NF-κB transcription factor family activates a variety of inflammatory and cellular survival mechanisms and is upregulated in many primary human malignancies. Viral associated lymphomas in particular, express high levels of NF-κB and its inhibition induces apoptosis in both EBV+ and HHV-8+ lymphoma lines. Inhibition of NF-κB has recently been shown to disrupt gamma herpes viral latency. Azidothymidine (AZT) has had remarkable clinical activity in combination with other anti-virals in some patients with EBV+ lymphomas. We report here a mechanism of AZT's anti-tumor activity. Primary EBV+/LMP-1- Burkitt lymphomas (BLs) express lymphotoxin beta (LTβ), the NF-κB subunits c-Rel, RelB, p52, p50 but not p65. Treatment of these cells with AZT results in rapid intracellular phosphorylation of the anti-viral and inhibition of NF-κB (and AP-1). This is followed by upregulation of the EBV viral lytic program including EBV TK (as measured by a novel whole genome RT-PCR array and Western blots) and apoptosis. AZT blocked LTβ mediated non-canonical mediated activation of NF-κB in BLs while p65+ subclones were resistant. We have recently treated three patients with EBV+ HIV related lymphomas, two with PCNSL (both of whom had detectable EBV TK RNA in their cerebrospinal fluid) and one with relapsed BL with intravenous AZT alone. In each case there was near total regression of their tumors without the addition of any other anti-cancer therapy or change in CD4+ lymphocyte count. We have now developed an animal model to further investigate this therapy. AZT therapy exploits the presence of EBV by paradoxically inducing the lytic program and is a promising, specific agent for certain lymphomas associated with this virus.

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## LIPOSOME ENCAPSULATED DOXORUBICIN (TLC D-99, MYOCET™) WITH CYTOXAN, VINCRISTINE AND PREDNISONE FOR AIDS-RELATED LYMPHOMA: THERAPY RESULTS AND CORRELATES OF RESPONSE

A Tulpule, BM Espina, D Dharmapala, A Sherrod, BN Nathwani, WD Boswell, AM Levine. USC Keck School of Medicine, Los Angeles, CA

Front-line, anthracycline-containing regimens for the treatment of AIDS-related lymphomas (ARL) have a complete response rate of around 50%. We evaluated the safety and efficacy of liposomal doxorubicin (TLC D-99) when substituted for doxorubicin in the CHOP regimen, in patients with newly diagnosed ARL. We also assessed the impact of HIV viral control on response and survival, and correlated MDR-1 expression with treatment outcome. TLC D-99 at doses of 40, 50, 60 and 80 mg/m$^2$ were given with fixed doses of cyclophosphamide, vincristine, and prednisone every 21 days. All patients received concurrent highly active antiretroviral therapy (HAART). Lymphoma tissues were evaluated for multidrug resistance (MDR-1) expression. Twenty-four patients were accrued. 67% had high or high-intermediate IPI scores; the median CD4 lymphocyte count was 97/mm$^3$ (range 19-743). No dose limiting toxicities were observed at any level, with myelosuppression the most frequent toxicity. Overall response rate was 88%: 75% complete responses (CRs); 13% partial responses. The median duration of CR is 15.6+ months (range 1.7-43.5+). Effective HIV viral control during chemotherapy was associated with significantly improved survival ($p=0.027$) and a trend toward decreased likelihood of relapse ($p=0.09$), but CRs were attained independent of HIV viral control. MDR-1 expression did not correlate with response, suggesting that the TLC D-99 liposome may evade this resistance mechanism. We conclude that TLC D-99 in combination with cyclophosphamide, vincristine, and prednisone is active in ARL with complete remissions achieved in 75%, independent of HIV viral control or tissue MDR-1 expression. HIV viral control is associated with a significant improvement in survival.

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## PHASE I TRIAL OF ANTISENSE OLIGO AGAINST VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF-AS, VEGLIN) IN RELAPSED AND REFRACTORY MALIGNANCIES

AM Levine, G Gorospe, BM Espina, R Mocharnuk, A.Tulpule
Univ Southern Calif Keck School of Medicine, Los Angeles, CA

**Background:** Vascular endothelial growth factor (VEGF) is critical for tumor angiogenesis. Elevated tumor or serum VEGF levels predict for poor survival in cancer patients (pts). A novel VEGF-antisense (VEGF-AS, Veglin) oligonucleotide has been developed, targeting VEGF-A, -C and -D. **Methods:** In the first 4 dose levels of this phase I dose escalation trial, pts received a single course of Veglin given IV over 2 hours for 5 consecutive days at doses of 15, 22.5, 30 and 37.5 mg/m$^2$. Subsequent pts were given repeat cycles (max= 8) of Veglin given for 5 days every 2 weeks at doses of 47, 59, 74, and 85, 96, 125 and 150 mg/m$^2$. Cohorts of 3 pts were accrued to each dose level. **Results:** To date, 35 pts with relapsed/refractory malignancies have been accrued. Three had AIDS-KS: 2 at dose level 1 (15 mg/m$^2$) and one pt at dose level 2 (22.5 mg/m$^2$). All three had failed multiple prior therapies including liposomal anthracycline(s) and taxane(s). At baseline, all three had non-detectable HIV viral loads and had been on stable HAART regimens >1 year. CD4 counts ranged from 175-453/mm$^3$. Two pts had symptomatic lower extremity lymphedema with widespread cutaneous disease and one had only cutaneous involvement. Veglin infusions were well tolerated; no dose limiting toxicities have been observed. Non-hematologic side effects were all grade 1 or 2 in severity and included fatigue, hypotension, and perioral numbness, seen in less than 20% of pts. Plasma VEGF levels decreased post VEGF-AS in 14/25 (56%). Evidence of clinical activity included a biopsy confirmed CR in AIDS-KS pt with cutaneous disease, lasting 4 mos. Another AIDS-KS pt had clinical benefit, with transient reduction in lymphedema. **Conclusions:** Veglin is well tolerated at doses up to 150 mg/m$^2$. No dose limiting toxicities have yet been observed. Veglin has shown preliminary evidence of anti-tumor activity in AIDS-KS, even at the lowest dose studied.

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## FLARE OF AIDS-ASSOCIATED KAPOSI'S SARCOMA (KS) FOLLOWING HAART: A MANIFESTATION OF IMMUNE RECONSTITUTION SYNDROME (IRS)

Rom Leidner[1], David Aboulafia[1,2]. [1]Virginia Mason Clinic and [2]University of Washington

*Background:* IRS is recognized as a heterogeneous and sometimes fatal disorder of immune resurgence following initiation of HAART. AIDS-KS has rarely been reported to dramatically flare following initiation of effective HAART and may represent a little appreciated manifestation of this syndrome.
*Patients and Methods:* Following successful initiation of HAART, 7 patients with rapidly proliferating KS were evaluated and treated between 2001-04. Ten additional cases of IRS-KS have been reported in the literature. We review their presentation and clinical course.
*Results:* Of 17 patients, 13 were male, 3 female, and for 1 patient gender was not specified. At time of KS flare, 12 had recently initiated HAART, 3 had recently started a salvage HAART regimen and 2 were resuming HAART following a treatment interruption. HAART included a protease inhibitor in 12 of 17 cases (71%). At HAART initiation, mean HIV viral load (VL) for the group was 283,000 copies/mL (range, 2621 to >750,000) and mean CD4+ count was 142 cells/μL (range, 5 to 397). Median time to onset of KS flare was 4 weeks (range, 3 to 95 weeks), at which time mean HIV VL was 958 copies/mL (range, 47 to 6441) and mean CD4+ count was 220 cells/μL (range, <10 to 519). Six patients expired, 4 from progressive pulmonary KS, 1 from NHL and 1 from a pulmonary embolus. KS treatments were heterogenous and included chemo and radiotherapy, surgery, cryotherapy and experimental agents.
*Conclusion:* Worsening of AIDS-KS after HAART initiation may represent yet another protean manifestation of IRS. Dramatic proliferation of KS may occur within 3 weeks of beginning HAART. For the KS patient initiating, changing or resuming HAART, close clinical supervision is warranted. Predictive factors for the development and severity of IRS-KS have yet to be identified.

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## ASSOCIATIONS OF CLASSIC KAPOSI SARCOMA WITH HAPLOTYPES OF *IL8*, *IL8RB* AND *IL13*

Elizabeth E. Brown[1,2], Daniele Fallin[2], Ingo Ruczinski[2], Amy Hutchinson[1], Brian Staats[1], Francesco Vitale[3], Carmela Lauria[4], Lorenzo Gafa[4], Diego Serraino[5], Giovanni Rezza[5], Maurizo Montella[6], Nilanjan Chatterjee[1], Denise Whitby[1], Angelo Messina[7], Nino Romano[3], James J. Goedert[1] and Stephen J. Chanock[1]. [1]National Cancer Institute, NIH, DHHS. [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD. Collaborating institutions in [3]Palermo, [4]Ragusa, [5]Rome, [6]Naples and [7]Catania, Italy.

**Background**: Classic Kaposi sarcoma (CKS) is an inflammatory-mediated neoplasm primarily caused by Kaposi Sarcoma Herpesvirus (KSHV). KS lesions are characterized by an inflammatory infiltrate capable of producing pro-inflammatory cytokines and growth factors thought to regulate KSHV replication and CKS lesion development. **Methods:** We examined variants in pro- and anti-inflammatory cytokine genes as predictors of CKS by comparing 133 CKS cases (71% males; median age, 72 years; range, 29-91) to 172 population-based controls (69% males; median age, 75 years; range, 37-92) from Sicily, Rome and Naples who were KSHV latent nuclear antigen (LANA) positive. Twenty-eight single nucleotide polymorphisms (SNP) in 14 genes were determined by use of TaqMan PCR with allele-specific probes and primers for genomic DNA. Haplotypes were inferred from unphased genotype data for 9 genes (*IL1A*, *IL1B*, *IL4*, *IL8*, *IL8RB*, *IL10*, *IL12*, *IL13* and *TNF*). We determined antibodies to LANA by immunofluorescence assay. Risk estimates were calculated by logistic regression. **Results**: Analysis of inferred haplotypes for *IL13* revealed an increased risk of CKS. Two haplotypes of *IL13*, both containing the 130A allele, were significantly over-represented (OR=1.5 [1.1-2.2] and OR=1.7 [1.0-2.8]), whereas a haplotype of *IL8RB* was significantly underrepresented among CKS cases (OR=0.6 [0.5-0.8]). Similarly, a 3-locus haplotype of *IL8* was associated with a modest decreased risk of CKS (OR=0.8 [0.6-1.0]). Risk estimates did not vary by age, sex, prevalent disease or severity.**Conclusions:** Our data are the first to provide evidence for genetic variations in cytokines that could influence the risk of CKS in HIV-negative individuals. Among KSHV seropositive Italians, risk of CKS is associated with haplotypes of *IL8*, *IL8RB* and *IL13*, corroborating laboratory evidence of $T_H1$-mediated pathogenesis.

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## KSHV INDUCED ANGIOGENESIS AND DIFFERENTIATION TO LYMPHATIC ENDOTHELIUM

Patrick Carroll, Elizabeth Brazeau and Michael Lagunoff
Department of Microbiology, University of Washington, Seattle WA 98195

Kaposi's Sarcoma (KS) is a highly vascularized tumor predominated by spindle cells, a cell of endothelial origin. Kaposi's Sarcoma-associated herpesvirus (KSHV) is an essential co-factor for KS formation and is present in all of the spindle cells in advanced tumors. To examine the involvement of KSHV in the induction of angiogenesis we have identified endothelial cell lines in which we can achieve high-level initial infection by KSHV. In KSHV infected tert-immortailized microvascular endothelial cells (TIME) as well as in primary microvascular endothelial cells greater than 90% of the cells are latently infected by 24 hours while a low percentage of the cells are undergoing lytic replication, similar to the percentages seen in spindle cells in vivo. We used a 13,000 gene microarray to analyze changes in TIME cells at 24 to 96 hours post infection. We identified many changes potentially important to KS biology that we have confirmed with other methods and in primary endothelial cells. Following KSHV infection a number of genes involved in angiogenesis are up-regulated including VEGF receptors 1 and 3 and two hypoxia induced transcription factors (HIF1 and HIF2). Interestingly, HIF-3 (IPAS) a negative regulator of the hypoxia response factors is strongly inhibited after KSHV infection, indicating an exquisite regulation of the hypoxia induced transcription factor response. To determine if HIF activation after KSHV is functional we cloned HIF response elements upstream of luciferase and found that HIF is functionally activated after infection. We also confirmed that a number of markers of lymphatic endothelial cells are significantly up-regulated after KSHV infection. Since many studies have found that spindle cells in the KS tumor express markers of lymphatic endothelium, KSHV may infect blood endothelium and drive differentiation to lymphatic endothelial cells. This potentially important process for KS formation will be discussed.

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## KSHV AND RRV CAPSID STRUCTURE AT ≤10 Å RESOLUTION AND IDENTIFICATION OF RRV-ASSOCIATED PROTEINS BY TANDEM MASS SPECTROMETRY

C.M. O'Connor[1], M. Liang[3], Z. H. Zhou[3], and D.H. Kedes[1,2]
[1]Departments of Microbiology and [2]Internal Medicine, University of Virginia, [3]Department of Pathology and Laboratory Medicine, University of Texas at Houston Medical School

We have previously determined the structure and protein composition of capsids from both KSHV and its phylogenetic relative, RRV. We now have refined these structures to ≤10Å resolution using cryoelectron microscopy and computer reconstruction, approaching the resolution of earlier studies of HSV capsids. Nevertheless, the protein components in the tegument layer lying outside of the capsid remain less well defined. To address this issue we have subjected purified virions to tandem mass spectrometry (MS/MS). Our goal is to develop a better understanding of the repertoire of proteins that comprise the intact gammaherpesviruses. The proteins within the tegument and envelope layers no doubt have critical functions in the early stages of infection, helping to evade the cell's rapid deployment of anti-viral defense. From MS/MS analysis of gradient-purified and delipidated RRV virions, we have identified at least 17 virus-associated proteins. These include three glycoproteins, nine tegument protein candidates, and five capsid components. Two of the putative tegument proteins are gammaherpesvirus-specific but have no known function. In parallel experiments, we extracted RRV virions with detergent and subjected the resultant particles to similar analysis. In addition to the expected capsid structural proteins, MS/MS also surprisingly detected eight tegument protein fragments that resisted both detergent treatment and sonication. These data suggest that a subset of tegument proteins may interact more directly with the underlying capsid components and, in turn, may play a role in the assembly/attachment of additional overlying proteins during virion assembly.

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## LANA MEDIATED INTRANUCLEAR SHUTTLING OF KSHV GENOMES

Magdalena Weidner[1], Masahiro Fujimuro[2], Meir Shamay[2] and S. Diane Hayward[1, 2]. [1]Department of Pharmacology, School of Medicine and [2] Kimmel Cancer Center, The Johns Hopkins University, Baltimore, MD, USA

The Kaposi's sarcoma herpesvirus, KSHV, is associated with cancers that have increased incidence in patients who are also HIV positive. The KSHV encoded LANA protein is expressed in Kaposi's sarcoma and primary effusion lymphoma (PEL) cells where it colocalizes with KSHV episomes and mediates their persistence. LANA binds to the replication origin of KSHV and, during latent infection, links viral genomes to cell chromosomes via MeCP2 and DEK. However, KSHV genomes are located adjacent to PML Oncogenic Domains (PODs) during the KSHV lytic cycle. We were interested in a possible role for LANA in the transfer of KSHV genomes from chromosomes to PODs.

Testing of LANA interacting proteins from a yeast two-hybrid screen identified Sp100-HMG as a participant in trafficking to PODs. Sp100-HMG is an Sp100 splice variant that contains an HMG DNA binding domain and localizes to PODs. Indirect immunofluorescence assays (IFA) showed that LANA colocalized with Sp100-HMG in PEL cells as well as in cell transiently transfected with LANA and Sp100-HMG. IFA in combination with chromosome spreads revealed that Sp100-HMG forms foci on mitotic chromosomes. Transfected LANA painted mitotic chromosomes. However, in the presence of Sp100-HMG, LANA relocalized to the Sp100-HMG foci. LANA could also be visualized in PODs in the following experimental conditions: 1) In cells arrested in G1 and transfected with Sp100-HMG. 2) In a PML overexpressing cell line. 3) When a LANA mutant was used that had lost chromosome tethering.

In conclusion, a new LANA partner, Sp100-HMG, has been identified. We are further investigating LANA mediated transfer of KSHV genomes from chromosomes to PODs during the transition from latency to the KSHV lytic cycle.

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## KSHV/HHV-8 REPLICATION AND TRANSCRIPTION ACTIVATOR (RTA) REGULATES BOTH VIRAL AND CELLULAR GENES BY BINDING TO INTERFERON-STIMULATED RESPONSE ELEMENT (ISRE)

Jun Zhang [1], Jinzhong Wang [1], Charles Wood [1,2], and Luwen Zhang [1,2]

Nebraska Center for Virology[1], School of Biological Sciences[2], University of Nebraska, Lincoln, Nebraska 68588

Kaposi's sarcoma-associated herpesvirus (KSHV or HHV8) replication and transcription activator (RTA) is necessary and sufficient for a switch from viral latency to lytic replication. The ORF K14 (viral OX-2 homologue) and ORF74 (viral G-protein-coupled receptor homologue or vGPCR) genes are expressed from a bicistronic messenger RNA in the lytic cycle. Our data show that RTA may regulate K14-ORF74 genes through an interferon-stimulated response element (ISRE)-like sequence, namely K14 ISRE, in their promoter region. RTA activated viral K14 ISRE-containing K14-ORF74 promoter construct strongly as well as a heterologous promoter reporter construct containing K14 ISRE. RTA could bind to K14 ISRE and other ISREs, activate promoter reporter constructs from interferon-simulated genes (ISGs), and selectively induce two endogenous ISGs in primary endothelial cells. In addition, a region in RTA has been identified with certain similarity to the DNA binding domains of interferon regulatory factor (IRF) family. Mutation in one conserved amino acid within this region greatly reduced the ability of RTA to bind to DNA, to activate RTA responsive promoters, and to induce viral lytic gene expression. The mutation at the same conserved amino acid residue in IRF-7 drastically reduced its ability to bind to ISRE and to activate IFN-β promoter. The sequence and functional similarities between RTA and IRFs suggest that HHV8 RTA may usurp cellular IRF pathway.

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## A SECOND SELF-REINFORCING LOOP INVOLVING JUN/FOS, RTA AND RAP DRIVES KSHV LYTIC CYCLE REACTIVATION IN PEL CELLS AT EARLIER STAGES THAN THE C/EBPA, RTA, RAP LOOP

Shizhen Emily Wang, Frederick Y. Wu, Gary S. Hayward
Viral Oncology Program, Sidney Kimmel Comprehensive Cancer Center; The Johns Hopkins University School of Medicine

We have previously described a positive self-reinforcing regulatory loop during the KSHV lytic cycle in which the cellular C/EBPα and p21 promoters and protein levels, as well as the viral RTA, RAP and MTA promoters and protein levels are all strongly activated by both C/EBPα itself and by promoter bound complexes of C/EBPα with RTA and RAP. This pathway induces both direct transcriptional effects and protein stabilization mechanisms that also lead to G1/S cell cycle arrest. We now report that a similar but earlier positive self-reinforcing regulatory loop involves AP1 DNA-binding activity formed by cJUN and cFOS heterodimers. Either TPA alone or cotransfected cJUN/cFOS alone activates the RTA, RAP and MTA promoters through specific API promoter binding sites and the two act synergistically on all sites. Cotransfected RTA and RAP also enhance the effect of cJUN/cFOS. TPA treatment alone leads to JNK mediated phosphorylation of cJUN and detectable AP1 DNA-binding activity within 1 h and RTA alone increases the levels of overall cJUN/cFOS protein. Both RTA and RAP bind to cJUN/FOS complexes by *in vitro* GST assays and by co-immunoprecipitation from TPA-treated PEL cells, and cJUN associates with the RAP, RTA and MTA promoters by ChIP assays within 12 h. Therefore, just the subset of TPA-treated PEL cells that are induced to express RTA go on to express high levels of RAP, cJUN/cFOS, C/EBPα and p21. These same cells also display high levels of pRb protein, but not of E2F protein, and all fail to incorporate BUdR. Furthermore, either RAP alone or RTA alone can induce the G1/S cell cycle arrest phenotype. Evidently, once RTA expression is triggered in a subset of PEL cells, it initiates two distinct cascades of both viral and cellular gene expression that result in high levels of RAP, cJUN/cFOS, C/EBPα and p21 proteins followed by G1/S cell cycle arrest and activation of the full viral lytic cycle.

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## SEROLOGIC EVIDENCE FOR EXPOSURE TO SIMIAN VIRUS 40 (SV40) IN ZOO WORKERS

Eric A. Engels[1], William M. Switzer[2], Walid Heneine[2], Raphael P. Viscidi[3]. [1] National Cancer Institute, [2] Centers for Disease Control and Prevention, [3] Johns Hopkins University School of Medicine.

*Introduction*: The macaque polyomavirus SV40 contaminated pre-1963 poliovirus vaccines used in the U.S.. It remains unclear if humans are exposed to SV40 via other routes. SV40 epidemiology is relevant since some laboratories, though not all, report detecting SV40 DNA in various human tumors, including AIDS-associated lymphomas. We studied zoo workers, a group at risk for occupational infection with primate viruses, including SV40. *Methods*: 254 North American zoo workers (109 w/direct primate contact, 145 w/other jobs) participated in an anonymous serosurvey. We tested sera for antibody to SV40 and related human polyomaviruses (BK and JC) using validated virus-like particle (VLP) ELISAs. To evaluate the specificity of any SV40 seroreactivity, SV40 seropositive sera were tested in competitive inhibition experiments with BK and JC VLPs. *Results*: 25 primate workers (23%) and 15 other workers (10%) were SV40 seropositive ($p=0.01$). SV40 reactivity was generally low-level and correlated with BK and JC reactivity ($R = 0.32$, $p<0.01$; $R = 0.23$, $p=0.01$). Of 29 SV40 seropositive workers evaluable in competitive inhibition experiments, only 14 (48%) had SV40-specific reactivity (i.e., blocked by SV40 but not by BK or JC VLPs). Thus, prevalence of SV40-specific reactivity was 10% in primate workers vs. 3% in other workers ($p=0.04$). *Conclusions*: Although most SV40 antibody reactivity represents cross-reactivity with human polyomaviruses, some SV40-specific reactivity in zoo workers may reflect SV40 occupationally acquired from primates. We could not distinguish between limited, abortive SV40 infection following exposure vs. persistent infection. Further study with a larger population of primate workers is needed to better estimate exposure risk, identify routes of occupational SV40 transmission, and determine whether there are health effects of exposure.

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## LUNG CANCER IN HIV POSITIVE AND HIV NEGATIVE WOMEN: WOMEN'S INTERAGENCY HIV STUDY (WIHS)

AM Levine[1], EC Seaberg[2], N Hessol[3], S Preston-Martin[1], M Cohen, M Glesby, M Young, H Minkoff, H Watts[4], J Black[5]. [1]USC Keck School of Medicine, LA, CA; [2]Johns Hopkins School of Public Health, Baltimore, MD; [3]UCSF, San Francisco, CA; [4]NICHD, Bethesda, MD; [5]NCI, Bethesda, MD; and other sites

**Background**: The incidence of lung cancer (Ca) has increased among HIV infected (+) individuals. Characteristics and risk of lung Ca in HIV+ women have not been defined. **Methods:** The WIHS, an ongoing cohort study, has enrolled 2059 HIV+ and 569 HIV- women seen every 6 mos. Self-reported cancers are confirmed by chart abstraction, cancer registry and/or national death index matches. Diagnostic path reports are reviewed. **Results:** As of 3/03 (median F/U of 7.4 years), 11 lung Ca cases have occurred: 9 in HIV+ and 2 in HIV-. Among HIV+, median age at lung Ca dx was 55.8 yrs (range 36-63). All had history of smoking, median of 20 pack-years (range 9 to 94 PYs). Median CD4 count prior to dx of lung Ca was 376/$mm^3$ (range 0 to 842); median HIV-RNA level=2,800 copies/cc (range 550 to 310,000). Prior AIDS defining illness was present in 7 (78%). Pathology included adenocarcinoma in 2, poorly differentiated non small cell in 2, and squamous cell in 3. One pt was on HAART at time of lung Ca dx, 5 were on no ART, and 3 were on combination ART. Stage IIIB/IV disease was present in 6. Median survival was 15.7 months (range 3.7 to 48+ mos). The 2 HIV- women with lung Ca were 46 and 56 yrs, with 25 and 29 PY hx of smoking and survivals of 1.6 and 8 mos. Adjusted for sex, age, and race, the incidence of lung Ca in HIV+ women was significantly higher than the general population (SEER registry), with a standardized incidence ratio (SIR) of 4.7 (95% CI: 2.1-8.1). Among HIV- women, the incidence of lung Ca was elevated at SIR = 4.4 (95% CI: 0.5-12.4, P=NS). Overall, lung Ca incidence rates among the HIV+ (77.8 per 100,000 PYs) and HIV- (63.3 per 100,000 PYs) women were not different (Rate ratio = 1.2, 95% CI: 0.3-11.7). **Conclusions**: The incidence of lung cancer is increased in HIV+ women compared with SEER registry expectations. There is no difference in the incidence of lung cancer among HIV + and HIV- women at risk in the WIHS. A history of heavy smoking appears more strongly related to the development of lung Ca than HIV related immunocompromise.

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## ANAL CANCER INCIDENCE IN RELATION TO HUMAN IMMUNODEFICIENCY VIRUS (HIV) EPIDEMIOLOGY

Chiao, E.[1], Krown, S.[1], Schrag, D.[1] [1]Memorial Sloan Kettering Cancer Center

**Background**: Like Cervical Cancer, Squamous Cell Carcinoma of the Anal Canal (SCCA) is etiologically linked to Human Papilloma Virus (HPV). HIV-related immunosupression likely contributes to the increased incidence of SCCA.We sought to describe secular trends in SCCA in relation to the HIV epidemic. **Methods**: We used data from the Surveillance, Epidemiology and End Results (SEER) database to calculate trends in age-adjusted incidence of invasive SCCA from 1973-2000.We analyzed the data according to three separate periods during the HIV epidemic: the Pre-HIV era (cases reported from 1973-1984), HIV era (cases reported from 1985-1995), and the HAART era (cases reported from 1996-2000.) **Results**: A total of 4,580 cases of invasive SCCA were reported from 1973-2000.The incidence per 100,000 of SCCA increased from 0.6 (0.6,0.7) in the pre-HIV era to 0.8 (0.8,0.8) in the HIV era to 1.0 (1.0,1.1) in the HAART era. The gap between SCCA incidence in women compared to men decreased from 1.6:1 Pre-HIV to 1.2:1 in the HAART era. There was a statistically significant increase in the incidence rate among men aged 35-54 from 0.6 (0.5,0.7) in the HIV era to 1.3 (1.1,1.4) in the HAART era. There was also a statistically significant increase in incidence of SCCA in men aged 65-74 from the HIV era to the HAART era. Among women there in the 35-54 age group, the incidence increased significantly from 0.8 (0.7,0.9) in the HIV era, compared to 1.2 (1.1,1.4) in the HAART era. Percentage of cases diagnosed early (In Situ or Localized disease) increased from 44% pre-HIV to 51% pre-HAART and to 60% with HAART. .Five-year relative survival increased from 53% pre-HIV era to 56% in the HIV era to 59% in the HAART era. **Conclusions:** Despite the advent of HAART therapy for HIV, the incidence of SCCA continues to increase.The incidence of SCCA has particularly increased in men and in cases diagnosed in the 35-54 age group.

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## HIGH RATE OF PERSISTENT CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN) DESPITE STANDARD THERAPY IN HIV-POSITIVE WOMEN

Isabelle Heard[1], V. Potard[2], H Foulot[3], C. Chapron[3], D. Costagliola[2]

[1]Dept of Immunology, Hospital Pompidou, [2]INSERM E 0214, [3]Dept of Gynecology, Hospital Cochin, Paris, France.

**Background**: Previous studies of HIV+ women have demonstrated a high rate of CIN with frequent recurrence despite adequate surgical treatment. Our objective was to determine the rate of recurrence of CIN in a group of HIV+ women after standard surgical therapy. We sought to determine the efficacy of different specific surgical modalities. Of particular interest was the potential impact of positive surgical margin, immunosuppression and HAART on the recurrence rate. **Methods**: 121 HIV+ women enrolled in a gynecological survey of HIV+ women, were followed-up by cytology, histology and colposcopy after surgical treatment for CIN. Univariate and multivariate analyses of the association of recurrence and risk factors were performed using Cox's proportional hazard models. HAART and CD4+ cell counts were included as time-dependent variables. **Results**: Ablative laser therapy was used in 16 low-grade CIN (LGCIN) and 2 high-grade CIN (HGCIN). Excisional therapy with cold knife or laser conisation or LEEP was performed in 17 LGCIN and 85 HGCIN. Hysterectomy was performed in one HGCIN. Overall, 369 patient-years were followed-up. The recurrence rate was of 26.2 per100 patient-years of follow-up. One woman developed invasive cervical carcinoma during follow-up. In multivariate analysis, a positive margin (RR:4.0 95%CI, 1.5-10.9), severe immunosuppression (RR 9.1 95%CI, 2.7-30.7), and not being on HAART (RR 2.9 95%CI, 1.2-6.9) were associated with a significant risk of recurrence. Recurrence rate was similar by treatment modalities (p=0.31). **Conclusion**: We found that severe immunodeficiency and positive margin were predictors of recurrence. As cervical conisation is not an effective method to eradicate CIN in HIV-positive women, and as only a modest positive impact of HAART was observed on the recurrence of CIN, meticulous follow-up of cervical disease remains indicated in HIV-positive women.

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## IMMUNE STATUS OF HIV-POSITIVE PATIENTS WITH OCULAR SURFACE SQUAMOUS NEOPLASIA IN BOTSWANA

Bazghina-Werq Semo[1], Gregory Bisson[2], Oathokwa Nkomazana[1].
[1]Princess Marina Hospital, Gabarone, Botswana, [2]University of Pennsylvania.

**Introduction:** Ocular surface squamous neoplasia (OSSN) has been associated with HIV infection in several countries in Africa, and the incidence of OSSN has increased markedly as the HIV epidemic has evolved in these regions. No studies to date have documented the immune status of HIV-infected patients with OSSN. Moreover, there are no data on the burden of this disease in Botswana, where approximately 37% of adults are HIV positive. **Methods:** In order to evaluate the CD4 counts of patients presenting with OSSN in Botswana, we performed a cross-sectional study at the Princess Marina Hospital (PMH) Department of Ophthalmology. Patients who agreed to be tested for HIV had CD4+ T cell counts determined using flow cytometry at the time of initial presentation. Data were described using STATA, version 8.0. The analysis of trend of new cases of OSSN in Botswana was done via a review of medical records of patients seen in the Department of Ophthalmology between the years of 1993 and 2003. **Results:** A total of 21 HIV+ patients with OSSN were evaluated for the study. Of these, 3 were on antiretroviral (ARV) therapy at the time of diagnosis. Overall the median CD4+ T cell count at diagnosis was 192 cells/mm$^3$ (range, 20 to 627 cells); excluding the patients who were on ARV therapy at diagnosis the median CD4+ T cell count was 154 cells/mm$^3$. Excluding patients on ARV therapy, 78% of patients presented with CD4+ T cell counts <200. The number of new cases of OSSN seen at PMH increased from 6 cases from 1993 to 1996 to 127 cases seen between 1997 and 2003. **Conclusion:** The HIV epidemic in Botswana is associated with a large increase in the number of new cases of OSSN. Individuals who present with OSSN usually do so with CD4 counts < 200 cells/mm$^3$, consistent with a diagnosis of AIDS.

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# TRENDS IN INCIDENCE OF CANCERS IN RELATION TO AIDS PANDEMIC IN SWAZILAND

Longengo S. Okonda[1], Mfokololo Dlamini[1],Nomvula Dlamini[1]
[1]Swaziland Cancer Registry, Ministry of Health & Social Welfare

## Introduction

The study compares the patterns and the incidences of malignancies before (1979-83) and after (1996-99) HIV pandemic in Swaziland in order to measure the burden of AIDS-related malignancies.

## Method

Diagnostic method used by the first cancer registry (1979-83) was basically clinical. It was completed for the last two years by histology reports, which acted as an independent source of notification of cancer cases. The second cancer registry (1996-99) is located in the sole Department of Pathology in the country. It is based on the population, and uses all diagnostic methods including histology, cytology, clinic, and death certificates.

## Results

It is difficult to make comparisons between the two studies because of the differences in case ascertainment. Nevertheless, many of the changes reflect the epidemic of AIDS, which is severe in Swaziland: prevalence of HIV infection in adults is constantly increasing: from 25.2% in 1999 to 33.4% in 2001. Thus, the frequency of Kaposi's sarcoma has increased enormously to reach 16.8% of all cancers in males (ASR 17.2 per 100 000) and 10.4% in females (ASIR 9.5 per 100 000). In females, the picture is dominated by the extraordinary high rate of cervix cancer: 41.7% of all cancers (ASIR 59.3%). In childhood cancers, the principal cancers recorded was Burkitt's lymphoma 12.9% (ASIR 7.6 per million) and Kaposi's sarcoma 7.5% (ASIR 4.4 per million).

ASIR: age standardized/adjusted incidence rate

## Conclusions

Further studies measuring accurately the burden of AIDS-related malignancies and their risk factors in developing country settings are required in order to comprehend their clinical manifestations.

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## HUMAN PAPILLOMAVIRUS E2 PROTEIN INTERACTS WITH TRANSCRIPTIONAL COFACTOR TAFII250 AND MODULATES ITS ENZYMATIC ACTIVITIES

Xiaohua Zhang[1] and Kenneth A. Alexander[1,2]
From the [1]Department of Molecular Genetics & Microbiology, and the [2]Department of Pediatrics, Duke University Medical Center, Durham, North Carolina 27710

Human papillomaviruses (HPVs) DNA replication and transcription regulation require the virus encoded E2 protein. The E2 protein is comprised of a transactivation domain, a sequence-specific DNA binding domain and a proline-rich hinge region. In this study, we demonstrate that E2 interacts with TAFII250, a cellular transcription coactivator carries phosphorylation, histone acetylase, and ubiquitin activating/conjugating activities. E2 inhibits TAFII250 autophosphorylation and inhibits TAFII250-catalyzed phosphorylation of TFIIA, but activates TAFII250-mediated RAP74 phosphorylation. E2 acetylation by TAFII250 occurs at the E2 hinge/DNA-binging domain. E2 inhibits TAFII250-mediated histone acetylation in a dose-dependent manner. TAFII250 mediates E2 transactivation upon E2 binding to distal E2 binding site in HPV long control region. E2 also stimulates TAFII250-mediated MHC class I gene transcription.

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## CHARACTERIZATION OF BPV E2 INTERACTIONS THROUGH THE CELL CYCLE

Maria McPhillips, Alison McBride
Laboratory of Viral Diseases, National Institute of Allergy and Infectious Disease

For bovine papillomavirus type 1 (BPV-1), it has been shown that viral genomes are tethered to cellular mitotic chromosomes via a protein-protein interaction mediated by the viral protein E2. In doing so, the viral genomes are efficiently segregated to daughter cells following mitosis ensuring longevity of the viral infection. The E2 protein also functions as a transcriptional activator of viral genes and, in complex with the E1 protein, initiates viral DNA replication.

To determine if E2 exists in different multiprotein complexes throughout the cell cycle, E2 was extracted by differential salt concentrations from both interphase and mitotic cells and analyzed by immunofluorescence and Western blotting. A fraction of the E2 protein was extracted with increasing salt but a highly salt-resistant protein population remained in both interphase and mitotic cells. To correlate protein extraction with function, a series of well-characterized mutated E2 proteins was assayed with the same extraction procedures. The results obtained indicated that salt retention of the E2 protein in interphase cells correlated with transactivation ability, possibly as a result of interactions with nuclear matrix bound transcription factors. However, all E2 proteins that were able to bind mitotic chromosomes were retained on mitotic chromosomes up to 600mM NaCl, even if they were transactivation defective and easily eluted from interphase cells. This suggests that the viral E2 protein forms different multiprotein complexes through the cell cycle, with the strongest complexes forming in mitosis to ensure efficient segregation of the viral genome.

The E2 protein has been shown to interact with numerous cellular proteins. To determine the functional significance of these interactions the techniques described above are being used to characterize these complexes throughout the cell cycle.

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## SEGREGATION ROLE OF HUMAN AND ANIMAL PAPILLOMAVIRUS E2 PROTEINS

Jaquelline Oliveira and Alison McBride - Laboratory of Viral Diseases (LVD) - DNA Tumor Virus Section NIAID - National Institutes of Health (NIH)

Upon infection of basal epithelial cells, papillomavirus genomes are established as episomes in the nucleus. As infected cells divide, the replicated viral genomes must utilize mechanisms to ensure segregation to daughter cells following mitosis. In the case of bovine papillomavirus type 1 (BPV-1), it has been shown that E2-mediated attachment of viral genomes to mitotic chromosomes ensures equal partitioning. However, it has not been established if other papillomaviruses employ a similar segregation mechanism. To determine whether other papillomavirus E2 proteins are also localized on mitotic chromosomes, we have expressed FLAG-tagged E2 proteins from several human (HPV-1a; HPV-4; HPV-8; HPV-11; HPV-16; HPV-57 and HPV-31) and animal (CRPV; ROPV; COPV, EEPV and DPV) papillomaviruses. The E2 genes were expressed from the inducible vector, pMEP4, and good nuclear expression in interphase cells was observed with most E2 proteins. Expression was increased for HPV4, HPV11, HPV16 and HPV31 E2 proteins by partial or complete codon optimization. All of the E2 proteins were functional as assayed by an E2-dependent reporter assay. Chromosomal association has been detected for the Supergroup B, C and D papillomaviruses. The E2 proteins from the Supergroup A mucosal viruses were also found in close association with mitotic chromosomes but the E2 protein does not appear to be stable throughout mitosis. Studies are currently underway examining whether viral genomes and/or other viral proteins are required to stabilize both E2 expression and chromosomal interaction in mitosis.

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## THE HPV16 E2 PROTEIN FUNCTIONS AS A TRANSCRIPTIONAL REPRESSOR IN PRIMARY HUMAN KERATINOCYTES

Emiko Soeda and Alison McBride
Lab of Viral Diseases, NIAID, NIH, Bethesda, MD 20892

In HPV16 associated cancers, viral DNA is often integrated in such a way as to disrupt the viral E2 gene. In cotransfection experiments the E2 protein can repress the promoter that controls expression of the E6/E7 oncoproteins and so its loss is predicted to increase E6/E7 gene expression. However, other models have been proposed to explain why integration promotes malignant progression. The E2 protein is required for viral DNA replication and so genetic analysis of E2 in an episomal HPV is impossible. To circumvent this we have developed a vector containing the HPV16 regulatory region and early genes that is maintained episomally by the EBV EBNA1 protein and oriP element. HPV expression was assessed by real time QRT-PCR and the transcription and splicing pattern of HPV genes expressed from these episomes was identical to that of the full-length HPV genome in primary human keratinocytes. To determine which functions of E2 are required for regulation of E6/E7 expression, we generated a series of mutations that disrupted specific functions of E2. Higher levels of E6/E7 expression were detected shortly after transfection in the absence of E2, indicating that E2 repressed transcription. Mutations that abrogated the transactivation, but not the replication function of E2 relieved repression of the E6/E7 promoter. Mutation of either E2 binding sites also alleviated repression by E2. These studies suggest that when E2 is expressed from its own promoter in primary human keratinocytes, it functions as a transcriptional repressor and that this repression requires both DNA binding and transactivation functions of the E2 protein.

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## GENE CLONING AND EXPRESSION OF HPV 6 L1/L2 AND IMMUNOGENICITY OF THE ASSEMBLED VIRUS-LIKE PARTICLES

Wang M, Wang LL, Chen LF, Si JY, Lee K, Liu SD, Song GX
Institute of Basic Medical Sciences, Peking Union Medical College, China

Introduction: Exophytic genital wart, is caused mainly by human papillomaviruses(HPVs) type 6 and 11 infection. HPVs are non-enveloped viruses with the capsid composed of major capsid protein L1 and minor capsid protein L2. This study is focus on the immunogenicity of L1 and L2.

Methods and Results: Based upon L1 and L2 overlapping sequence two sequences were assembled and classified into HPV-6b by phylogenetic analysis. Compared with prototype sequence, four missense mutations were found. When expressed in baculovirus expression system, VLPs self-assembled in Sf9 nucleus by L1 alone (L1-VLPs) and by L1 plus L2 (L1+L2-VLPs) were purified and further characterized. Both types of VLPs were spherical particles with a diameter of approximately 50 nm . As characterized by ELISA , the L1+L2 VLP was found to be reactive with both H6E51 and H6K57 , demonstrating the L1+L2 VLP possessed HPV-6 L1 VLP immuno-reactivities.

BALB/c mice were used for testing immunogenicity of recombinant VLPs . The titers of serum antibodies in groups vaccinated with adjuvant L1-VLP and L1+2-VLP were above 1:10000 as detected by ELISA, which was higher than groups without adjuvant (1:2000). In vitro re-stimulating with HPV-6 L1 VLP was observed $^{3}$H-TdR incorporation test for immunized mice ($P<0.01$).The stimulation indexes (SI) were 6.4, 6.2 and 1.1 for L1-VLP, L1+2-VLP and control group.

In conclusion, The prepared recombinant HPV-6 VLPs are highly immunogenic, inducing both cellular and humoral immunity in mice. The antibodies possess neutralizing capability.

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## MOLECULAR CYTOGENETIC CHARACTERIZATION OF HUMAN PAPILLOMAVIRUS POSITIVE CELL LINES

Eva M. McGhee, Philip D. Cotter and Joel M. Palefsky.
University of California San Francisco, San Francisco, CA 94131

**Background:** Anogenital cancers are closely associated with human papillomavirus (HPV). HPV-infected individuals, particularly those with high-grade dysplasias, are at increased risk for cervical and anal cancer. Although genomic instability has been documented in HPV-infected keratinocytes, the full spectrum of genetic changes in HPV-associated lesions has not been fully defined. **Methods:** Examination of four HPV-positive epithelial cell lines, Caski, SiHa, Hela, and MT-16, by G-banding and 24-color fluorescence in situ hybridization revealed multiple numerical, complex and cryptic chromosome rearrangements. **Results:** Translocations of chromosomes 12 and 14 involving specific sites were identified in MT-16 and Caski cell lines. All four cell lines showed loss and gain of DNA due to unbalanced translocations involving complex rearrangements on the short arms of chromosomes 1, 3, 9, 10, and 17. **Conclusions:** These regions contain known tumor suppressor genes. Chromosomal damage in these regions might help to explain the increased risk of cancer associated with HPV.

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## KERATINOCYTE GROWTH CONDITIONS MODULATE TELOMERASE EXPRESSION, SENESCENCE, AND IMMORTALIZATION BY HUMAN PAPILLOMAVIRUS TYPE 16 E6 AND E7 ONCOGENES

Baojin Fu[1], Astrid Baege[1], Xuefeng Liu[2], Richard Schlegel[2], and Carl C. Baker[1]

[1]Laboratory of Cellular Oncology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892. USA and [2]Department of Pathology, Georgetown University Medical School, Washington, DC 20007. USA

Induction of telomerase is thought to be an important step in the immortalization of keratinocytes by high risk HPVs. We have previously demonstrated the reversible induction of telomerase activity when human foreskin keratinocytes (HFKs) are transferred from culture on plastic in K-SFM to feeder fibroblasts and F medium. This is true for both primary HFKs as well as HPV-16 E6 and E7 transduced HFKs (HFK/16E6E7 cells). However, the feeders are only essential for telomerase induction in primary HFKs. Telomerase levels in HFKs grown in the feeder environment were sufficient to maintain telomere length for at least 43 PDs. Beyond this point, however, both telomerase activity and telomere length decreased until the cells ceased growing at 81 PDs, probably due to replicative senescence. We also demonstrated previously that expression of both E6 and E7 in HFKs cultured in the feeder environment lead to a superinduction of telomerase levels. These cells maintained telomere length out to at least 56 PDs. In contrast, HFK/16E6E7 cells cultured in K-SFM showed significant erosion of telomeres at least as early as 35 PDs. In a squamous epithelium in vivo, telomerase activity is present only in the undifferentiated basal cells. We assessed the differentiation status of the cells in the experiments described above and found that the feeder system does not induce telomerase by blocking differentiation. Further studies are currently being carried out to identify the mechanisms by which culture conditions and E6 and E7 induce and/or repress telomerase.

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## IDENTIFICATION AND ISOLATION OF PUTATIVE CERVICAL STEM CELLS BASED ON CELL SURFACE PHENOTYPE

Astrid Baege[1], Richard Schlegel[2], Carl Baker[1]. [1] NCI, NIH and Department of Pathology, Georgetown University Medical Center.

Persistent HPV infections are much more likely to progress to cervical displasias and cancer than transient productive viral infections. The crucial factors for persistence are still unknown. However, it has been proposed that viral persistence only occurs upon targeted infection of specific cervical cells such as stem cells. Cervical epithelial stem cells remain poorly characterized. Here we present the identification of a candidate cervical epithelial stem cell based on the combined use of the cell surface markers integrin α6 (CD49) and transferrin receptor (CD71). We demonstrate evidence that a population of cervical cells expressing undetectable to low levels of CD71 in combination with high levels of CD49 show stem cell properties. These cells represent a minor subpopulation of small quiescent cells, expressing keratin (K)14, but not K1. Colony formation assays demonstrated that these cells have a much higher potential to proliferate in vitro and to form colonies when compared with any other keratin expressing subpopulation within the cervical epithelium. Suprabasal, transient amplifying cells were enriched in a major subpopulation which expressed slightly lower CD49 and profoundly increased CD71, comprised of larger and actively cycling cell with a decreased ability to form colonies. Binding assays on isolated cervical cells with virus like particles (VLPs) revealed a small subpopulation within the putative stem cell population that has a clearly increased VLP binding capacity. Furthermore, these binding assays identified another population within the cervical epithelium that binds VLPs with high capacity. Interestingly, these cells do not express K1 or 14 and show a high colony formation capacity. These results represent an important step in the characterization and isolation of cervical epithelial cells, providing the base for the determination whether targeting of a specific cell type by HPV is required to obtain persistent infection.

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## 4,4'-DIHYDROXYBENZOPHENONE-2,4-DINITRO-PHENYLHYDRAZONE (A-007) – AN IMMUNOMODULATOR OF CD45+ T-LYMPHOCYTES: RESPONSES IN HPV ASSOCIATED ANOGENITAL CANCERS

L.R. Morgan[1], C.L. Hooper[1], A. Rodgers[1], V.J. Culotta[2], J. H. Bellina[3], R. W. Kelly[3], Travis Harrison[4] and Barry Sartin[4]. [1]DEKK-TEC, INC, New Orleans, LA, [2]East Jefferson Hospital, Metairie, LA, [3]Omega Institute, Metairie, LA and [4]Memmorial Hospital, New Orleans, LA.

A-007 is an aryl hydrazone that has immune modulating properties when applied topically, intravaginal or intra/perianal, as a 0.25% gel to pts with cancer of anogenital origins. In other studies, A-007 has demonstrated >25 % objective responses when applied topically to patients with metastatic breast cancer, melanoma and lymphomas [NCI-EORTC, Abst. 477, 1998 and AACR, Abst. 4825, 2002]. In the current study, 18 patients with cancers of the cervix (11), anal (5) or vagina (2) were treated daily for 5-days with 2 g of a 0.25% A-007 gel (4 pts were treated with 2 courses of drug). 14/18 patients were HPV+ and 1/18 HIV+. Pre- and post-A-007, tissue biopsies, complete blood counts, chemistries, urine analyses and plasma levels for A-007 were obtained. Biopsies of A-007 treated anogenital sites revealed increased infiltrations of CD3+/ CD4+/ CD8+/CD45+ T-lymphocytes after only 5-days of treatment with no local toxicity. To date - 12/CR, 2/PR and 4/NR have been noted. One anal cancer responded well with a CR. 10 Patients developed negative tissue titers for HPV. No related acute contact dermatitis (ACD) or neutrophilic or eosinophilic infiltrates were noted. A-007 was not detected in any plasma samples, nor were changes noted in CBCs, chemistry profiles or urines for any pt during or after treatments. The most obvious consistent immunohistochemical change noted (for tissue and blood) was an up-regulation of CD45+ T-lymphocyte receptors – RA to RO. A-007 exists in resonating electronic states capable of inducing dimerization of the CD45 catalytic receptor, with up-regulation and activation of the T-cell cascade. Interactions with CD45+ surface receptors and T-lymphocyte activation-cascades will be discussed. Future plans to use A-007 as an immune modulator and/or a co-modulator of HPV/HIV associated anogenital cancers will be discussed. Supported by NCI grants CA49310 (SBIR) & CA89772 (FLAIR).

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## THE EFFECT OF HIV ON CERVICAL MUCOSAL IMMUNE MICROENVIRONMENT AND FUNCTION IN HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS

Kobayashi A[1], Anastos K[2], Minkoff H[3], Massad S[4], Young M[5], Levine A[6], Ahdieh L[7], Teresa Darragh[1], Weinberg V[1], Greenblatt R[1,10] and Smith-McCune K[1]

[1]University of California at San Francisco, [2]New York City/Bronx Consortium, [3]Brooklyn, NY, [4]Cook County Hospital, Chicago, [5]Washington DC Metropolitan Consortium, [6]Southern California Consortium, [7]WIHS Data Coordinating Center, [10]the Connie Wofsy Study Consortium of Northern California

The role of the mucosal immune response in human papillomavirus (HPV)-related cervical diseases is poorly understood. Our goal was to characterize the local mucosal immune response to HPV in high-grade dysplasia (HSIL) and to determine the effects of HIV infection. Samples from women with normal cervix, HIV seronegative women with HSIL ($HIV^{neg}$HSIL) and HIV seropostive women with HSIL ($HIV^{pos}$HSIL) were compared. Cervical immune cell densities indicated that CD4+ and CD8+ T cells, macrophages, B cells, NK cells and neutrophils were significantly elevated in $HIV^{neg}$HSIL compared with normal cervix ($p = 0.0002$, 0.04, 0.04, 0.01, 0.01 and 0.001, respectively). There were significant increases in CD4+ and CD8+ T cells, mast cells and macrophages in $HIV^{pos}$HSIL compared to normal cervix ($p = 0.03$, 0.003, 0.002, and 0.05, respectively). CD4+ T cells, macrophages Neutrophils and NK cells in $HIV^{pos}$HSIL were significantly decreased ($p = 0.03$, 0.02, 0.03, and 0.03 respectively) compared to $HIV^{neg}$HSIL. Functionally, expressions of IL-2R and T bet were detected in both HSIL groups. However, IFN-γ positive cells in $HIV^{pos}$ HSIL were significantly reduced compared to $HIV^{neg}$HSIL ($p = 0.0004$). Interestingly, TGF-β in CD25+, CD4+, CD68+, and/or CD1a+ cells, and IL-10 in CD1a+ cells, appeared to be more abundantly expressed in $HIV^{neg}$HSIL than $HIV^{pos}$HSIL, indicating that regulatory cytokines are unlikely to explain the reduction of IFN-γ in $HIV^{pos}$HSIL. In addition, we found CD1a+ dendritic cells with immature morphology which express both IL-10 and TGF-β, suggesting a novel form of mucosal immune regulation in the cervix. These results imply that HIV impairs mucosal lymphocyte differentiation and alters mucosal immune regulation in cervical HSIL.

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## HPV TESTING OF ASCUS PAP SMEARS IN HIV POSITVE WOMEN

Gregory Taylor[1], Tracy Wolfe[2], Lori Fantry[2]. [1]Institute of Human Virology, [2]University of Maryland Baltimore

**Background**-Women infected with the Human immunodeficiency virus (HIV) are at an increased risk for infection with and persistence of human papillomavirus (HPV). This renders them more susceptible to abnormalities on screening Papanicolaou smears including findings of atypical squamous cells of undetermined significance (ASCUS). Currently, women with ASCUS and HIV are referred directly for colposcopic examination because of the high rate of dysplasia in this population. In contrast, according to the 2001 consensus guidelines, women with ASCUS and without HIV are triaged to colposcopy or routine follow-up based on presence or absence of high risk HPV. Our study evaluated whether this strategy would be appropriate in women with HIV. **Methods-**Information was obtained from a database that included records of 508 HIV-infected women referred for colposcopic examination at an urban, university-based infectious disease clinic. Data from women who had undergone colposcopic examination because of an ASCUS Papanicolaou smear (n=108) were analyzed. Digene hybrid capture II was used to detect the presence of HPV DNA and was collected at the time of colposcopy. Colposcopy was performed in the standard manner approximately 2 months from the date of the ASCUS result. The positive and negative predictive values were calculated for the use of high-risk HPV to predict dysplasia on biopsy obtained at colposcopy. **Results-**Eighty-three of the 108 samples (77%) tested positive for any HPV (high and low risk types) and 76/108 (69%) tested positive for high-risk HPV types. Of the 108 samples, 16 had any dysplasia: 10 had mild dysplasia, 4 had moderate dysplasia, 1 had cancer, and 1 had an "upgradeable dysplasia." High-risk HPV DNA was detected in 14/16 of cases with any dysplasia and in 60/92 cases without dysplasia. The positive predictive value was 0.19 and the negative predictive was 0.94. All of the 7 cases of high-grade dysplasia or cancer were positive for high risk HPV. Thus the sensitivity of HPV testing was 88% and the specificity was 35%. **Conclusion-**The overall rate of HPV-positive women in this sample was much higher than the 31-60% rate of high-risk HPV reported in HIV-negative women. The use of HPV screening of ASCUS Papanicolaou smears for triage to colposcopy is a very sensitive but not very specific tool for genital dyplasia in this population. Because of the low specificity and the difference in prevalence and persistence of HPV infection in HIV-positive women, we do not recommend using HPV testing on screening ASCUS Papanicolaou smears as a triage method for referral to colposcopy. Further large-scale, longitudinal studies are needed to determine the role of HPV testing in this population.

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## KNOWLEDGE AND ATTITUDE OF WOMEN WITH HIVTOWARDS CERVICAL CANCER AND CERVICAL CANCER SCREENING LAGOS NIGERIA-WEST AFRICA

[1]RI Anorlu, [1]CI Igwillo, [2]AS Akanmu, [3]AAF Banjo. [1]SI Dim [1]Dept Ob-Gyn, [2]Dept Hematology [3] and Dept. Morbid Anatomy, all University of Lagos Teaching Hospital

Introduction: There are about 6-7 million people living with HIV/AIDS in Nigeria (pop. 120million). Over 50% of them are women. Two million people have died of the disease since it was first reported in the country in 1986. Cervical cancer is the commonest genital cancer in Nigeria with most women presenting in the very late and untreatable stage. Several studies have shown HIV disease is associated with an increased risk of cervical dysplasia and cervical cancer in women. There are no published studies yet on this association in Nigeria. We determined the knowledge and attitude of women with HIV towards cervical cancer and cervical cancer screening as a preliminary to studies in cervical cancer and HIV. Methods: We interviewed consecutive consenting women attending HIV clinics in two centers in Lagos-Nigeria between January 12th 2004 and February 16th to assess their knowledge and attitude to cervical cancer and cervical screening. These centres have been accredited by the government for treatment of HIV patients at subsidized rate. Results: Two hundred and four patients were interviewed, their mean age was 36.3± 8.9 years (range 20-65 years). The parity was 2.2 ± 2.1 (range 0-9). 105 (51.4%) were married, 42 (20.6%) single, 41 (19.6 %) were widowed (in 39 husbands died of HIV) and 8.2% separated (because of HIV). 69 (33.8%) had had tertiary education, while 47.1% and 19.1% had only secondary and primary school education respectively. The mean age at sexual intercourse was 17.1±. 3.32 years (range 9-35 years) and the mean number of sexual partners 2.5±1.7 (range 1-10). Only 6 (2.9%) had had a Pap smear, 33 (16.2%) knew about cervical cancer and 24 (11.8%) were aware of Pap smear. 197 (96.6%) were willing to have a Pap smear if offered, reason is they were ready for anything that would prolong their lives. The remaining seven were fed up and would not want any further tests. Conclusion: Knowledge of cervical cancer and cervical cancer screening among women with HIV in Lagos Nigerian is poor. Nonetheless many are willing have a Pap smear if offered.

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## PLASMABLASTIC LYMPHOMAS (PBL): DIVERSE LYMPHOMAS ASSOCIATED WITH IMMUNO-DEFICIENCY AND HIV

[1]Lluís Colomo M.D., [2]Florence Loong M.D., [1]Susana Rives M.D., [2]Stefania Pittaluga M.D., [1]Antonio Martínez M.D., [1]Armando López-Guillermo M.D., [1]Jesús Ojanguren M.D., [1]Vicens Romagosa M.D., [2]Elaine S Jaffe M.D. and [1]Elías Campo M.D. (1) Univ of Barcelona, Barcelona, Spain; (2) NCI, Bethesda, MD.

PBL was 1st described as a variant of diffuse large B-cell lymphoma (DLBCL) involving the oral cavity of HIV+ pts. However, other lymphomas may exhibit similar features. To determine the significance of plasmablastic differentiation in DLBCL, we examined 50 DLBCL with low or neg CD20/CD79a & an immunophenotype indicative of terminal B-cell differentiation (MUM1/CD38/CD138/EMA-+). We defined several subgroups. 23 tumors were classified as PBL of the oral mucosa type, composed of monomorphic blastic cells without plasmacytic differentiation. 16 (70%) pts were HIV+ and 5 pts had other causes of immunosuppression: post-transplant (2), steroids (1), and age >80 yrs (2). EBV was + in 74%. 11(48%) cases presented in the oral mucosa, but the remaining presented in other extranodal (39%) or nodal (13%) sites. 16 cases were classified as PBL with plasmacytic differentiation. 5 pts (33%) were HIV+; 1 patient had Crohn's disease. EBV was detected in 62%, and 44% had nodal presentation. Only 2 cases presented in the oral cavity. 9 cases, morphologically indistinguishable from the previous group, were secondary extramedullary plasmablastic tumors occurring in pts with prior or synchronous plasma cell neoplasms. 2 additional neoplasms were an ALK+ DLBCL and an HHV-8+ extracavitary variant of primary effusion lymphoma. HHV-8 was examined by immunohistochemistry in 39 additional cases, and was negative in all. In conclusion, DLBCLs with plasmablastic differentiation are a heterogeneous group of neoplasms often associated with immunodeficiency and HIV-infection. The spectrum extends beyond the original definition of PBL of the oral mucosa.

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**IS EBNA1 A SURVIVAL AGENT?**

Allon Canaan[1], Izhak Haviv[2], Myung-Soo Kang[3] and Raymond P. Baumann[1].

[1]Yale University School of Medicine, New Haven, CT 06520. [2]Peter MacCallum Cancer Institute, East Melbourne, Vic 3002 Australia. [3]Harvard University School of Medicine, Boston, MA 02155.

Normal immunocompetent patients rarely develop EBV associated lymphomas. However, in the settings of congenital or acquire immunodeficiency syndrome EBV immortalized B-cells may give rise to lymphoproliferative disorders or lymphomas. The majority of lymphomas occurring in HIV infected patients are EBV positive. It is likely that the continued proliferation of EBV-associated lymphomas is dependent on expression of EBV genes. EBV nuclear antigen 1 (EBNA1) initiates the latent viral replication in dividing lymphoma cells, maintains the levels of viral genome copy number, and regulates transcription of other EBV oncogenes. EBNA1 is expressed in all EBV associated tumors and is required for latency and transformation. To further elucidate the role of EBNA1 in transformation, we have examined the effect of EBNA1 on cellular gene expression by cDNA microarray analysis using the 293 and the BJAB cell lines transfected with EBNA1. Analysis of our data revealed that EBNA1 affects on cellular gene expression is both tissue specific and EBNA1 specific. Among the later, was the Myc oncogene which was found to be down-regulated by EBNA1, as was confirmed by QR-PCR and northern blot analysis. Since Myc is involved in both apoptosis and neoplastic growth, we examined cellular death and proliferation of the EBNA1/BJAB cell line in comparison to the BJAB cell line, under various serum levels. We have found that for serum levels ranging from 2.5-0.3%, EBNA1/BJAB demonstrated better growth and reduced level of apoptosis. These data suggest that under limiting growth conditions, EBNA1 may function as a survival/rescue agent for the host cell. In addition, latency type I might be kept by EBNA1 down-regulation of Myc.

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## CIRCULATING EPSTEIN-BARR VIRUS (EBV) DNA AND RNA IN PATIENTS WITH ASYMPTOMATIC HIV-INFECTION OR AIDS-RELATED NON-HODGKIN LYMPHOMA (ARNHL)

Servi JC Stevens[1], Sandra AWM Verkuijlen[1], Paul HM Smits[2], Jaap M Middeldorp[1]. [1]Vrije Universiteit Medical Center and [2]Slotervaart Hospital, Amsterdam, The Netherlands.

Quantitative monitoring of circulating EBV-DNA has little diagnostic value for predicting EBV-driven ARNHL, as approx. 25% of asymptomatic HIV-infected persons (carriers) show elevated EBV-DNA loads in whole blood (WB), even on HAART. Therefore, this study aims 1) to determine the nature of elevated EBV loads in HIV-carriers and 2) to investigate differences in EBV mRNA transcription in WB of ARNHL versus HIV-carriers as putative diagnostic marker. The nature of EBV-DNA was investigated by quantifying EBV loads in simultaneously obtained WB and plasma samples from 14 HIV-carriers using a 213bp and 99bp EBNA1 amplicon in Light Cycler PCR. EBV DNA loads ranged from 2,800-89,400 copies/ml WB. Only the HIV carrier with highest EBV load in WB had detectable EBV-DNA in plasma (4,200 copies/ml). All other plasma samples were below cut-off. This indicates that circulating EBV-DNA is not derived from virions or damaged cells. Using RT-PCR and NASBA, expression of (non-coding) BamH1-A rightward transcripts (BARTs) was found in 11/14 WB samples from HIV-carriers. One also had mRNA encoding EBNA1 and LMP2, but mRNA for the EBV oncogene LMP1 was never found. In 16 follow-up WB samples from 3 ARNHL patients (EBV-DNA loads ranging from 2,000-120,000 copies/ml), BARTs were detected in 6/16 samples, 2/16 had EBNA1 expression and 2/16 showed LMP2 expression. Again, LMP1 mRNA was not present. We conclude that 1) circulating EBV-DNA in HIV-carriers is cell-associated, 2) (qualitative) EBV mRNA monitoring in WB has no diagnostic value for ARNHL prediction and 3) despite elevated EBV-DNA loads, EBV latent mRNA expression in blood of HIV-carriers and ARNHL-patients is restricted, with only frequent expression of BARTs. This resembles EBV mRNA expression in healthy EBV-carriers, where circulating cells are transcriptionally quiescent but abundantly express non-coding BARTs.

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## IN VIVO IMAGING OF HERPESVIRUS LYTIC GENE ACTIVATION

Yvette C. Tanhehco[1], Catherine A. Foss[2], Yuchuan Wang[2], James Fox[2], Martin G. Pomper[2], Richard F. Ambinder[1]. [1]Viral Oncology Program of the Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, [2]Department of Radiology, Johns Hopkins Hospital.

Expression of Epstein-Barr virus (EBV) lytic genes can be monitored *in vivo* by planar gamma imaging. EBV expresses viral kinases during lytic infection that can serve as reporter genes for the [$^{125}$I] 1-(2-deoxy-2-fluoro-beta-D-arabinofuranosyl)-5-iodouracil (FIAU) reporter probe. *In vitro* cell uptake studies were performed with a stable cell line (TK143b) that constitutively expresses the EBV thymidine kinase (TK) gene and with Rael cells (Burkitt's lymphoma cell line) induced into the lytic cycle with 5-aza-2'-deoxycytidine (azadC) in order to show that the EBV kinases can phosphorylate FIAU. Tumors were generated in SCID mice (6-7 weeks old) by injecting 5 x $10^6$ cells subcutaneously anterior to the right flank and allowed to grow until the tumors reached 1cm in diameter. Lytic infection in Rael tumors was induced with azadC (5mg/kg) administered intraperitoneally at 3-hour intervals (total dose, 15mg/kg/mouse). EBV lytic gene expression in the tumors was confirmed by immunoblotting. Planar gamma imaging was then performed with an X-SPECT camera by injecting mice with 4.44MBq (120μCi) of $^{125}$I-FIAU intravenously. *In vitro* cell uptake studies show an increase in the phosphorylation and retention of FIAU in EBV kinase expressing cells over time. Planar gamma imaging of TK143b and V143b (negative control cell line that does not express the EBV TK) xenograft-bearing mice showed uptake in TK143b tumors as early as 6 hours post injection (p.i.) but not in V143b tumors. Rael xenograft-bearing mice treated with azadC showed tumor uptake at 72 hours p.i. These results show that EBV lytic gene expression *in vivo* can be monitored repeatedly and noninvasively by planar gamma imaging with [$^{125}$I]FIAU.

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## SYNTHESIS OF CEPHALOSTATIN ANALOGS WITH ANTI-LYMPHOMA'S ACTIVITY

M. Nawasreh [1] and E. Winterfeldt [2]
[1]Basic Sciences Department, Faculty of Engineering Technology, Al-Balqa Applied University, Amman-Jordan. [2]Institut fur Organische Chemie, Uni-Hannover, Schneiderberg 1b, D-30167, Hannover, Germany.

Cephalostatin 1 is one of the most potent anti-cancer agents ever been tested in the National Cancer Institute, belongs to the cephalostatins family. This family consists from 19 compounds, which have been isolated from *Cephalodiscus gilchristi*, a microorganism living in the Indian Ocean, by Pettit's group at the Arizona State University.

As part of our work, we are concerned in the synthesis of analogs to cephalostatins, hoping to prepare potent anti-cancer pro-drugs. One of the routes, which have been selected, was the hydroboration of the exo-cyclic double bond at carbon-12 of the steroid moiety. This resulted in a mixture of diastereomers of hydroxymethylene derivative with a large enhancement of the biological activity. The efforts were concentrated on diastereoselective hydroboration using different borane complexes. This process gave, in the cases of catechol-borane complex and D-(N-tosyl) valine-borane complex, F-ring opened products instead of hydroborated products.

In this study we have developed a method with chemo-, regio- and stereoselctive F-Ring opening. Several bis-steroids have been synthesized and the biological activity for some compounds was evaluated against 60-cell lines (NCI-Testing). Others were tested against three-selected cancer cell lines (stomach adenocarcinoma, hepatocellular carcinoma and breast adenocarcinoma), tested in the Medizinische Hochschule Hannover (MHH)/ Germany. Some of these compounds have shown a remarkable activity against different cell lines and others will be tested later.

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## MULTICENTER AIDS COHORT STUDY (MACS) "LYMPHOMA RISK SCORE": DEVELOPMENT AND PLANS FOR VALIDATION

Elizabeth Crabb Breen[1], W. John Boscardin[2], Roger Detels[2], and Otoniel Martínez-Maza[1]. [1]David Geffen School of Medicine, and [2]School of Public Health, University of California, Los Angeles.

Adults with HIV/AIDS have a greater than 70-fold increase in risk of developing non-Hodgkin's B cell lymphoma (AIDS-lymphoma) compared to the U.S. population overall. Archival serum samples from the Multicenter AIDS Cohort Study (MACS), which has followed homosexual men at six-month intervals since the early 1980s, have provided us with an unique opportunity to evaluate multiple markers of B cell activation *prior to* the clinical recognition of AIDS-lymphoma (soluble CD27 [sCD27], sCD23, interleukin 6 [IL6], IL10, sCD44, sCD30, IgA, IgG, IgM, and IgE,), to determine if there are relevant immunological changes preceding the development of B cell lymphoma. Some of the markers are at least moderately correlated with each other (sCD27 with sCD30 and sCD44, sCD30 and sCD44, sCD30 and sCD23, and IgG with sCD30 and sCD27). Many of these also appear to contribute independent information about the likelihood of lymphoma onset, with significant increases or decreases in men who go on to develop lymphoma (n=50), compared to AIDS, HIV+, or HIV- controls. Overall, approximately 20% of subjects are missing one or two marker measurements. A combination of multiple imputation and logistic regression was used to develop a risk stratification scoring algorithm for predicting lymphoma. Applying this algorithm to the set of lymphoma cases and AIDS controls used to develop it, we achieve cross-validated sensitivity and specificity of 80% for discriminating between lymphoma and AIDS (n=44). The Lymphoma Risk Score will be tested when immunoassays are completed on a validation set consisting of pre-lymphoma serum samples from all HIV-infected MACS participants reported with AIDS-lymphoma through December 2002 (n=181), and HIV+ (n=181) and AIDS (n=100) controls.

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## ASSOCIATION OF CYTOKINE POLYMORPHISMS WITH PTLD IN PEDIATRIC LIVER TRANSPLANT RECIPIENTS

Timothy C. Lee[1], Barbara Savoldo[2], Neal R. Barshes[1], Cliona M. Rooney[2], Helen E. Heslop[2], Lisa J.Bristow[1], Jayme D. Scott[1], and John A. Goss[1]

[1]Department of Surgery, Baylor College of Medicine, Houston, TX, [2]Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX

**Background:** Post-transplant lymphoproliferative disorder (PTLD) is a leading cause of morbidity and mortality in pediatric orthotopic liver transplant (OLT) recipients. We hypothesized that a genotypic predisposition for a low cellular immune response would be associated with an increased incidence of PTLD in pediatric OLT recipients. **Methods:** From 5/1992 to 1/2004, 77 patients underwent OLT and were followed for EBV infection by clinical exam and monthly serum EBV titers. Patients were divided into: low EBV titers (<4000 copies/ug DNA), high EBV titers with no PTLD (>4000 copies/ug DNA over two months), and biopsy proven PTLD. Cytokine polymorphism genotyping was performed for IL-2, IL-6, IL-10, TNF-α, TGF-β, and IFN-γ. Data was calculated as a mean with a range. Categorical variables were compared using a chi-square test. A p-value $< 0.05$ was considered significant. **Results:** DNA samples and consents were obtained from 49 patients. There were 5 patients with PTLD, 9 patients with high EBV titers but no PTLD, and 35 patients with low EBV titers. The low IFN-γ secreting polymorphism was more prevalent in the PTLD group (4/5) then in patients with no PTLD (15/44) (p-value$< 0.05$). When comparing the high EBV group with the PTLD group, there was a trend towards increased frequency for the higher IFN-γ secreting polymorphism (6/9, p-value <0.10). **Conclusion:** This study demonstrates an association between the low IFN-γ secreting polymorphism and PTLD development. These polymorphisms can be used as a clinical tool in helping to predict PTLD development in pediatric OLT recipients and allow for pre-emptive treatment in preventing PTLD.

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## EFFECTS OF ORAL CHEMOTHERAPY ON CD4 LYMPHOCYTE COUNT AND HIV-1 PLASMA RNA (VL) IN PATIENTS WITH AIDS-RELATED NON-HODGKIN'S LYMPHOMA (AR-NHL) IN EAST AFRICA AS PART OF A CLINICAL TRIAL

J Orem[1], MW Otieno[2], P Fu[3,4], C Banura[1], E Katongole-Mbidde[1], J Johnson[4], A Ness[3], S Reynolds[3], J Black[5], E Feigal[5], C Whalen[4] and SC Remick[3,4]. [1]Uganda Cancer Institute, Kampala, Uganda; [2]Kenyatta National Hospital, Nairobi, Kenya; [3]CASE Comprehensive Cancer Center and [4]Center for AIDS Research at Case Western Reserve Univ., Cleveland, OH; and the [5]NCI, Bethesda, MD.

There are no data on the effect of cytotoxic chemotherapy (CT) on CD4 lymphocyte and HIV-1 plasma RNA levels (VL) in pts with AR-NHL in East Africa. It is critical to have an understanding of the immunologic effects of anticancer therapy on underlying HIV infection in this setting, since the majority of these pts do not have access to antiretroviral therapy (ARV). The first 27 pts (10 Uganda & 17 Kenya; 13M/14F) registered to an oral CT study are reported. At entry, median CD4 count was 212/μL (range 14 to 384) and median VL was 99,741 copies/mL (range 646 to 2.25 x $10^6$). A total of 22 pts (5 pts excluded: 3 registered not treated; 2 received ARV at start of CT) are eligible for full analysis of changes of CD4 count and VL over time during CT. Five additional pts received ARV upon completion of CT (26% had access to ARV). Taken together, these preliminary observations suggest that there are minimal adverse effects on CD4 count (trend down) and VL (trend up) with dose-modified oral chemotherapy. [Supported in part by NIH grants nos.: CA83528, TW0001 and AI36219.]

| Time Period | No. Pts. | Mean CD4 (cells/μL) | Median (range) | SEM | P-value |
|---|---|---|---|---|---|
| Baseline | 22 | 198 | 213 (14, 384) | 24.7 | — |
| 1 to 70 days | 14 | 140 | 123 (4, 310) | 24.4 | 0.181* |
| 70 to 90 days | 4 | 118 | 111 (25, 222) | 49.8 | 0.177* |
| | | | | | |
| **Time Period** | **No. Pts.** | **VL Mean (copies/mL)** | **Median (range)** | **SEM** | **P-value** |
| Baseline | 20 | 349,558 | 101,910 (646, 2250000) | 121,311 | — |
| 1 to 70 days | 13 | 442,939 | 308,800 (527, 1813400) | 148,668 | 0.384* |
| 70 to 90 days | 3 | 3,003,494 | 750,000 (78683, 8181800) | 2,596,395 | 0.171* |

[* P-value reported was from the comparison between baseline and different time period.]

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## RITUXIMAB AND INFUSIONAL CYCLOPHOSPHAMIDE, DOXORUBICIN AND ETOPOSIDE (CDE) IN COMBINATION WITH HAART: A SAFE AND HIGHLY ACTIVE REGIMEN IN HIV-RELATED NON-HODGKIN'S LYMPHOMAS (NHL)

Michele Spina[1], Cecilia Simonelli[1], Emanuela Vaccher[1], Giuseppe Rossi[2], Ulrich Jaeger[3], Joseph Sparano[4], Umberto Tirelli[1]. [1]National Cancer Institute, Aviano, Italy, for the GICAT; [2]Spedali Civili, Brescia, Italy; [3]University of Vienna, Austria; [4]Montefiore Medical Center, NY, USA.

The combination of Rituximab plus chemotherapy (CT) is more effective than CT alone in the treatment of high grade NHL. With the aim to evaluate the efficacy and activity of combining infusional CDE plus Rituximab in HIV-NHL, we started a phase II study using infusional CDE (Cyclophosphamide 187.5 mg/m$^2$/day, Doxorubicin 12.5 mg/m$^2$/day and Etoposide 60 mg/m$^2$/day) administered by continuous intravenous infusion for 4 days every 4 weeks and Rituximab 375 mg/m$^2$ i.v. on day 1. HAART was given concomitantly with CT. From June 1998 to December 2002, 74 patients (pts) have been enrolled. The median CD4+ cell count was 161 (range 3-691) and the median performance status was 1 (range 0-3). Seventy per cent of pts had advanced stage (III-IV) disease and 49% had B symptoms. Fifty-two out of 74 pts (70%) achieved a complete remission (CR), 4/74 (5%) had a partial remission and 18 (25%) progressed. Only 7/52 pts (13%) in CR relapsed and 48/74 (65%) are alive. Grade 3-4 neutropenia, anemia and thrombocytopenia were observed in 78%, 32% and 24% of pts respectively. Twenty-six per cent of pts developed bacterial infections during neutropenia. The actuarial overall survival and time to treatment failure (TTF) at 2 years were 62% and 64%, respectively. In a Cox model, Burkitt subtype was significantly associated to a shorter survival in comparison with diffuse large B-cell NHL. Our data show that the combination of Rituximab and CDE in HIV-NHL treated concomitantly with HAART is safe, feasible and active. CR rate (70%) and TTF at 2 years (64%) are comparable to those observed in high grade NHL of the general population even if a more aggressive treatment should be evaluated for Burkitt subtype. Supported by ISS and AIRC grants.

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## KSHV-ENCODED VIRF-3/LANA2 BINDS TO C-MYC MODULATOR 1 (MM-1), AND INCREASES C-MYC TRANSCRIPTIONAL ACTIVITY

Barbora Lubyova, Rana Dutta, Augusto J. Frisancho, and Paula M. Pitha. Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins School of Medicine, Baltimore, MD

KSHV infection is linked to development of Kaposi's sarcoma, Castleman's disease and primary effusion lymphoma (PEL). Cell lines established from AIDS-PEL constitutively express only five viral genes vFLIP, vCYC, LANA1, vIRF-2 and vIRF-3/LANA2. KSHV-encoded vIRF-3 is a 566 amino acids nuclear protein expressed during the latent stage of KSHV infection. In this study we show that vIRF-3 can associate with a novel tumor suppressor, c-Myc modulator 1 (MM-1). MM-1 binds to c-Myc and represses its E-box-dependent transcriptional activity via recruitment of the HDAC complex. This causes the active form of c-Myc to change to an inactive form by translocation into an inactive structure within chromatin. Employing a transient transfection assay with cdk4 and 4xE-box reporter constructs we show that vIRF-3 can significantly increase the c-Myc-mediated transcription even in the presence of MM-1. Co-immunoprecipitation experiments indicated that vIRF-3 competes with MM-1 for binding to c-Myc, thus releasing the c-Myc from MM-1-mediated inhibition. We have also used RNA interference to directly reduce expression of vIRF-3 in KSHV-infected BCBL-1 cells. Interestingly, BCBL-1 cells, which had significantly reduced levels of vIRF-3 expression, had fewer cells entering the S phase than control BCBL-1 cells. The composition of the enhanceosome which is established on the cdk4 promoter is currently being examined. The results of these studies will be presented. Our data indicate that vIRF-3 has an oncogenic potential and therefore may contribute to KSHV-induced tumorigenesis.

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## LYTIC VIRAL INFECTION OF HERPESVIRUS 8 (HHV8) BUT LOW EXPRESSION OF CD20, IN PATIENTS WITH MULTICENTRIC CASTLEMAN'S DISEASE (MCD)

I. Oehri, M. Baumann, G. Cathomas
Laboratory for Pathology of Infectious Diseases, Cantonal Institute of Pathology, Liestal, Switzerland

**Background.** In AIDS patients, multicentric Castleman's disease (MCD) is associated with human herpesvirus 8 (HHV8), Similar to other immunosuppression-associated lymphoproliferative diseases it has been suggested that anti-CD20 antibodies may be useful in the therapy of MCD. Aim of the study was the analysis of CD 20 expression in MCD and the association with lytic viral infection.

**Material and methods.** Formalin fixed, paraffin embeded lymph nodes were analyzed by a double immunofluorescence assay using antibodies against CD20 and three HHV8 proteins: The latent viral protein LANA (ORF 73), the viral interleukin 6 (vIL-6) and the ORF74, both associated with lytic HHV8 infection.

**Results**. 11 lymph nodes of 7 HIV patients with MCD were analyzed. In the mantle zone of the lymphoid follicles, 8,4%, 2,8% and 1% of cells show expression of LANA, vIL-6 and ORF74, respectively ($p < 0,02$). 91% of HHV8 infected cells showed co-expression of LANA/vIL-6 and LANA/vORF74 and 96% showed co-expression of LANA/vlL-6, respectively. There was a statistical significant correlation between vIL-6 and LANA as well as vIL-6 and vORF74 ($p < 0,01$). Of the HHV8 infected cells, 6,5% showed CD20 expression and no difference between the three viral proteins analyzed were observed (5,5%, 7,4% and 6,6% of LANA, vIL-6 and vORF 74, respectively).

**Conclusion**. The present data support the concept that HHV8 runs in MCD, in contrast to Kaposi's sarcoma, a lytic viral infection. Furthermore, only a small subset of infected cells show co-expression of CD20, independent of the state of viral replication. This small number of infected cells expressing CD20 argues against a major therapeutic effect of anti-CD20 therapy in MCD. Based on the evidence of lytic HHV8 infection, however, anti-viral drugs inhibiting HHV8 replication may be more efficient.

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## KSHV (HHV8) DNA IN PERIPHERAL BLOOD OF PATIENTS WITH AIDS-NHL AND THE IMPACT OF CHEMOTHERAPY

Lan Lin[1] and the AIDS Malignancy Consortium
Sidney Kimmel Cancer Center, Johns Hopkins Medical Institute

We investigated KSHV loads in PBMC and plasma of patients with AIDS-NHL and AIDS-KS. KSHV load was measured with real-time PCR. AIDS-NHL patients were randomized 2:1 to receive CHOP with rituximab (CHOP-R) or CHOP alone. Baseline KSHV DNA in PBMC was detected in 18% of 45 AIDS-NHL patients and 60% of 45 AIDS-KS patients. Baseline KSHV in plasma was detected in 18% of 51 AIDS-NHL patients and 65% of 71 AIDS-KS patients. Patients with detectable KSHV DNA in PBMC were 4 folds more likely to have KSHV DNA detectable in plasma. No correlation between KSHV loads and EBV loads. No correlation between KSHV loads and HIV loads or CD4 counts. With either CHOP or CHOP-R therapy, KSHV loads fell dramatically in both PBMC and plasma. Among 6 AIDS-NHL patients with KSHV detectable at baseline in PBMC (2 on CHOP-R, 4 on CHOP), KSHV DNA dropped to undetectable the first post-treatment measurement (before cycle 4) in 5 patients and from baseline 60625 to 22 copies/million PBMC in the other one (on CHOP). Among 7 AIDS-NHL patients with KSHV detectable at baseline in plasma (4 on CHOP-R, 3 on CHOP), KSHV DNA dropped to undetectable levels in plasma before cycle 4 in six patients and KSHV load dropped from 730625 to 255 copies/100 ul plasma in the other one (on CHOP).The percentage of B cells in peripheral blood also fell dramatically to almost undetectable level in both patients on CHOP-R and CHOP, with the drop more rapidly in patients on CHOP-R. Analysis of protection from DNase digestion and DNA fragment size distribution are consistent with the presence of KSHV in virion particles in plasma. CHOP-R and CHOP lead to decreased B cell number and decreased KSHV load in PBMC and plasma. The possible significance of these changes for therapy of KSHV-associated diseases is unknown.

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## HHV-8 PLASMA VIRAL LOAD (VL) IN LYMPHOPROLIFE-RATIVE DISEASES ASSOCIATED WITH HIV INFECTION

Cecilia Simonelli, Rosamaria Tedeschi, Renato Talamini, Maria Teresa Bortolin, Michele Spina, Emanuela Vaccher, Aldo Morra, Paolo De Paoli, Antonino Carbone, Umberto Tirelli. National Cancer Institute, Aviano Italy

We evaluated the HHV-8 VL and HIV infection in: 8 patients (pts) affected by Multicenter Castleman Disease (MCD), 4 with primary effusion lymphoma (PEL) and 3 with HHV-8 positive solid lymphomas (SL) diagnosed and treated in our Institute. At the onset HHV-8 VL was measurable in all 3 groups and the median value was 4400 (range $6x10^2$-$1.6x10^6$), 12700 (range $2.9x10^2$-$8.0x10^4$) and 555000 cp/ml (range $5.1\ x10^5$-$5.4x10^6$) in MCD, PEL and SL, respectively. MCD pts had a median value of CD4 count of 327/$mm^3$ (range 55-424), PEL of 43/$mm^3$ (range 25-839) and HHV-8 of 104/$mm^3$ (range 20-282). HIV-viremia was undetectable in 3/7 pts in MCD group, in 3/4 PEL pts and only in 1/3 HHV-8 SL. All pts had positive serology for EBV. EBV-plasma DNA was measurable in all pts with PEL and SL, and only in 3/8 MCD pts. In PEL pts, HHV-8 had a significantly negative correlation with CD4 count (r=0.80). In MCD EBV plasma DNA was significantly lower level than in PEL and SL (p=0008, p=0.01). The HHV-8 VL was lower in MCD pts than in PEL pts (p= 0.07) and significantly lower than SL pts (p=0.02). Four out of 8 MCD pts were treated only with HAART and 5 with oral VP16 and HAART, the median overall survival (OS) was 44 mos, 3 PEL pts were treated with CHOP-like regimen and HAART and 1 with HAART alone the median OS was 18 mos. The 3 SL pts were treated with CHOP-like regimen with a poor outcome: OS <1 mo. We found that pts with HHV-8 >40000 cp/ml had a shorter OS when considering all diseases together. The same trend is present in each distinct diseases. MCD has the better prognosis and it is associated with higher CD4 count with a lower HHV-8 VL while SL have the worst outcome and the highest level of HHV-8 VL. HHV-8 VL might be a prognostic marker in this setting.

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## KAPOSI'S SARCOMA IN CHILDREN IN MALAWI.

T Hodgson[1], W Kondowe[2], E Molyneux[2], T Mhango[2], S Packer[1], F Mbopi-Keou[1,3], C-G Teo[3], C Scully[1], S Porter[1].
[1]Oral Medicine, Eastman Dental Institute for Oral Health Care Sciences, University College London, UK. [2]Department of Paediatrics, College of Medicine, University of Blantyre, Malawi. [3]Virus Reference Division, Health Protection Agency, London, UK.

Malawian National Cancer Registry data suggest an increased incidence of paediatric Kaposi's sarcoma (PKS) since the start of the HIV pandemic. However, the presentation of PKS in this geographical region is poorly documented. We report the features of 33 children with histopathologically confirmed PKS (median age 9 years, range 2-14, male:female 2:1) who presented to the Department of Paediatrics, Queen Elizabeth Central Hospital Blantyre, Malawi, over a 14 month period to September 2003. Eighteen of the 33 children had mucocutaneous disease, 12 had cutaneous disease alone and 3 had only lymph node involvement. Twenty three children had > 10 cutaneous lesions. Two children had aggressive mucocutaneous disease with marked oedema despite evidence of HIV-infection. Thirty one of the children proved to be HIV-infected (HIV RNA viral load mean 58300, range 16400-269000 copies/ml; CD4 mean 290, range 40 -700 $x10^3/mm^3$). On presentation the mean Lansky performance status was 67 (range 40-100) and the mean haemoglobin 9.4 g/dl (range 6.2 -13.3). It is concluded that children in Malawi present to health care professionals with severe PKS associated with undiagnosed HIV disease. *This work was supported by Grant DE12176-03 from the National Institute of Health.*

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**PROFILE OF PATIENTS WITH KS IN THE ERA OF HAART**

James Gallafent[1], Susan Buskin[1,2], Peter De Turk[2], David Aboulafia[1,3]. [1]University of Washington, [2]Public Health, Seattle & King County, HIV/AIDS Epidemiology Program, [3]Virginia Mason Clinic.

*Purpose:* Since the advent of HAART, the incidence of KS among AIDS patients has declined both nationwide and in King County, Washington. We sought to compare clinical parameters of patients diagnosed with KS in the pre-HAART (1990-1996) and HAART (1997-2002) eras.

*Methods:* We used patient data abstracted from the Adult/Adolescent Spectrum of HIV-related Diseases (ASD) study of Public Health-Seattle & King County.

*Results:* Patients diagnosed with KS in the HAART era (n = 40) were significantly more likely ($p < .05$) than pre-HAART era KS patients (n = 366) to be diagnosed with alcohol abuse (43% vs. 18%), non-injection drug use (45% vs. 18%), injection drug use (25% vs. 10%), psychosis (25% vs. 13%), and hypertension (13% vs. 2%). Although mean CD4+ count (107 vs. 110 cells/μL) and HIV viral load (232,000 vs. 349,000 copies/mL) at the time of KS diagnosis were not significantly different between the 2 groups, significantly fewer ($p < .01$) HAART era KS patients developed opportunistic illnesses (OIs) during their time of follow-up. The risk of dying among patients diagnosed with KS in the HAART era is significantly ($p < .01$) lower than if diagnosed with KS pre-HAART (Hazard Ratio 0.24).

*Conclusion:* Patients diagnosed with KS in the HAART era in King County survived longer and developed fewer OIs despite maintaining diminished CD4+ counts and elevated HIV viral loads at the time of diagnosis. They also had an increased prevalence of substance abuse and mental illness, contributing to a dynamic and changing KS clinical profile.

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## ADDITIONAL NOVEL GENOTYPES OF KSHV FROM CENTRAL AND SOUTHERN AFRICA AND FROM THE PACIFIC RIM

Jianchao Zong, Henry Kajumbula, Lynn Morris, William Boto, Edward Katangole-Mbidde, Ih-Jen Su and Gary S. Hayward
Viral Oncology Program, The Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins University School of Medicine

KSHV genotypes in KS, PEL and PBMC samples analyzed from different ethnic groups display significant differences and clustering patterns that largely reflect founder effects associated with the known global migration patterns of modern humans over the past 100,000 years. However, the subtype and divergence patterns at the LHS of the genome (VIP or K1), in the central constant region, and at the RHS (TMP or K15) of extant modern KSHV genomes have had different evolutionary histories displaying evidence for both ancient and recent recombination events and chimerism. We interpret that, although hypervariable (with up to 30% amino acid variation) all modern VIP or K1 genes, including newly recognized F and G subtypes from Central Africa diverged no more than 100,000 years ago. In contrast, the TMP genes exist as three alleles (P, N or M) that differ by 28% and 70% at the protein level and originally diverged as long ago as 2 million and 10 million years ago. Furthermore, both the P and M alleles of TMP have split into distinctive Sub-Saharan Africa, Pacific Rim and Eurasian branches. Finally, in the central constant regions, where specific selected loci display between 3 and 8% overall nucleotide variation, we now recognize up to ten distinct subtypes: three in Eurasia, one in the Pacific Rim, and six in Sub-Saharan Africa (with a very different distribution in Central versus Southern Africa). Evidently only very modern versions of the VIP gene survive in Africa, whereas the constant region more accurately reflects all older versions of modern humans, but several RHS segments containing very ancient or exotic TMP alleles still exist as chimeras with modern forms of the KSHV genome.

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## ACTIVATION OF PAK1-NFκB SIGNALING AXIS BY KAPOSI'S SARCOMA-ASSOCIATED HERPES VIRUS G-PROTEIN COUPLED RECEPTOR DURING CELLULAR TRANSFORMATION

Disha Dadke*, Benjamin H. Fryer, Erica A Golemis* and Jeffrey Field[+]

*Division of Basic Science, Fox Chase Cancer Center, Philadelphia, PA. [+]Department of Pharmacology, University of Pennsylvania, School of Medicine, Philadelphia, PA.

Kaposi's sarcoma-associated herpes virus (KSHV) contributes to the pathogenesis of Kaposi's sarcoma and primary effusion lymphomas. KSHV encodes a G-protein coupled receptor (KSHV-GPCR) that signals constitutively and transforms NIH3T3 cells. In this study, we show that KSHV-GPCR transformation requires activation of the small G protein Rac1 and its effector, the p21-activated kinase 1 (Pak1). Either transient or sustained expression of KSHV-GPCR activated both Rac1 and Pak1. Furthermore, expression of dominant negative mutants of Rac (RacN17) or Pak1 (PakR299, Pak-PID) inhibited KSHV-GPCR induced focus formation and growth in soft agar. We also demonstrate that signaling from Pak1 to nuclear factor-κB (NFκB) is required for cell transformation induced by KSHV-GPCR. KSHV-GPCR induced transcriptional activation by NFκB, which is inhibited by the PAK-PID. Reciprocally, expression of constitutively active Pak1 (PakL107F) activated NFκB comparably to KSHV-GPCR. The Pak-PID and RacN17 inhibited the KSHV-GPCR-induced phosphorylation of inhibitor of IκB kinase β (IKKβ) and inhibitor of κB – α IκBα, indicating that it is Pak1-dependent phosphorylation and subsequent destruction of the IκB proteins that allows NFκB activation. Also, experiments with the KSHV-GPCR inverse agonist IP-10, the $G_{\alpha i}$ inhibitor pertussis toxin (PTX), and an inhibitor of phosphatidylinositol-3-kinase (PI3K), wortmannin, indicate that signaling through the $G_{\alpha i}$ pathway and PI3K contributes to the cell transformation and NFκB activation induced by the KSHV-GPCR.

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## THE EFFECT OF LANA SUPPRESSION ON KSHV LATENCY AND PEL CELL GROWTH

Jianmeng C. Chen, Jae myun Lee, Richard Ambinder. Sidney Kimmel Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD21231

Kaposi's sarcoma-associated herpesvirus (KSHV) latency-associated nuclear antigen (LANA-1) is required for the persistence of extrachromosomal KSHV DNA. We characterized the effects of LANA suppression by siRNA in KSHV(+) cell lines. Using the oligoengine program, two sets of LANA specific siRNA were designed. A retrovirus-based vector (MF100) for delivering anti-LANA siRNA was constructed. MOCK/MF100 and VSV-G were cotransfected into phoenix cells to generate infectious retroviral particles. These were subsequently used to transduct BC2 cells with puromycin selection and a stable BC2 cell line expressing siRNA for LANA was established. Western blot confirmed that LANA expression was reduced to about 10% in BC2-MF100 cells. IFA indicated persistent low level LANA protein in each cell. KSHV and Epstein-Barr Virus (EBV) viral copy number were assessed by real-time PCR. Neither the absolute KSHV viral load nor the KSHV/EBV ratio was affected by LANA suppression. Fluorescence in situ hybridization (FISH) show that KSHV episomes were tethered to chromosomes in the LANA reduced cell line as in BC2 cells. With 10% LANA left, cell cycle and replication were not affected in BC2 cells.

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## HYPOXIC ACTIVATION OF KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS (KSHV) ORF 36, A PHOSPHOTRANSFERASE

Muzammel Haque and Robert Yarchoan.
HIV and AIDS Malignancy Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892-1868

KSHV is an etiologic agent for all forms of Kaposi's sarcoma (KS), for primary effusion lymphoma (PEL), and for multicentric Castleman's disease (MCD). We previously reported that KSHV is activated to lytic replication by hypoxia and that the promoter for ORF 34 (a late gene) contains several hypoxia response element (HRE) consensus sequences, at least one of which is functional for it (Haque et al., *J. Virol.*, 77, 6761, 2003). Downstream of ORF 34, KSHV contains a cluster of overlapping genes (ORF 35 to ORF 37) oriented in the same direction. Previous studies (Cannon et al., *J. Virol.*, 73, 4786, 1999) provided evidence that the RNA for two or more of these genes can be transcribed on a single transcript. We performed rapid amplification of cDNA ends (RACE) analysis for each gene in this cluster. This revealed that the predominant transcription start site of ORF 36 induced by tetradecanoylphorbol acetate (TPA) is upstream of ORF 35. ORF 36, an early gene, is a phosphotransferase that can phosphorylate ganciclovir to a toxic moiety. These findings suggested that ORF 36 might be activated by hypoxia. We found that exposure of PEL cell lines to hypoxia or to hypoxia-mimicking chemicals strongly induced ORF 36 mRNA as revealed by Northern blot analysis. A 2300-bp 5'-flanking region of ORF 36 was cloned to luciferase reporter vector. This region has four consensus HREs, three of them in the previously described ORF 34 promoter and one in the coding region of ORF 34. A reporter assay in Hep3B cells indicated that this promoter is activated by hypoxia. Sequential deletion analysis to find the active HRE element(s) in this region is ongoing. It is possible that activation of ORF 36 by hypoxia could be used to therapeutic advantage, especially with regard to PEL, which arises in a hypoxic environment (pleural effusions).

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**KSHV LYTIC DNA REPLICATION**

Yan Wang, Hong Li and Yan Yuan.
Department of Microbiology, University of Pennsylvania School of Dental Medicine, Philadelphia, PA

Herpersvirus lytic DNA replication requires both *cis*-acting element, a DNA replication origin, and *trans*-acting factors such as virally encoded origin binding protein and DNA replication enzymes. The KSHV genome contains two lytic DNA replication origins (*ori-Lyts*) located in the genome between K4.2 and K5 and between K12 and ORF71, respectively. The two *ori-Lyt*s share an almost identical 1.1-kb core sequence and 600 bp GC-rich repeats that are represented as 20-bp and 30-bp tandem arrays. The whole 1.7-kb DNA sequences are necessary and sufficient as a *cis*-acting signal for KSHV replication. Many interesting motifs were found in the *ori-Lyt* and some have been experimentally demonstrated to be essential for the *ori-Lyt*-dependent DNA replication. The most critical *cis*-acting elements are as follows. (i) An 18-bp AT-palindromic sequence; (ii) eight C/EBP binding motifs, arranged as four spaced palindromes; (iii) an ORF50/Rta responsive element and a TATA box. In addition, the 600-bp GC-rich tandem repeats were found to be required for efficient DNA replication. Two viral proteins, namely K8 bZip and ORF50/Rta, were found to bind to the *ori-Lyt* and shown to serve as origin binding proteins. Binding of K8 and Rta to an *ori-Lyt* and interaction between these two proteins lead to looping of the *ori-Lyt*, which appears to be a prerequisite for *ori-Lyt*-dependent DNA replication. In addition, Rta is also responsible for an *ori-Lyt*-associated transcription. The Rta responsive element and the downstream TATA box constitute an Rta-dependent promoter which directs a transcription of a 1.4 kb polyadenylated RNA. Our data showed that a transcription event across the 600-bp GC-rich tandem repeat sequence is critical for initiation of DNA replication.

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## RAPID SCREENING OF CHEMICAL INHIBITORS THAT BLOCK PROCESSIVE DNA SYNTHESIS OF HERPESVIRUSES

M Ciustea[1], X Chen[1], S Sei[3], R Shoemaker[4], R Ricciardi[1,2]Departments of [1]Microbiology School of Dental Medicine and [2]Biochemistry and Biophysics, School of Medicine University of Pennsylvania, and [3]Laboratory of Antiviral Drug Mechanisms, SAIC-Frederick, [4]Screening Technology Branch, Developmental Therapeutics Program, National Cancer Institute, Frederick

In order to successfully copy their genomes, DNA polymerases need to be processive, i.e., they need to be able to incorporate thousands of nucleotides without dissociating from the template. To accomplish this task, most DNA polymerases are dependent upon an accessory protein, often referred to as a processivity factor. The processivity factors encoded by herpesviruses are ideal targets for blocking viral replication, particularly because of their apparent specificity for their cognate viral DNA polymerases. We have developed a rapid mechanistic plate assay that has potential application to high-throughput screening of chemical libraries of tens of thousands of chemical compounds to identify inhibitors of processive DNA synthesis. We use the DNA polymerase (Pol-8) and processivity factor (PF-8) of KSHV (Kaposi's Sarcoma Herpesvirus) as an illustrative example of how this assay can be applied to identifying DNA synthesis inhibitors of any herpesvirus. A 250-base long oligonucleotide, biotinylated at the 5' terminus and primed at its other end, is fixed on the bottom of streptavidin coated microtiter plate and serves as template for DNA synthesis catalyzed by Pol8/PF8. DNA synthesis activity is analyzed by measuring the incorporation of chemically labeled nucleotides, such as digoxigenin labeled dUTP, which can be quantitated by ELISA using anti-digoxigenin-peroxidase conjugate. Following assay validation by testing known DNA polymerase inhibitors, a chemical library containing 2000 compounds was screened and potential 28 inhibitors were identified. These inhibitors were also tested to determine if they inhibited HSV-1 processive DNA synthesis. One of the compounds showed a strong preference for selectively inhibiting HSV-1. This approach provides the potential to discover drugs that will effectively block opportunistic herepesvirus infections that are associated with HIV and AIDS.

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## A NOVEL ENZYME LINKED IMMUNOSORBANT ASSAY FOR THE DETECTION OF ANTI-HERPESVIRUS-8 LYTIC IGG ANTIBODIES

AnnMarie Barrett[1], Shirley O'Keeffe[1], Des O'Leary[1], Gordon Elliott[1], Susan Reid[1]. [1]Biotrin International Ltd, 93, The Rise, Mount Merrion, Co. Dublin Ireland.

**Objective:** Human Herpesvirus-8 (HHV-8) also known as Kaposi's Sarcoma-associated Herpesvirus (KSHV) has been identified in skin lesions of classic, endemic, transplant associated and AIDS assocciated Kaposi sarcoma. The virus is classified as a gamma herpesvirus and resembles EBV in its tropism for B cells and ability to exist in a latent state. The Biotrin HHV-8 IgG EIA was developed as a screening tool for AIDS and organ transplant associated populations. **Method:** The immunoassay format incorporates a synthetic peptide mix coupled to a solid phase. The peptides are derived from ORF glycoportein K8.1 and capsid KS65. A combined African and European panel of 166 sera from AIDS associated Kaposi's sarcoma patients were analysed using the Biotrin HHV-8 IgG EIA to determine the clinical sensitivity of the kit. As confirmed by IFA a panel of HHV-8 negative specimens consisting of 114 normal human sera and 76 potential cross-reactives (including HIV, EBV, CMV and other Herpes sub-groups) were tested to evaluate the specificity of the new EIA. **Results**: Of the 166 AIDS associated KS samples 152 were found to be IgG seropositive for HHV8 lytic antigens, 2 specimens were negative, while 14 specimens were within the equivocal zone ($\geq$0.8 $\leq$1.2 index value); these figures translate to a clinical sensitivity value of 91.2%. Specificity of the assay, including the cross reactive specimens, was measured at 93.6%. Results reflect a clinical assay agreement of 91.9%. **Conclusion**: These results demonstrate high clinical sensitivity and specificity using the selected synthetic peptides derived from lytic HHV8 viral proteins.

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## DEVELOPMENT OF A FLUORESCENCE POLARIZATION-BASED SCREENING ASSAY FOR INHIBITORS OF KSHV PROCESSIVITY FACTOR

Dorjbal Dorjsuren[1], Andrew Stephen[2], Angela Brennan[1], Robert Ricciardi[3], Robert Shoemaker[4], Shizuko Sei[1]. [1]Laboratory of Antiviral Drug Mechanisms, [2]Protein Chemistry Laboratory, SAIC-Frederick, [3]Departments of Microbiology, and Biochemistry & Biophysics, University of Pennsylvania, [4]Screening Technologies Branch, Developmental Therapeutics Program, NCI-Frederick.

Kaposi's sarcoma-associated herpesvirus (KSHV) polymerase processivity factor (PPF) is indispensable for processive DNA synthesis mediated by its cognate DNA polymerase (KSHV POL). KSHV PPF not only interacts with KSHV POL as a specific cofactor, but also preferentially binds dsDNA over ssDNA without sequence specificity. The processive functionality of PPF is determined by both the POL- and dsDNA-binding activities. In the current study, we developed a simple and rapid fluorescence polarization (FP) assay to screen for inhibitors of PPF-dsDNA binding. Functionally active KSHV PPF was expressed and purified from recombinant baculovirus vector-infected insect cells. Increasing concentrations of PPF, ranging from 3 to 500 nM, were incubated with 10 nM fluorescein-labeled dsDNA (F-dsDNA) in HEPES buffer for 15 min at room temperature. A dose-dependent increase in FP was observed at PPF concentrations up to 50 ~ 100 nM, after which the FP values reached a plateau. In order to evaluate the assay validity and reproducibility, pilot-scale screening of the NCI Training Set, a library of ~ 230 compounds, against pre-assembled protein-DNA complex, comprising 25 nM PPF and 10 nM F-dsDNA, was conducted in 96-well microplates at 10 μM. With a reaction volume of 40 μL, the microplate based PPF-FP assay produced Z′ factors ranging from 0.779 ~ 0.927 (median 0.867), with a high degree of reproducibility ($r^2$ = 0.99). These results support the feasibility of high throughput screening of larger chemical libraries. (Supported in part by NCI Contract No. NO1-CO-12400)

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## THE ABILITY OF HHV8 ANTIBODY ISOTYPE ASSAYS TO IDENTIFY HHV8 INFECTION

Daniel Edelman[1], Brad Goebel[2], Manhattan Charurat[3], Niel T. Constantine[1]. [1]Department of Pathology, Univ. of MD Baltimore (UMB), [2] SAIC-Frederick, and [3]IHV, UMBI.

**Introduction:** Current HHV8 serological tests target IgG antibodies to one of three viral antigens: K8.1, Orf 65, or Orf 73. However, little information is available on the detection and utility of IgA and IgM isotypes as indicators of HHV8 infection. We present data describing the utility of all three isotype-specific serologic assays that incorporate all three antigens. **Methods:** The ORF 65 tests were developed at UMB while the K8.1 and Orf 73 tests were developed at SAIC-Frederick. For the IgA and IgM assays, samples were pretreated using GullSORB™ to remove any interfering IgG antibodies. Each isotype-specific assay used the corresponding anti-human antibody isotype conjugate; the ORF 65 ELISA utilized HRP and the K8.1 and ORF 73 ELISAs used AP. Between 155 - 210 Kaposi's sarcoma (KS) samples and 109 - 241 healthy controls were screened by all nine assays. Statistical analyses using the STATA software package determined ROC curves and p-values. **Results:** ROC analyses of K8.1, Orf 73, and Orf 65 antigens, showed that the area under the curve (AUC) for the IgG tests had the best sensitivity and specificity for differentiating between KS and healthy subjects, exhibiting values of 0.9975, 0.9863, and 0.9952, respectively. For K8.1 and Orf 73, IgA was the next best indicator (0.8808 and 0.8736, respectively) while IgM levels provided little value. Interestingly, Orf 65 IgM reactivities produced a large AUC (0.9414), although this was still statistically of lesser value than the Orf 65 IgG response. All AUC differences between antigens and isotypes were statistically significant ($p \leq 0.0002$). **Conclusions:** These results support previous studies that of the three antigens, K8.1 provides the best sensitivity and specificity. Our data also show that the use of IgM and IgA isotype detection will have minimal diagnostic impact. However with continued test development, Orf 65 IgM reactivities might be helpful in the detection of early HHV8 infection.

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## REAL-TIME QUANTITATIVE PCR ARRAYS FOR AIDS-ASSOCIATED MALIGNANCIES

Dirk P. Dittmer, Denise Whitby#, Wolfgang Vahrson, Michelle R. Staudt, and Rebecca Hines-Boykin

Department of Microbiology and Immunology, The University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, U.S.A., #Viral Epidemiology Section, AIDS Vaccine program, SAIC-Frederick and the National Cancer Institute, Frederick, MD 21702

We initially reported the transcription profile of the entire Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) genome in Kaposi's Sarcoma (KS) biopsies using our novel real-time quantitative PCR (QPCR) array (Cancer Research 63: 2010-2015, 2003). Since then we have developed similar arrays for other herpesviruses, and find differences in the degree of lytic reactivation in primary effusion lymphomas (PEL) (i) in culture, (ii) in mouse models and (iii) in clinical studies. Since real-time QPCR records a truly quantitative transcription profile, this technology will improve statistical analysis in clinical trials for AIDS-associated malignancies and solidify decision-making for lymphoma therapy.

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## NOVEL CELLULAR GENES ESSENTIAL FOR TRANSFORMATION OF ENDOTHELIAL CELLS BY KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS

Camilo Raggo, Rebecca Ruhl, Ashlee Moses, Klaus Früh. Vaccine and Gene Therapy Institute, Oregon Health and Science University, 505 NW 185th Ave, Beaverton, OR, 97006.

Kaposi's sarcoma-associated herpesvirus (KSHV, HHV8) is involved in lymphoproliferative diseases and Kaposi's sarcoma. The oncogenicity of this virus is reflected *in vitro* by its ability to transform B cells or endothelial cells. Infection of dermal microvascular endothelial cells (DMVEC) transforms the cells from a cobblestone-like monolayer to foci-forming spindle cells. This transformation is accompanied by dramatic changes in the cellular transcriptome. Known oncogenes, such as c-Kit, are among the KSHV-induced host-genes. We previously demonstrated that c-Kit is an essential component of the KSHV-mediated transformation of DMVEC. Here, we test the hypothesis that this transformation process can be used to discover novel oncogenes. After inhibiting a group of KSHV-induced cellular transcripts with antisense-oligomers, we observed inhibition of foci-formation and proliferation by antisense molecules to RDC-1 and neuritin. RDC-1 is an orphan G-protein coupled receptor that can also serve as co-receptor for HIV. Neuritin was previously described as a brain-specific growth-promoting protein mediating neurite outgrowth. Thus, KSHV-mediated transformation involves multiple cellular genes, some of which are likely to be novel oncogenes. The ability of RDC-1 and neuritin to transform cells is currently being investigated in NIH3T3 cells and DMVEC.

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## CO-EXPRESSION OF VASCULAR AND LYMPHATIC ENDOTHELIAL MARKERS IN KAPOSI'S SARCOMA

Liron Pantanowitz[1], Geraldine S Pinkus[2], Steven R Tahan[1], Bruce J Dezube[1]. [1]Beth Israel Deaconess Medical center, [2]Brigham & Women's Hospital, Harvard Medical School

**BACKGROUND:** The origin of the spindle cell in Kaposi's sarcoma (KS) is controversial and remains unresolved. It is unclear if they are derived from vascular endothelium, lymphatics and/or dermal dendrocytes. Therefore, we evaluated a series of KS lesions using immunohistochemical markers to determine the origin of the KS spindle cell. **METHODS:** Formalin-fixed tissue from 7 AIDS-related (1 patch, 4 plaque, 1 nodular stage) and 2 non-HIV classic (1 plaque, 1 nodular stage) cutaneous KS lesions were stained using (i) latent nuclear antigen-1 (LNA-1) of HHV8, (ii) dendritic cell markers CD21, CD23, CD35, and factor XIIIa, (iii) mixed dendritic-endothelial marker fascin, (iv) endothelial cell markers CD31, CD34, GLUT-1 and factor VIII-ra, (v) as well as with the monoclonal antibody D2-40 directed against a sialoglycoprotein that reacts with an epitope on lymphatic endothelium. **RESULTS:** All (100%) KS lesional cells stained strongly positive for HHV8, factor VIII, CD31, CD34, fascin and D2-40. Numerous (10-30% cellularity) admixed dendritic cells were immunoreactive for factor XIII-ra (100%), CD21 (89%), CD35 (44%) and CD23 (11%). All cases were negative for GLUT-1. No difference in staining between AIDS-related and classic KS or early and advanced lesions was observed. **CONCLUSIONS:** Our findings suggest that all HHV8-infected spindle-shaped cells in KS lesions exhibit a mixed endothelial cell immunophenotype, and that these lesional cells begin to co-express vascular and lymphatic endothelial markers at an early stage. The immunophenotypic profile also demonstrates that spindle-shaped KS cells are not derived from dendrocytes. Rather, hyperplasia of admixed dendritic cells may represent a reactive phenomenon that is important to an HHV8-induced angioproliferative process.

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## STUDY OF SEX-HORMONE RECEPTOR EXPRESSION IN KAPOSI'S SARCOMA

Liron Pantanowitz[1], Steven R. Tahan[1], Glen Bubley[1], Nalinee J. Upalakalin[1], Erich J. Schwartz[2], Sabine Kohler[2], Marie-France Demierre[3], Timothy P. Cooley[3], Bruce J. Dezube[1]
[1]Beth Israel Deaconess Medical Center, Harvard Medical School; [2]Stanford University Medical Center, Stanford School of Medicine; and [3]Boston Medical Center, Boston University School of Medicine.

**Background:** The incidence of Kaposi's sarcoma (KS) is higher among males than females, in both AIDS-related and non-HIV-related (e.g. classic) forms of KS. Early papers proposed that the marked predilection for male patients suggests a possible role of sex hormones in the pathogenesis of KS. However, to date there have been no systematic studies to evaluate the expression of sex hormone receptors in KS. The aim of this study was to determine the expression of androgen (AR), estrogen (ER) and progesterone (PR) receptors in different KS lesions. **Methods:** Archival cases (N=23) of formalin-fixed, paraffin-embedded lesions, including 18 (78%) AIDS-related and 5 (22%) classic KS specimens, were studied. Various cutaneous samples (11 from extremities; 4 trunk/face; 2 site not specified) at different histological stages (4 patch, 7 plaque, 8 nodular) and biopsies of visceral KS (3 nodal; 1 spleen) were included. Tissue sections (5μm thick) from all cases were stained using immunohistochemistry with antibodies to AR, ER and PR. For all stains appropriate positive and negative controls were evaluated. **Results:** Patients were of median age 42 years (range 32-92 years), including 16 (70%) males (median age 41 years) and 7 (30%) females (median age 73 years; 4 of whom had classic KS). AR and PR immunostaining was absent in all cases. ER staining was absent in all but one case, in which immunoreactivity was found to be present in scattered lesional KS cells (45-year-old HIV+ man with CD4+ T-lymphocyte count >800 cells/$mm^3$, undetectable HIV viral load on HAART and alpha-interferon therapy). **Conclusions:** The lack of sex hormone receptors in KS lesions argues against a direct effect of sex hormones on the pathogenesis of this angioproliferative tumor. KS in males may be related to the indirect effects of sex hormones (not mediated by hormone-specific receptors) or to (unknown) factors unrelated to these hormones.

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## LOCALIZATION OF HUMAN HERPESVIRUS-8 (HHV8) IN OROPHARYNGEAL TISSUES

Liron Pantanowitz[1], Steven R Tahan[1], Geraldine S Pinkus[2], Bruce J Dezube[1]. [1]Beth Israel Deaconess Medical center, [2]Brigham & Women's Hospital, Harvard Medical School

**Introduction:** Previous studies examining the shedding of HHV8 in saliva analyzed only oropharyngeal secretions and/or swabs. However, it is unclear if HHV8 in saliva is present within mucosal epithelium, salivary gland and/or inflammatory cells. Therefore, we performed immunohistochemical studies to localize HHV8 in oropharyngeal and salivary gland biopsies from HIV-infected and HIV-negative individuals. **Methods:** Archival biopsies of 280 salivary glands with overlying mucosa obtained over a 7-year period were evaluated. Only specimens from patients with a HIV test (positive or negative) were included. Tissue sections were incubated with anti-HHV8 Latent Nuclear Antigen-1 (LNA-1) monoclonal antibody. Immunoreactivity in the mucosa, stroma, inflammatory cells and salivary glands (acini and ducts) was recorded. **Results:** Four HIV+ and 7 HIV-negative patients were included. None had Kaposi's sarcoma at the time of biopsy. LNA-1 nuclear staining of mucosal epithelium was identified in 3/4 HIV+ cases and 0/7 control cases. Cytoplasmic reactivity was seen in rare mononuclear dendritic-type cells within lymphoid tissue in 1/4 HIV+ cases and in 2/7 control biopsies. No staining was observed in salivary gland epithelium, ducts, supporting stroma or vessels in all cases. **Conclusions:** HHV8 is present in oropharyngeal mucosal epithelium only in HIV+ patients. HHV8 is possibly ubiquitous in the general population, as both immunocompetent and HIV+ patients appear to harbor HHV8 within mononuclear cells of their oropharyngeal lymphoid tissue. These data further suggest that since mucosal epithelium is likely to be shed into saliva more easily than inflammatory cells, HHV8 may occur more frequently in the saliva of HIV+ persons.

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## REGRESSED KAPOSI'S SARCOMA (KS) LESIONS RETAIN ATROPHIC LESIONAL CELLS WITH THE POTENTIAL FOR RECURRENCE

Tahan SR, Dezube BJ, Pantanowitz L. Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

**Introduction:** Regression of KS can occur following appropriate pharmacotherapy. Clinical and histologic regression may be partial (residual lesional cells present) or complete (no lesional cells identified by light microscopy). It is unknown if completely regressed lesions retain any tumor cells, with the potential to redevelop. The aim of this study was to specifically monitor tumor cell regression in regressing AIDS-associated KS lesions.

**Methods:** HIV-infected men (n=3) with cutaneous KS were treated with KS-specific chemotherapy (Doxil in all cases with the addition of imatinib or Col-3 in 2 patients). KS lesions were biopsied prior to therapy, after 1 cycle (3 weeks later) and after 2 cycles (6 weeks later) of therapy. Immunohistochemistry using the lymphatic endothelial marker D2-40, a proven marker for KS cells, was performed. All specimens were evaluated by light microscopy and the number of D2-40 positive lesional cells compared. D2-40 stained biopsies of normal skin (from HIV- negative individuals) were used as controls.

**Results:** All patients (age range 43-50 years, CD4 cell count 5-512 cells/μL and HIV viral load 15,000 to >100,000 copies/mL) were maintained on HAART and had plaque stage KS lesions prior to treatment. Clinical and microscopic KS regression occurred after the first (2 partial, 1 complete) and second (2 complete, 1 partial) treatment cycles. D2-40 immunoreactive cells diminished in quantity and size in all cases, but not to control levels, even in lesions that had undergone complete regression.

**Conclusion:** Regression in Kaposi's sarcoma is accompanied by reduction in number as well as atrophy of spindle cells, however a population of cells remain in an atrophic state even in completely regressed lesions. This suggests that regressed KS lesions retain a reservoir of cells that have the potential to recur.

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## PAIN AND APPEARANCE ARE RELATED TO RESPONSE TO TREATMENT IN AIDS-RELATED KAPOSI'S SARCOMA

Jeannette Lee for the AIDS Malignancy Consortium, University of Alabama at Birmingham

Kaposi's Sarcoma (KS), the most common malignancy among HIV-infected individuals, is characterized by pigmented cutaneous lesions that can adversely effect quality of life. Lesion related symptoms include pain, diarrhea, edema, decreased ambulation and discomfort with physical appearance. Few quality of life instruments are focused on the assessment of KS-related symptoms.

The AIDS Malignancy Consortium has completed two studies with quality of life evaluations in AIDS-KS patients with limited disease. In a phase II study of 9-*cis* retinoic acid (AMC002), 43 patients completed a General Health Self-Assessment questionnaire including the module for Kaposi's Sarcoma. In a phase III placebo-controlled study of IM862 (AMC013), 184 patients were evaluated using a clinical benefit instrument. For both instruments, measures were transformed to the 0-100 scale with higher scores reflecting a better quality of life.

On AMC002, pain scores (mean $\pm$ SD) declined from 82.6 $\pm$ 24.1 at baseline to 66.3 $\pm$ 24.1 during treatment, and satisfaction with appearance scores improved from 49.4 $\pm$ 28.1 at baseline to 53.6 $\pm$ 22.1 during treatment. Non-responders had a marked increase in pain-related interference with activities from baseline while responders showed little change. Appearance scores improved from baseline in responders while little change was observed in non-responders. Pain scores on AMC013 declined from 96.2 $\pm$ 10.1 at baseline to 93.5 $\pm$ 12.5 on treatment. The treatment pain score was significantly higher in responders than non-responders reflecting more severe pain in the nonresponders.

These results show that appearance improves with response and that pain increases for non-responders. It is essential that information on these measures be captured on studies of AIDS-KS.

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## ANGIOGENESIS INHIBITOR COL-3 FOR THE TREATMENT OF HIV-RELATED KAPOSI'S SARCOMA (KS): A PHASE II AIDS MALIGNANCY CONSORTIUM STUDY

Bruce J Dezube, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA

**Background:** Angiogenesis is critical to the pathogenesis of KS. Matrix metalloproteinases (MMP) are involved in tumor invasion and metastasis. Col-3, a chemically modified, non-antimicrobial tetracycline, is an inhibitor of matrix metalloproteinases. The primary aim of this Phase II study was to evaluate the tumor response rate of Col-3 at 2 different dose levels. We had previously reported (Phase I) that a statistically significant difference in change in MMP-2 levels (gelatinase A) existed between responders to Col-3 and non-responders.

**Methods:** Patients were randomized to either 50 mg or 100 mg orally, once daily. Patients were required to have at least 5 measurable lesions. Patients with symptomatic visceral KS or severe tumor-associated edema were excluded. Antiretroviral therapy was permitted, but not required.

**Results:** 75 patients received Col-3 at a daily dose of 50 mg (36 patients) or 100 mg (39 patients). The mean age was 42 years. Most (99%) were male and 43% were from minority groups. The median CD4+ cell count at entry was 357 cells/microliter. Preliminary results show that there were 12 responses [1 complete (CR), 11 partial (PR)] (33%) in the 50 mg arm and 11 PR (28%) in the 100 mg arm. The most common adverse event attributed to drug was photosensitivity of any grade, which was reported in 12 (33%) 50 mg and 12 (31%) 100 mg patients. Grade 3 dermatologic adverse events were reported only in the 100 mg arm (photosensitivity, N=2; rash/desquamation, N=1; pruritis, N=1).

**Conclusions:** Col-3, when administered orally once daily in patients with HIV-related KS, is well tolerated. The response rates in the two arms were similar, but less severe phototoxicity was seen at the lower dose.

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## PERSISTANCE OF MITOCHONDRIAL TOXICITY IN HEARTS OF FEMALE B6C3F1 MICE EXPOSED IN UTERO TO 3′-AZIDO-3′-DEOXYTHYMIDINE

Dale Walker[1], Miriam Poirier[2], Matthew Campen[1], Dennis Cook, Jr.[1], Rao Divi[2], Kunio Nagashima[2], Vernon Walker[1]. [1]Lovelace Respiratory Research Institute, [2]National Cancer Institute

Cardiac toxicity has been associated with HIV infection and exposure to nucleoside reverse transcriptase inhibitors (NRTIs), but the role of the latter in the development of cardiac disease of HIV-infected patients is uncertain. To investigate the cardiotoxicity of transplacentally administered zidovudine (AZT) or AZT plus lamivudine (3TC) in the absence of HIV infection, we evaluated several biomarkers of cardiac mitochondrial ultrastructure and cardiac function in a B6C3F1 mouse model. In utero exposure to AZT-3TC resulted in ultrastructural pathology, loss of mitochondria, and altered echocardiographic measurements in newborn mice. Cardiac pathology and dysfunction persisted into adult life of female mice exposed in utero to AZT, as evidenced by significant dose-dependent heart enlargement, clusters of atypical mitochondria and myofibril alterations, significantly increased cytochrome *c* oxidase activity, and significantly higher numbers of mutations in mitochondrial tRNA genes compared with unexposed controls at 18 to 24 months of age. These data led to the hypothesis that the long-term pathology of perinatal exposure to these NRTIs is related to persistent mitochondrial DNA mutations in cardiac tissue, i.e., the primary damage during drug treatment is mutational (as opposed to affecting polymerase γ and/or other mitochondrial elements) and leads over time to delayed, progressive cardiotoxicity.

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## HIV INTEGRATION SITES FOUND IN CARDIAC MYOCYTE NUCLEI AND ADJACENT PCNA POSITIVE MACROPHAGE NUCLEI FROM AIDS CARDIOMYOPATHY HEART TISSUE

Leona W. Ayers[1], Xia Jin[2], John Anthony Bauer[3], Michael McGrath[4]. The AIDS and Cancer Specimen Resource (ACSR) [1,4], SLIL Biomedical Corporation, Menlo Park, CA[2], Center for Cardiovascular Medicine, Children's Research Institute[3], The Ohio State University, Columbus, OH 43210 .

Production of reactive nitrogen species and DNA damage have been observed in HIV/AIDS related complications but their roles in cardiac myocyte infectivity and related cardiomyopathy have not been defined. Here we asked if myocyte nuclei have high levels of DNA repair, could the peroxynitrite likely present facilitate HIV viral insertions into myocyte nuclear DNA? Could this question be pursued in the same formalin fixed paraffin embedded tissue used to demonstrate the protein nitration? (AIDS cardiomyopathy cases from the NCI funded ACSR). Methods: Myocyte nuclei and PCNA stained nuclei of adjacent macrophages from two AIDS cardiomyopathy cases and one non-AIDS cardiomyopathy were harvested using an Arcturus® Pixcell II LCM with CapSure HS (7.5 um laser spots). The DNA was surveyed for HIV proviral integration sites using inverse PCR (IPCR) with sensitivity of 1:100. HIVbase software was used to analyze results. Results: Clonal IPCR products were present in the myocyte and macrophage nuclear DNA with HIV insertion sites mapping to the first intron of specific genes in one of the AIDS cardiomyopathy cases; the control cardiomyopathy was negative. Conclusions: HIV insertions into the DNA of actively DNA repairing cardiac myocytes were identified using formalin fixed, paraffin embedded tissues in a relatively insensitive IPCR assay. LCM harvested DNA provided the ability to localize the identified integration sites to myocytes or macrophages. Many questions are raised by these findings. Further study is required. Information about the national ACSR and the types of tissues banked is available at http://acsr.ucsf.edu.

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## TISSUE MICRO-ARRAY DATA USING TMA STANDARD AND PUBLIC TOOL FOR LINKED LEGEND AND CLINICAL DETAILS AT ACSR.MID-REGION.ORG

David G. Nohle, Barbara Hackman, Leona W. Ayers. The Mid-Region AIDS and Cancer Specimen Resource (ACSR), The Ohio State University, Columbus, OH 43210.

The AIDS and Cancer Specimen Resource (ACSR) offers Tissue Micro-Array (TMA) sections with de-identified clinical data to approved researchers. Each of the hundreds of tissue cores has separate data so an organized approach to producing, navigating and publishing data is necessary. The April 2003 Association for Pathology Informatics (API) proposed XML TMA Data Exchange Standard (TMADES) was tested. Methods: Our tool consists of an Extensible Stylesheet Language Transformations (XSLT) file that uses one of several block style XML data files that specifies: layout of the TMA block (number of rows and columns, cell designations, gaps, etc.), legend cell color coding, data items to include in the legend cells and data items to have details table columns. An automatically generated Hypertext Markup Language (HTML) web page for each TMA block contains a details table and a legend table. The details table has user specified core specific demographic data copied from the export file. The data items contain links to that core's place in the legend table. Results: The tool was successfully applied to different block formats. Eleven Common Data Elements (CDE) from the TMADES standard were used and eight more were created for site-specific data. Conclusions: This tool encourages the use of TMADES. Information about the national ACSR and the types of tissues banked is available at http://acsr.ucsf.edu. Interest in this software can be directed to the OSU-ACSR, Leona W. Ayers, M. D., M352 Starling Loving Hall, 320 W. 10th Avenue, Columbus, Ohio 43210, (614-293-8106).

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## RELATIONSHIPS BETWEEN EXPOSURE CONCENTRATION, EXPOSURE DURATION AND MUTAGENIC EFFECTS IN HUMAN LYMPHOBLASTOID TK6 CELLS TREATED WITH AZT, 3TC, AND AZT-3TC

Salina Torres[1,2], Vernon Walker [2], Meghan Carter[2], Dennis Cook, Jr.[2], Ofelia Olivero[3], Miriam Poirier[3], Dale Walker[2]. [1]University of New Mexico, [2]Lovelace Respiratory Research Institute, [3]National Cancer Institute.

Zidovudine (AZT) and lamivudine (3TC), a combination of nucleoside analogues (NRTIs) that is highly effective in reducing transmission of HIV-1 from mother to infant, increases the frequency of mutations in reporter genes during fetal life and may impose a risk for cancer in exposed children. The current study was designed to characterize the relationships between exposure concentration, exposure duration, and mutagenicity in human lymphoblastoid cells exposed in culture to AZT, 3TC, or AZT-3TC. Using a cell-cloning assay to measure mutant frequencies at the *HPRT* and *TK* loci, combined exposures to AZT-3TC were found to have synergistic mutagenic effects greater than the additive responses to equimolar exposures of AZT or 3TC alone. Cells exposed to a combination of 10 μm AZT-3TC for 30 days, which mimics standard clinical practice, showed *HPRT* and *TK* mutant frequencies that were significantly increased over control values (*HPRT*: control, $26.5 \pm 15.6 \times 10^{-6}$; treated, $36.7 \pm 7.6 \times 10^{-6}$; *TK*: control, $21.0 \pm 3.4 \times 10^{-6}$; treated, $64.0 \pm 1.6 \times 10^{-6}$). Comparisons between AZT-3TC induced mutant frequencies showed that the mutagenic responses were remarkably similar for each reporter gene following the same cumulative dose achieved by long-term (30 days), plasma-level exposures or short-term (3 days), high-level exposures. These findings suggest that AZT-3TC coexposure has a synergistic mutagenic effect in human cells and support the hypothesis that the increased frequencies of mutations previously found in the *HPRT* and glycophorin *A* genes of exposed newborn infants is related to the duration of *in utero* exposure.

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## ANTI-HIV ACTIVITY OF THE CHEMOPREVENTIVE DRUG, N-(4-HYDROXYPHENYL) RETINAMIDE (4-HPR)

Catherine M. Finnegan, Satinder S. Rawat, Anu Puri, Frank Ruscetti, and Robert Blumenthal. National Cancer Institute, Frederick, MD, 21702.

Studies of ceramide metabolism and function in a wide range of biological processes have revealed a role for this lipid in regulating key biologic responses. N-(4-hydroxyphenyl) retinamide (4-HPR, fenretinide) has been reported to cause large increases of ceramide levels in tumor cell lines and induce cell death with minimal toxicity to normal lymphocytes, fibroblasts and myeloid progenitors. The compound is under investigation in clinical trials as a cancer preventive and therapeutic agent. Our research on the role of sphingolipids in HIV entry has led to the hypothesis that modulation of ceramide levels in target cells affects their susceptibility to HIV infection by rearranging HIV receptors. Ceramide levels were determined following incorporation of [$^3$H] sphingosine for 48 hours in the presence of the appropriate agent. Infectivity assays were performed using a HeLa-derived indicator cell line TZM-bl, CD4+ lymphocytes and monocytes. We observed a dose-dependent inhibition by 4-HPR of infection of TZM-bl cells by a broad range of HIV-1 isolates at low μM concentrations with an IC50 less than 1μM for most isolates tested. Near complete inhibition was seen at 1 μM, a dose that enhanced ceramide levels by 50-100%, yet was non-toxic to the cells. Treating cells with other pharmacological agents that cause enhanced ceramide levels, or with sphingomyelinase, or exogenous addition of long chain ceramide also resulted in inhibition of HIV-1 infection. Enhancing ceramide levels in CD4+ lymphocytes had no effect on susceptibility to HIV-1 infection. By contrast treatment of human monocytes with 4-HPR or sphingomyelinase reduced infectivity to less than 10% of control without toxicity. Our data suggest that the drug may be exceedingly suitable as a combined chemopreventive and anti HIV therapeutic agent.

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## HIV-ASSOCIATED GAMMOPATHY IN THE HAART ERA: ASSOCIATION WITH HIV VIREMIA

L. Pantanowitz, B.A. Beckwith, G.L. Horowitz, B.J. Dezube. Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

**Introduction:** Monoclonal gammopathy in HIV+ patients is being increasingly reported. Some paraproteins appear to exhibit high-titer anti-HIV activity. Qualitative immunoglobulin abnormalities have not been studied in HIV+ patients in the HAART era. We aimed to characterize serum protein electrophoresis (PEP) patterns in the HAART era, determine the incidence of monoclonal gammopathy in HIV+ patients and correlate M-spikes with HIV viral load and CD4 cell counts. **Methods:** PEP, and immunofixation for suspected abnormalities, were performed on 322 consecutive HIV+ blood samples. PEP findings were correlated with HIV viral load and CD4+ cell count. **Results:** Patients, of mean age 43 years (range,7-67) including 255 males and 67 females, had a median CD4+ count of 520 cells/uL. HIV viral load was detectable (>50 copies/mL) in 176 (55%) patients (median 6,100). PEP in 270 (84%) samples were classified as normal, 6 (2%) each as hypo- or hyper-gammaglobulinemic, 26 (8%) with oligoclonal bands and 14 (4%) with monoclonal bands. Monoclonal bands were all of IgG class and represented less than 5% of total protein. Only one M-protein had concomitant hypogammaglobulinemia. Patients with M-proteins were of median age 45.5 years (8 males). Among patients with either oligoclonal or monoclonal bands, 80% had a detectable viral load, as compared to 50% of patients with a normal PEP pattern. There was no correlation between the occurrence of monoclonal and/or oligoclonal bands and CD4+ count. **Conclusions:** PEP was abnormal in 16% of HIV+ blood samples. Abnormalities were subtle compared to the striking hypergammaglobulinemia and oligoclonal bands described in the pre-HAART era. Monoclonal bands were identified in 4% of patients of relatively young age. However, no samples showed high concentration bands suggestive of myeloma. Chronic HIV antigen stimulation may explain why more patients with bands had detectable HIV viral loads.

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## INACTIVATION OF HIV BY IN VIVO GENETIC MODIFICATION

William Taylor and David J. Segal. Department of Pharmacology and Toxicology, University of Arizona, Tucson, AZ, USA, 85721

There are currently many therapies for HIV infection, but with these treatments the virus is not eliminated and a reservoir of latently infected cells remains. Our project aims to create HIV-resistant hematopoietic stem cells with the potential to restore a functional immune system in infected individuals. Our approach will be an intracellular immunization strategy involving the construction of custom HIV-specific endonucleases. There are four possible ways such endonuclease proteins can inactivate HIV: excision, recombination, mutation, and apoptosis. This approach differs from vial suppression strategies in that the HIV would be permanently inactivated. The DNA form of the HIV genome represents a novel target for therapeutic intervention, and structural features of the genome make it ideally suited for the modifications described. For the initial stages of the project the endonuclease will be the isolated catalytic domain of the FokI enzyme. The endonuclease is linked via an amino acid linker to zinc finger proteins, which bind DNA. These zinc fingers are designed to bind specifically to selected, conserved regions of the HIV genome. A functional zinc finger/endonuclease chimeric protein will deliver the enzyme to the targeted nucleotide sequence and induce double-strand cleavage. We are currently evaluating their ability to specifically cleave their target sites in vitro and in cell culture assays. The net result of this study will be an increased understanding of inducing genomic modifications in cells, insights into the structure/function relationship of DNA modifying enzymes, and the development of a new class of molecular therapeutics that should find broad application in the study and treatment of HIV/AIDS and other diseases.

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