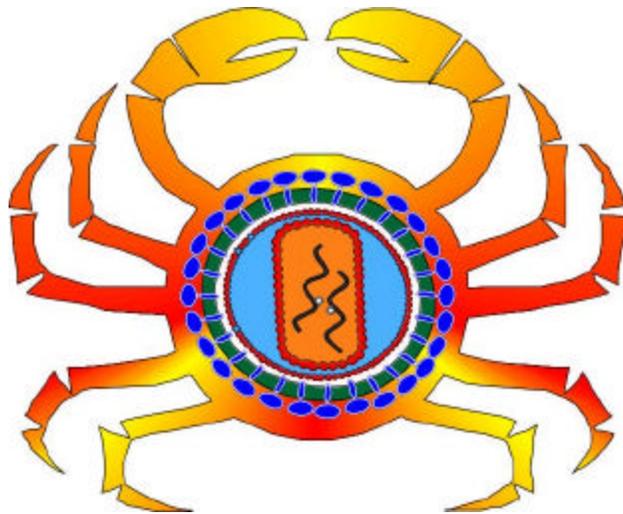


# **AIDS-ONCOLOGY RESOURCES HANDBOOK**

**MAY 2003**



<http://ctep.cancer.gov/resources/aids.html>

**DIVISION OF CANCER TREATMENT AND DIAGNOSIS  
NATIONAL CANCER INSTITUTE  
NATIONAL INSTITUTES OF HEALTH  
DEPARTMENT OF HEALTH AND HUMAN SERVICES  
BETHESDA, MARYLAND**

**NATIONAL  
CANCER  
INSTITUTE**

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## INTRODUCTION

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The National Cancer (NCI) Institute is committed to enhancing clinical and laboratory research opportunities in AIDS malignancies. Our objective is to encourage communication on the breadth and depth of research opportunities and facilitate rapid development of tools to pursue crucial research questions in this area. The AIDS Oncology Resources Handbook facilitates this objective by providing information on the current scope of NCI activities in AIDS malignancy research. The first Handbook was presented in 1996 and this version is the annual update. The scope of the Handbook was expanded from the original version to include relevant information on activities within other institutes of the National Institutes of Health (NIH), including the National Institute of Dental and Craniofacial Research and the National Institute of Allergy and Infectious Diseases.

Web site availability allows broad access of valuable information to the research community at large and provides an accessible and comprehensive listing of the array of clinical and laboratory research resources that receive NCI AIDS funding. The NCI Intramural AIDS/AIDS Malignancy Activities section provides brief synopses of intramural research studies and recent accomplishments. The broad research questions and major highlights of the extramural research programs are summarized in the NCI Extramural AIDS/AIDS Malignancy Activities section, including a description of the Fogarty International Center activities that enhance research and training opportunities in resource-poor countries. The intramural and extramural research efforts of the National Institute of Dental and Craniofacial Research can be found in the National Institute of Dental and Craniofacial Research section. To facilitate interactions and collaborations, a contact person, telephone, email address and related Web site follows each research summary. Information on clinical trials and NCI budgets is also included in two separate sections.

**Navigation Tips:** Direct links to Web sites appear in underlined blue text. Clicking on either the orange titles on the Organizational Cross Reference page, or the page numbers on the Contents page navigates the reader directly to those sections.

Our thanks and appreciation to the many individuals who contributed to this Handbook. We encourage readers of this Handbook to let us know how we can make future editions more useful.

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#### DIVISION OF CANCER BIOLOGY (DCB)

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Chief & Program Director - Roy Wu, Ph.D.

##### **Developmental Therapeutics Program (DTP)**

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Program Director - George Johnson, Ph.D.

##### **Screening Technologies Branch (STB)**

Chief - Robert Shoemaker, Ph.D.

##### **Laboratory of Antiviral Drug Mechanisms (LADM)**

Head – Shizuko Sei, Ph.D.

## **ORGANIZATIONAL CROSS-REFERENCE**

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### **NCI INTRAMURAL AIDS/AIDS MALIGNANCY ACTIVITIES**

#### **CENTER FOR CANCER RESEARCH (CCR)**

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**Chief - Silvio Gutkind, Ph.D.**

## **CANCER ETIOLOGY BRANCH**

### **Division of Cancer Biology**

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The [Cancer Etiology Branch](#) plans, develops, and directs a national extramural research program dealing with biological, chemical, and physical agents that are possible etiological factors or co-factors in cancer and with the control of these agents and their associated diseases. Specific agents of interest include infectious agents such as viruses and bacteria and chemical carcinogens such as polycyclic aromatic hydrocarbons and hormones. Investigations include studies of the agents themselves and their properties, mechanisms of oncogenesis and carcinogenesis, and interactions of oncogenic microbiological agents with their hosts as well as basic studies to identify possible targets for preventive or therapeutic measures.

#### **Areas of Investigation and Interest**

- Human and animal DNA viruses as etiologic agents in specific cancers and basic studies of the molecular mechanisms of malignant transformation.
- Retroviruses and oncogenes in human and animal cancer, and hepatitis viruses associated with human liver cancer.
- AIDS-associated virology and malignant sequelae: HIV and AIDS; KSHV and Kaposi's sarcoma; animal models of AIDS-related cancers.
- Chemical carcinogenic agents and their metabolism, identification of tumor initiators and promoters, study of mechanism(s) of action, and signal transduction pathways.
- Metabolism, toxicity, and carcinogenic mechanisms of chemicals and identification of biochemical and molecular markers of chemical carcinogenesis.
- Investigations of the hormone-related biochemistry of cancer and hormonal perturbations in malignancy.
- Studies of the etiologic mechanisms of bacteria and other microbial species associated with human cancer.
- The synthesis and distribution of chemical carcinogens and mutagens as well as experimental tumor inhibitors to chemical carcinogenesis investigators.

#### **Important Study Findings/Highlights**

- The development of genomic instability is a hallmark of human papillomavirus (HPV)-associated cervical cancer. HPV-16 E7 has been shown to rapidly induce abnormal centrosome duplication resulting in multipolar, abnormal mitoses and aneuploidy. Recent findings demonstrate that both HPV E6 and E7 oncoproteins can trigger anaphase bridge formation, which typically develops after chromosomal breaks and alterations of chromosomal structure. Furthermore, the expression of both high-risk E6 and E7 oncoproteins causes DNA damage. These studies show that HPV 16 E6 and E7 oncoproteins can independently induce various mitotic abnormalities and trigger the complex chromosomal changes that are observed in high-risk HPV-associated cancers.
- Cervical cancer cells express high-risk HPV E6 and E7 oncoproteins, and repression of HPV gene expression causes these cells to cease proliferation and undergo senescence. Repression of the E7 oncoprotein efficiently triggers Rb-dependent senescence without activating p53 or inducing p21, whereas E6 expression triggers p53-dependent senescence and apoptosis without activating the Rb pathway. High-level telomerase, cyclin-dependent kinase activity, and c-myc expression require continuous expression of both viral oncoproteins. Thus, continuous expression of both E6 and E7 oncoproteins is required for optimal proliferation of cervical carcinoma cells, and the two viral proteins exert distinct effects on cell survival and proliferation.
- Constitutive STAT activation has been detected in a wide variety of human cancers, including Epstein-Barr virus (EBV)-associated tumors, implicating these molecules in tumor formation and progression. STAT members 3 and 5 have been found to be constitutively activated in nasopharyngeal carcinoma. LMP1, which is essential for EBV-induced transformation of primary B cells, has been shown to be primarily responsible for STAT activation that occurs on EBV infection of epithelial cells. This activation of STAT 3 appears to be mediated through IL-6. STATs may play a

dual role by regulating EBV latent infection as well as directly contributing to the tumorigenic cell phenotype.

- The LMP2A protein of EBV is the only viral protein consistently identified in latently infected B cells, suggesting that it plays a key role in viral persistence and in the development of EBV-associated malignancies. DNA microarray technology and the transgenic mouse model were both used to study changes in gene transcription induced upon LMP2A expression in murine B lymphocytes. LMP2A alters the expression of critical transcription factors involved in normal B-cell development. In particular, the transcription factors E2A, EBF, and Pax-5 are each down-regulated in bone marrow and splenic B cells from LMP2A transgenic mice. The ability of LMP2A to interfere with B-cell transcription factor regulation may be important in maintaining EBV latency.
- Attachment and entry of Kaposi's sarcoma-associated herpes virus or human herpes virus 8 (KSHV/HHV8) particles into host target cells require binding to an extracellular receptor followed by endocytosis of the virus particle. The envelope glycoprotein B (gB) of KSHV/HHV8 has been identified through *in vitro* studies to bind to  $\alpha_3\beta_1$  integrin molecules on the surface of host cells. Initial attachment of gB to the  $\alpha_3\beta_1$  integrin occurs through an RDG amino acid motif at the amino terminal end of the gB molecule. Attachment of the KSHV/HHV8 particle to the integrin molecule induces phosphorylation of focal adhesion kinase (FAK), which then initiates endocytosis of the particle into the host cell.
- Viruses have mastered mechanisms to escape immune surveillance. One mechanism to disrupt the initial host response to viral infection employed by KSHV/HHV8 is blocking the induction of interferon. The immediate-early protein ORF45 exerts its effect by preventing the phosphorylation of the cellular interferon regulatory factor 7 (IRF-7) that must be activated to induce the transcription and translation of type 1 interferon genes. Other evasion strategies include the down-regulation of the immunomodulatory molecules MHC class 1, B7-2, and ICAM, the molecules from the cell surface of KSHV/HHV8-infected cells. The KSHV/HHV8 K3 protein induces endocytosis of MHC class 1 molecules, which prevents CTL-mediated lysis of infected cells. The cell surface molecules B7-2 and ICAM are also rapidly removed from the surface of the cell by the HHV8 protein K5. K5 disrupts normal natural killer cell responses and humoral helper T-cell responses.

### Program Announcements

- [Models for HIV Disease and AIDS-related Malignancies](#)

The purpose of this Program Announcement (PA) is to encourage investigator-initiated grant applications for the development of useful and predictive biochemical, cellular, *in vivo*, and mathematical models for the preclinical evaluation of new therapies against HIV and AIDS-related malignancies. The availability of well-characterized *in vitro* and *in vivo* models would accelerate the pace of evaluation of different paradigms of disease progression and would facilitate the discovery of successful treatments, including drugs, vaccines, gene therapy, and immune modulators. R01 and R21 applications are accepted.

- [Therapeutics Research on AIDS-associated Opportunistic Infections and Malignancies](#)

The purpose of this PA is to encourage research grant applications aimed at novel approaches to discovery and preclinical development of therapeutic agents against opportunistic infections and malignancies in people with AIDS. The intent of this PA is to seek investigator-initiated grant applications that involve creative and original preclinical research using state-of-the-art technologies necessary to propel advances in new or improved therapies. No clinical trials will be supported under this PA. R01 and R21 applications are accepted.

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## **CANCER IMMUNOLOGY AND HEMATOLOGY BRANCH**

### **Division of Cancer Biology**

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#### **Broad Research Questions Currently Under Study**

The areas under study include hematologic malignancies, specifically those that occur as a result of congenital, iatrogenic, or acquired immunodeficiencies. There are three program areas: cellular, genetics/molecular aspects of immunology, and hematology in relationship to the genetics of hematologic malignancies. AIDS lymphoma is located within both the cellular immunology and hematology program areas. Research covers areas such as the role of cytokines, B and T cell ontogeny, lymphocyte activation and deactivation, the effect of somatic mutations on lymphomagenesis, immunodeficiencies, antigen receptors on B and T cells, the Natural/Innate Immune system and use of transgenic animals. All aspects of immunology and its relevance to cancer are included in the Cancer Immunology and Hematology Branch.

#### **Important Study Findings/Highlights**

Studies of B cell proliferation in HIV/AIDS patients indicate varying levels of “polyclonal B cell activation”. The data suggest that this “activation” is antigen driven. As long as there are sufficient CD4+ T cells, there is a lower risk of B lymphomagenesis. With the decrease in CD4+ T cells in those patients who progress to AIDS, the risk of lymphomagenesis increases. In addition, the role of CD40/CD40L signaling has been shown to be a major contributor to aberrant B cell behavior. Preliminary results further suggest the role of mutated co-stimulatory molecules facilitating B lymphomagenesis.

AIDS non-Hodgkins lymphoma (AIDS-NHL) is a complicating entity of AIDS patients when their CD4 counts drop below 200mm<sup>3</sup>. This puts them at high risk for lymphomagenesis. HIV/AIDS patients demonstrate polyclonal B cell activation/proliferation during their infection with the concomitant danger of B lymphomagenesis because of chronic B cell proliferation and prolonged B cell survival. To prevent malignant B cells from developing, apoptotic signals are normally sent through the BCR complex. However, recent evidence has shown that mutations in genes coding for the BCR-complex prevent apoptotic signaling with the following consequences: (1) prolonged B cell survival, (2) accumulation of mutations, and (3) lymphoma development. Current research in laboratories supported by CIHB continue to elucidate the genetic mutations that lead to B cell malignancies. Additional studies have shown that patients with less advanced disease can be divided into two groups based on the frequency of malignant B cells that express the cell-surface marker CD38. Those patients whose malignant B cells have less than thirty percent expressing CD38 may have longer survival. Other studies have focused on the emerging Innate/natural immune system. Specific focus is on dendritic cells – regarded by all immunologists as the professional antigen presenting cells (APCs), natural killer (NK) cells, and the particularly novel NKT cells that possess both NK and T cell functions. NKT cells were shown to influence the prevention and reduction of certain cancers. It will be important to assess the role of Innate immunity during HIV infection.

#### **Total Number of Grants and/or Contracts Funded Within CIB**

FY 2002: 137 with 87 (100% AIDS) and 50 (<100% AIDS)

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The [Division of Cancer Control and Population Sciences](#) is interested in interdisciplinary studies of the molecular epidemiology of pre-neoplastic conditions and cancers among persons infected with, or at high risk for, HIV/AIDS. The cancers of primary interest are those associated with concomitant infection with viruses conferring latency, including the Epstein-Barr virus (EBV), human herpes virus 8/Kaposi's sarcoma-associated herpes virus (HHV8/KSHV), human papillomavirus (HPV), and hepatitis B and C. Since these infections are apparently necessary, but not sufficient, to cause cancer, both endogenous and exogenous cofactors must be involved in the etiology. Epidemiologic variables of interest include host susceptibility, age at first acquisition of the oncogenic virus, timing of acquisition of the oncogenic virus relative to that of HIV infection, effect of circulating viral load of the oncogenic virus, measurements of immune response, role of lifestyle factors such as tobacco use and diet, and behavioral characteristics. Relevant topics also include the progression and clinical course of cancers and pre-neoplastic changes; the interplay of the route of acquisition of the DNA virus, particularly HHV8/KSHV, and other cofactors on the molecular epidemiology and natural history of oncogenesis; and the role of psychosocial processes in infection, disease detection, and progression.

### **Important Study Findings/Highlights**

Co-infection with HIV and hepatitis B virus (HBV) is very common. However, liver-related mortality in persons with HIV/HBV co-infections is not well characterized. Data from a prospective cohort study of 5,293 co-infected men from the Multicenter AIDS Cohort Study (MACS) were examined for the risk of liver-related mortality. Study findings revealed the overall liver-related mortality rate as 1.1/1000 person years, with mortality rates higher in those co-infected with both HIV and HBV (14.2/1000 person years). The mortality rate was also highest in those having low CD4 counts. In addition, liver-related deaths seem to increase after the introduction of HAART, suggesting that comprehensive management of HIV patients co-infected with HBV is important in controlling liver toxicities and death. (Thio et al. *Lancet*, 2002;360:1921-26)

A new ELISA for detection of virus-like particles (VLP) of HPV was investigated. Although current assays have proven to be useful, they do not produce high sensitivities and there is low signal-to-noise ratios. Two vinyl polymers, PVA-50 and PVP-360, were used in the assay for detection of sensitivity as well as specificity of antibodies to the VLP. Study results indicated that PVA-50 reduced the amount of nonspecific binding of the antibodies to VLPs and that PVP-360 increased the sensitivity of antibody detection. The new ELISA demonstrated increased sensitivity and specificity for the detection of HPV type 16. (Burk et al. *J Clin Microbiol*, 2002;40(5):1755-60)

The functional role of IL-10 was investigated to determine whether increased serum levels and high genotype presence were associated with the development of AIDS-lymphoma. In this study, patients who developed AIDS-lymphoma had significantly higher levels (21%) of detectable IL-10 in their serum samples than did controls ( $p \leq 0.002$ ). Similarly, there was a significant increase in IL-10 expressor genotype in subjects who developed lymphoma versus subjects who did not ( $p = 0.007$ ). Data suggest that increased levels of IL-10 in HIV-positive persons are associated with the development of AIDS-associated B-cell lymphoma. Elevated IL-10 slows the progression to AIDS and can activate isotype switching, which may result in an increased risk of AIDS-lymphoma. (Breen et al. *Pharmacol Ther*, 2002;95:295-304)

HIV infection and associated immunodeficiency are known to alter the course of human papillomavirus (HPV) infections. Data from two large prospective studies, the Women's Interagency HIV Study (WIHS) and the HIV Epidemiology Research Study (HERS), were used to examine the relationship between HIV, HPV and genital warts. A total of 2,930 HIV-positive and 1,008 HIV-negative women were enrolled from both studies. HPV 6 or 11 was 5.6 times higher among HIV-positive WIHS women and 3.6 times higher among HERS women. Genital wart prevalence in the HIV-positive women was increased by 3.2 in the

WIHS and 2.7 in the HERS. HIV infection and immunodeficiency synergistically modified the relation between HPV 6 or 11 infection and genital wart prevalence. (Shah et al. Sex Transm Dis, 2002) In Zambia, primary infection with HHV-8 is reported among 50% of the adolescents and childbearing women. Current knowledge regarding the route of transmission and risk factors that influence transmission is limited. Investigators have found that *in utero* transmission occurs but is infrequent. Postnatal transmission is most likely the route of HHV-8 infection in Zambian infants. To investigate whether HHV-8 might be transmitted vertically from mother to infant, a cohort of women and infant pairs in Zambia was studied prospectively. Polymerase chain reaction (PCR) analysis of the infant's PBMCs revealed HHV-8 DNA sequences in two infants (17%) born to HHV-8-positive/HIV-1-negative mothers. This finding of HHV-8 DNA in neonates at birth indicates vertical transmission, but other routes such as horizontal transmission remain the most likely means of HHV-8 transmission. (Wood et al. J Infect Dis, 2003;187:559-68)

### **Description of Portfolio**

This extramural research portfolio includes cooperative agreements with several NIH Institutes and Centers to support natural history studies of HIV-associated cancers in U.S. cohorts of HIV-infected and high-risk, HIV-uninfected women and men. For example, supplemental funds have been provided to support the malignancy agendas in both the WIHS, the largest U.S. study of HIV infection in women, and the MACS, a longitudinal study of the natural and treated history of HIV infection in homosexual and bisexual men. In addition, there is a research portfolio of investigator-initiated research grants, including international studies. There is an open PA, [Molecular Epidemiology of Cancers Associated with Acquired Immunodeficiency \(PA-03-024\)](#).

### **Total Number of Grants and/or Contracts Funded Within DCCPS**

FY 2002: 15 (100% AIDS) and 59 (<100% AIDS)

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**OFFICE OF THE DIRECTOR**  
**Division of Cancer Treatment and Diagnosis**

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The AIDS Malignancy Program (AMP), Office of the Director (OD), [Division of Cancer Treatment and Diagnosis \(DCTD\)](#) supports pre-clinical and clinical studies for treatment of cancer in HIV+ and immunocompromised patients. The AMP also supports resources for preclinical and translational/interdisciplinary studies including a tissue repository of well characterized specimens collected from HIV+ and HIV- patients, and infrastructure at institutions that receive significant AIDS funding.

**Broad Research Areas Currently Under Study**

- Identifying hypothesis-driven therapeutic approaches for the treatment of malignancies associated with AIDS, including but not limited to non-Hodgkin's lymphoma, primary central nervous system lymphoma, Kaposi's sarcoma (KS), anogenital dysplasia, and cancer. The therapeutic approaches include, but are not limited to, biologic therapy (e.g., IL-2, IL-12, IFN-alpha, stem-cell factor), immune-based therapy (e.g., Mabs directed against B-cell targets, CTLs directed against viral-mediated targets, immune system stimulation by IM862, stem-cell transplantation), angiogenesis inhibitors (thalidomide, COL-3), and therapeutic vaccines. Cytotoxic chemotherapy in combination with immune based or biologic therapies remains an active area of investigation.
- Identifying the effects of anti-tumor therapy on the underlying HIV infection and impact on the immune system.
- Identifying the effects of anti-HIV therapy on the underlying tumor, the complex issues of drug-drug interactions, and overlapping toxicities.
- Identifying the virologic (HPV, EBV, KSHV, and HTLV-1), immunologic, and molecular markers that may be important in the pathogenesis of AIDS malignancies, and defining their potential use as therapeutic targets.
- Identifying the optimum regimen for malignancies associated with AIDS through clinical trials.
- Developing and providing access to a repository of tumor tissue, relevant biological fluids, and controls with associated demographic and clinical outcome information.
- Identifying unique scientific opportunities in the international arena.

**[AIDS Malignancy Consortium \(AMC\)](#)**

The AMC was developed to facilitate the rapid evaluation of hypothesis-driven Phase I, II, and III clinical trials that utilize the expertise of both NCI- and National Institute of Allergy and Infectious Diseases-sponsored scientists. The AMC consists of 14 Clinical Trials Members and one Data Management, Operations, and Statistical Center. They develop and conduct innovative clinical trials aimed at improving the treatment and prevention of AIDS-associated cancers and related conditions, and identify and develop clinical and laboratory correlates. The AMC has activated a total of 23 clinical trials with six currently open to patients with AIDS-malignancies (refer to the Clinical Trials section of this handbook). Current therapeutic approaches include combination chemotherapy with Mabs directed against B-cell targets, compounds that inhibit angiogenesis and restore/improve immune function, therapeutic vaccines directed against viral targets (HPV, EBV), and stem cell transplantation. In addition to assessing potential anti-tumor activity and drug-drug interactions, the AMC members collaborate with investigators outside of the network to develop and utilize laboratory correlates that reveal the pathophysiologic basis of drug activity. This includes evaluating the impact of therapy on viral load, underlying immune function, and angiogenesis. Additional information about the AMC mission and details of trials may be obtained at <http://www.amc.uab.edu> .

Important clinical information resulting from completed AMC trials:

- Oral 9-cis-retinoic acid was shown to be an active anti-tumor drug for AIDS-related KS with an overall response rate of 37%.
- CHOP or a modified dosage of CHOP chemotherapy is an effective and tolerable treatment for NHL in HIV+ patients on concurrent HAART.
- IFN-alpha2a administered to HIV+ KS patients on protease inhibitors was well tolerated with an overall response rate of 39%.
- Oral COL-3 administered once daily to HIV+ KS patients is well tolerated, with an overall response rate of 44% in a phase I trial. A phase 2 trial of COL 3 recently completed accrual and analysis is underway.
- Results of a randomized placebo controlled Phase III trial of IM862 indicated that IM862 is ineffective against AIDS-KS.
- Preliminary results of a randomized Phase III trial of CHOP with or without rituximab for patients with HIV-associated NHL indicate no response benefit from the addition of rituximab to CHOP. However, a high incidence of documented neutropenic infection and death in those receiving rituximab raises concern regarding the safety of this approach in this patient population.

### **The AIDS and Cancer Specimen Resource (ACSR)**

The AMC investigators further contribute toward the research effort in AIDS-associated malignancies by collecting and donating specimens to the ACSR, formerly known as the AIDS Malignancy Bank. The ACSR consists of three main member institutions, approximately 30 affiliated institutions, and a Central Operations and Data Coordinating Center. The ACSR was established to identify and improve access to well-characterized tissue, fluids, and associated demographic and clinical data collected from HIV+ and HIV- controls, and to encourage and facilitate AIDS-related cancer research. The ACSR contains over 100,000 specimens collected from cohort studies, clinical trials, and other research, including international sources. Samples have been distributed worldwide at no cost to investigators. Specimen types made available include tumor and matched control frozen tissues, multi-site autopsy, blood cells/serum, frozen/fixed biopsies, AIDS-related lymphomas, KS, lung tissue, and anogenital samples. An updated database, application forms, and additional information about the ACSR samples are available at <http://acsr.ucsf.edu/>.

Examples of studies performed using ACSB samples:

- Searching for specific or novel viruses in cancer tissue
- Examining the role of chemokines, cytokines, and growth factors in cancer
- Studying the contribution of chronic B-cell stimulation to B-cell lymphomas
- Searching for genomic/chromosomal changes (some virally mediated) or changes in gene-expression profiles
- Developing and validating diagnostic assays
- Searching for markers of oncogene activation
- Examining the effect of HIV integration site on cancer development
- Identifying mechanisms of resistance and susceptibility to antiviral agents
- Identifying viral CTL epitopes

### **Centers for AIDS Research (CFARs)**

The DCTD AMP further supports the translation of basic laboratory results to clinical application by cofunding the CFAR infrastructure support program along with five other NIH institutes. The CFAR program provides administrative and shared research support to synergistically enhance and coordinate high-quality AIDS research projects. CFARs accomplish this through core facilities that provide expertise, resources, and services not otherwise readily obtained through more traditional funding mechanisms. There are 19 sites across the US using the P30 mechanism. Additional information about the CFAR program is available at <http://www.niaid.nih.gov/research/cfar/>.

### **Investigator-initiated Translational Research**

Several investigators are working on viral-targeted therapies for AIDS malignancies. Dr. Michael Caligiuri developed a reproducible preclinical model of EBV+ PCNSL in the nude rat. The safety and efficacy of radiation and antiviral agents to induce apoptosis of tumor cells was assessed in this model. A combination of AZT + GCV + low-dose XRT, previously shown to induce apoptosis of an EBV+ cell line, extended rat survival time when compared to the antivirals or XRT alone. Up-regulation of EBV thymidine kinase (TK) was detected after exposure to XRT in the triple regime. The novel observation that XRT induces viral TK, which further sensitized tumor to AZT and GCV, led to the development of a Phase 1 clinical trial. A renal transplant recipient with aggressive EBV+ PCNSL unresponsive to immunosuppressive taper achieved a complete response after treatment with this regime and remains disease free after three years (Caligiuri and colleagues, Ohio State University, Cancer Research, 63:965-971, 2003).

Dr. Shannon Kenny showed that GCV enhances tumor cell killing induced by both chemotherapy and irradiation. Since both are known to induce the lytic form of EBV replication and only a small percentage of tumor cells are infected with the virus, she developed a model for this synergistic effect. Induction of EBV lytic replication by XRT or chemotherapy results in GCV phosphorylation to its active form by the EBV lytic gene TK. The active form of GVC can then be transferred to adjacent tumor cells and kills them as well. This novel hypothesis led to the development of a phase I/II trial to determine whether the combination of conventional irradiation and GCV is more efficacious (or toxic) than irradiation alone for the treatment of AIDS-related CNS lymphoma.

Investigations into the mechanisms of cell death induced by AZT and IFN- $\alpha$  in EBV+ lymphomas and HHV-8+ primary effusion lymphomas (PEL) indicated that there were two distinct pro-apoptotic effects of these agents in primary lymphoma cell lines derived from AIDS patients. EBV+ Burkitt's lymphoma (BL) cell lines underwent apoptosis in the presence of AZT alone, but HHV-8+ PEL cell lines required the addition of IFN- $\alpha$ . Investigation into the mechanisms of IFN- $\alpha$ -induced apoptosis indicated that IFN- $\alpha$  induces the soluble death receptor ligand TRAIL, which, when combined with AZT, blocks expression of the anti-apoptotic NF $\kappa$ B resulting in initiation of a suicide program in these HHV-8-infected lymphomas. In contrast, IFN- $\alpha$  did not increase the apoptotic effect of AZT in EBV+ BL. This novel, targeted therapeutic approach was used to treat an HIV+ PEL patient, and led to complete resolution of the malignant effusion in five days (Harrington and colleagues, University of Miami, Oncogene, 20:7029-7040, 2001; Blood, 101:2321-2327, 2003).

Additional studies are aimed at improving therapy for HTLV-1-associated adult T-cell leukemia/lymphoma, examining the biological basis for tumorigenesis and tumor remission, developing therapies designed to enhance the innate immune response in HIV+ patients, and developing therapies targeting the latent phase of EBV for EBV+ NHL.

**Total Number of Funded AIDS-related Grants and/or Contracts in Office of the Director, DCTD**  
FY 2002: 52, 100% AIDS (R01, U01, R21, P01, P30)

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## **INTERNATIONAL ACTIVITIES, AIDS MALIGNANCY PROGRAM**

### **Office of the Director, Division of Cancer Treatment and Diagnosis**

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The AIDS Malignancies Program (AMP), Office of the Director (OD), [Division of Cancer Treatment \(DCTD\)](#) is working to facilitate research on AIDS malignancies in the international arena. Both the [AIDS-Associated Malignancies Clinical Trials Consortium \(AMC\)](#) and the [AIDS and Cancer Specimen Resource \(ACSR\)](#) are participating in this endeavor (see the OD, DCTD section under the Extramural Activities tab for a description of the AMC and ACSR).

The DCTD AMP funds a clinical trial in Africa to treat AIDS-related non-Hodgkin's lymphoma (NHL) using an oral based combination chemotherapeutic regime. RO1CA853528 was awarded to Dr. Scot Remick of Case Western Reserve University, and African colleagues Dr. Edward Mbidde (Uganda) and Dr. Otieno Mwanda (Kenya) to conduct this multi-center trial. Patient accrual is ongoing and expected to be completed in the next year.

Supplemental funding was awarded to the AMC to conduct two international studies. The aim of the first project entitled "T-cell Response to KSHV in Mother to Child Transmission" is to analyze the immunologic and virologic aspects of human herpesvirus 8/Kaposi's sarcoma-associated herpesvirus (HHV-8/KSHV) infection in infants. The study is a joint effort between the AMC and investigators at the University of Natal in Durban, and the National Institute for Virology in Johannesburg, South Africa. Using samples collected from an existing cohort of mother-child pairs, they will investigate whether HHV-8/KSHV viral load and concurrent HIV infection in the mother are predictors of HHV-8/KSHV transmission and the immune response of the infant infected with this herpesvirus.

The goal of the second study entitled "International Infrastructure" is to develop international collaborative efforts between the AMC and areas of the world severely affected by HIV disease including Brazil, South Africa, Zambia, and Zimbabwe. This project will test the feasibility of establishing international sites in resource poor areas for the conducting of clinical trials of AIDS-related malignancies. It will also provide paradigms for infrastructure and training needs and establish teams of clinical investigators in these countries.

The AMP continues to partner with the [Fogarty International Center \(FIC\)](#) on the [AIDS International Training and Research Program \(AITRP\)](#). This initiative was re-issued in 2003 and the announcement can be viewed at <http://grants1.nih.gov/grants/guide/pa-files/PA-03-018.html>. The AITRP supports HIV/AIDS international training and research for foreign health scientists, clinicians, and allied health workers from developing countries and emerging democracies. The primary goal of this program is to build biomedical and behavioral research capacity for the prevention of HIV/AIDS. Rates of HIV-associated malignancies have increased in developing countries as a result of the HIV epidemic, thus the AMP is helping to strengthen the capacity for research in AIDS-related malignancies in these areas. Investigators from Johns Hopkins University, University of Washington, Case Western Reserve University, and the University of California at Los Angeles received supplements to build capacity for AIDS-related malignancy research including KS, NHL, and human papillomavirus (HPV)-related anogenital disease in India, Uganda, Brazil, Kenya, Malawi, and Thailand as follows:

- Foster the development of a Center for Excellence in HHV-8/KSHV research in Kenya;
- Build capacity for research and clinical trials in HIV-associated malignancies (KS and AIDS-related lymphomas) of health-care professionals in Uganda;
- Train investigators in the diagnosis, treatment, and prevention of cervical cancer in Uganda;
- Train investigators in the detection, early treatment, and prevention of cervical carcinoma in India and in evaluating the effects of HIV on HPV acquisition, persistence, and risk for cervical carcinoma;
- Provide research-training opportunities in AIDS-associated malignancies to investigators to enhance their ability to conduct research in Brazil.

The AMP is also partnering with FIC on the [International Clinical, Operational, and Health Services Research Training Award for AIDS and Tuberculosis Program \(ICHORTA-AIDS/TB\)](#). The purpose of the ICHORTA program is to foster collaborative, multidisciplinary research in developing

country sites where AIDS, TB, or both are significant problems. This first phase of the ICOHRTA program consists of one-year planning grants to the foreign site to provide support to organize, plan for, prepare, and assemble an application for a Comprehensive ICOHRTA cooperative agreement (Phase II). Partnering with domestic institutions with recognized HIV expertise is required. The overall goal is to build capacity for integrated clinical, operations, and health services research encompassing the full range of conditions and issues related to AIDS, including malignancy. The following proposals chosen for cofunding had relevance to current program studies and interests, and established geographic diversity in areas with endemic virus-associated malignancies.

- Investigators from the Federal University of Bahia propose to partner with collaborators at Cornell University, the University of Miami, and the University of Nebraska to develop a Phase II application with an emphasis on viral related malignancies in the context of HIV in Bahia, Brazil. The US collaborating investigators are currently funded by NCI to conduct epidemiologic, basic, and clinical research in viral malignancies in the context of HIV infection. Participation in ICOHRTA will build an additional bridge between the foreign and US sites for research and treatment studies on malignancies associated with endemic viruses in an area with high HIV prevalence.
- Investigators from the University of Natal propose to develop a Phase II application with investigators at Columbia University. The AMC is interested in expanding into this region and is currently funded to conduct a clinical trials feasibility study and a study to measure the cellular immune response to HHV-8 as part of a mother-to-child transmission study in South Africa. AMC investigators have proposed conducting a trial in this region using HAART as a treatment for KS, where 80% of the KS patients are HIV positive. Additional clinical trials capacity building at the University of Natal, Durban, will facilitate future interactions with the NCI cooperative groups.
- Investigators involved with the Joint Clinical Research Center, Kampala, Uganda, propose to collaborate with investigators at Case Western Reserve University. Results of a successful Phase 2 application will build additional bridges between JCRC and CWRU for ongoing clinical studies, facilitate the ACSR tissue-banking endeavor, and facilitate NCI expansion into this region.
- Investigators from the Instituto de Medicina Tropical, Lima, Peru, proposes to develop a comprehensive Phase 2 application in partnership with investigators at the University of Alabama. An aim of this application is a systematic evaluation to determine the extent of HIV seroprevalence in Lima with an emphasis on coinfection with the endemic virus HTLV-1. The unique problem of HTLV-1 coinfection and development of ATLL is an important factor to study relevant to HIV.
- Investigators from the All India Institute of Medical Services (AIIMS), New Delhi, plan to develop a Phase II application in collaboration with investigators at the University of California at Los Angeles. The AIIMS is a well-established institution with extensive clinical facilities. HPV-associated cervical cancer is the leading cause of cancer death in women in this region. There are 3.8 million HIV-infected persons living in India.

Since 1997, the NCI has presented an annual conference on malignancies in AIDS and other immunodeficiencies. The objectives of this international conference are to showcase progress and to stimulate research across diverse disciplines. In 2003, the AMP, DCTD sponsored the [7th International Conference on Malignancies in AIDS and Other Immunodeficiencies \(ICMAOI\)](#). Summaries from the 2002 ICMAOI were made available for CME credit on Medscape, <http://www.medscape.com/cmecenterdirectory/hiv>.

**Total Number of Funded AIDS-related International Grants and/or Contracts in OD DCTD FY 2002:**  
13, 100% AIDS [(10) D43, (2) UO1 supplements, (1) RO1]

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## CLINICAL GRANTS AND CONTRACTS BRANCH

### Cancer Therapy Evaluation Program, Division of Cancer Treatment and Diagnosis

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#### **Broad Research Questions Currently Under Study**

- Maintaining a database on pretransplant HIV status of allogeneic and autologous bone marrow transplant (BMT) patients and the clinical outcomes of HIV+ bone marrow transplant patients
- Developing treatment regimens against viral infections
- Developing treatment regimens against fungal infections
- Developing treatment regimens for AIDS-related malignancies

#### **Important Study Findings/Highlights**

Dr. Mary Horowitz, principal investigator of a resource cooperative agreement (5U24CA76518-04) has been maintaining a database on pretransplant HIV status of allogeneic and autologous BMT patients and the clinical outcomes of HIV+ BMT patients. Seventy-nine patients out of 42,904 allograft recipients were HIV+. Twenty-seven patients out of 21,628 autologous graft recipients were HIV+. Five HIV+ patients were transplanted for their HIV disease (no new additional cases reported).

Several projects in Dr. Fred Appelbaum's program project (5P01CA18029-28) have AIDS relevancy. Project 5 headed by Dr. Lawrence Corey has made substantial progress in all the specific aims related to infectious complications post-transplantation (CMV, aspergillosis). In specific aim 1, a randomized double-blind multicenter trial of valganciclovir vs. placebo for the prevention of late CMV infection has been started in five centers (Fred Hutchinson Cancer Research Center, University of Florida, Mayo Clinic, Duke University, Memorial Sloan-Kettering Cancer Center, City of Hope National Medical Center). Thirty-six patients have been randomized with no major problems. Samples are being collected and stored for measuring CMV-specific immune responses and ganciclovir resistance. This randomized trial will demonstrate if the drug is effective and if long-term toxicity is manageable. Data will be obtained on the development of resistance with long-term prophylaxis on the impact of CMV-specific immune reconstitution. In specific aim 2, efforts were directed toward standardization of the Aspergillus-specific T cell assay (intracellular cytokine method). To date, CD4 T cell responses to a variety of Aspergillus cellular antigens have been tested and results have suggested that hyphal products or live conidial inocula stimulate the strongest Th1-type CD4 response. A small pilot study was initiated to determine variability of responses using the intracellular cytokine method. Responses of five healthy volunteers to hyphal and conidial antigens were measured and were highly variable (ranging from 0.05% to 0.77%) but responses to either antigen formulation were consistently "positive" or "negative." A large study of responses in healthy volunteers, and a longitudinal study in human stem cell transplant recipients are in development. Aim 2 will provide important data on the significance of aspergillus-specific T-cell immunity in the control of post-transplant aspergillosis. In specific aim 3, a protocol for a randomized double-blind multicenter trial of valacyclovir vs. placebo has been developed to examine whether prevention of primary CMV with valacyclovir among D+/R- human stem-cell transplant recipients will reduce the subsequent development of invasive bacterial and fungal infections. All assays for the laboratory correlative studies are in place and operative (quantitative plasma PCR for CMV DNA and the aspergillus galactomannan assay). This randomized trial of valacyclovir in patients at risk for primary CMV infection is aimed at examining the questions of whether indirect effects of CMV occur and whether these effects can be prevented by antiviral prophylaxis. Core C of the program project supports an infectious disease research database of transplant patients.

Several AIDS-related studies have been completed or are on-going. A retrospective cohort study of risk factors and the impact of adenovirus infections on survival in more than 5,000 patients over a 20-year period showed that adenovirus infection is increasing and is associated with poor survival in non-T cell depleted patients, and that ganciclovir given for CMV prevention has a protective effect. Ongoing studies include: 1) a retrospective study of risk factors of invasive aspergillosis in myeloablative transplant recipients, 2) a retrospective study of risk factors of invasive bacterial and fungal infections in non-myeloablative transplant recipients, 3) a retrospective analysis of the impact of pre-transplant

aspergillosis on post-transplant outcome, 4) a retrospective study of the impact of donor HSV serology on post-transplant HSV infection and disease, and acyclovir resistance, and 5) studies of factors determining reconstitution of CMV-specific immunity.

The [Blood and Marrow Transplant Clinical Trials Network](#), co-funded by the NCI and National Heart, Lung and Blood Institute (NHLBI) on September 30, 2001, has activated the first phase III clinical trial of the Network entitled “A Randomized Double-blind Trial of Fluconazole vs. Voriconazole for the Prevention of Invasive Fungal Infections in Allogeneic Blood and Marrow Transplant Patients.” The protocol development team involved the staff of the NCI, NHLBI, and National Institute of Allergy and Infectious Disease (NIAID), principle investigators from 16 Network clinical centers, staff from the Central Operations Office of the Network, a patient advocate, and a representative from the transplant investigators-at-large (not an investigator associated with any clinical center of the Network). This trial will not only determine which of the two drugs made by Pfizer is more efficacious, but will be the pivotal licensing trial for a clinical assay for *Aspergillus* (galactomannan assay) developed by BioRad Laboratories. A total of 600 patients will be accrued (300 to each arm) over three years. The primary objective is to compare the fungal-free survival rates between the two study arms. The secondary objectives are to compare the frequency of invasive fungal infection, time to invasive fungal infection, survival rate, duration of amphotericin B therapy for possible invasive fungal infection, time to neutrophil and platelet engraftment, time to and severity of acute and chronic GVHD, and utility of the galactomannan assay in detection of *Aspergillus*. The relative safety of the two antifungals will also be assessed through the collection of adverse events and routine laboratory monitoring. The multi-disciplinary nature of this trial involving three NIH institutes demonstrates the collaborative nature of the study and the coordination required to manage this Network.

Vaccination using CTL epitopes is a widely used immunization strategy. Dr. Donald Diamond (5R01CA77544-05) has shown that two different CTL epitopes from CMV-pp65 (HLA A2.1 or HLA A11) and three alternative T-help epitopes can be combined to form different fusion peptide vaccines. The fusion peptides, when administered intranasally with CpG single-stranded DNA, caused a powerful systemic response against CMV antigens in HLA transgenic mice. Dr. Diamond has successfully applied to the Rapid Access to Intervention Development mechanism of the DTP/NCI to manufacture GMP lots of vaccine peptides. Once the vaccine peptides are manufactured and available for clinical use, a Phase I safety trial will be initiated to establish safe dose levels in CMV-seropositive and CMV-seronegative subjects. He also has developed a new technology of enumerating CD8 lymphocytes with HLA tetramers. This HLA tetramer technique can be used to monitor the effectiveness of immunizations. As Project 3 Leader of Dr. Steve Forman’s P01 grant of (5P01CA30206-21), Dr. Diamond studied CD8+ T cell responses to three human CMV pp65 epitopes in panels of healthy seropositive HLA-A\*02/HLA-B\*07 individuals, and HLA-A\*02 donors mismatched for HLA-B\*07. The majority of the latter had significant responses to an HLA-A\*02-restricted epitope within the CMV pp65 antigen. By contrast, the strongest responses to CMV in the first group were to HLA-B\*07-restricted epitopes. Similar immunodominance of HLA-B\*07 over HLA-A\*02 was found in two immunocompromised HIV-infected patients, and in the reconstituting immune system of a stem cell transplant recipient. *In vitro* stimulation of PBMC from two immunocompetent HLA-A\*02/HLA-B\*07 individuals indicated that CTL precursors specific for both HLA-A\*02- and HLA-B\*07-restricted epitopes were present and could be expanded by stimulation with a cognate peptide. However, if stimulation was performed by antigen presenting cells infected with recombinant vaccinia, expressing full-length native CMV pp65, only HLA-B\*07 epitope-specific cells were seen. In one case, the HLA-B\*07 dominance was partially broken by using recombinant vaccinia expressing ubiquitinated CMV pp65, suggesting that enhanced protein processing can reveal weaker immune responses. These results indicate that CMV-specific cellular immune responses restricted by HLA-B\*07 dominate those restricted by HLA-A\*02 in both immunocompetent and immunocompromised individuals.

Several other projects in the Forman P01 involve the treatment of lymphoma in HIV- and HIV+ patients. A Phase I/II study of high dose radioimmunotherapy with Zevalin in combination with high-dose VP-16 and cyclophosphamide was completed. Eighteen patients with follicular lymphoma diffuse large-cell lymphoma and mantle cell lymphoma were treated with a median dose of Zevalin delivered with 74.9 millicuries and a median CD34 dose infused with 7.2 million cells/kilogram. The treatment was well tolerated. The most common acute toxicities were mucositis, neutropenic fever, and skin rash. All patients engrafted and the median time to reach ANC was 10 days and 18 days for platelets. Both one-year estimated overall survival and disease-free survival were 92%. The initial impressions of this trial are that high-dose Zevalin can be given safely in combination with high-dose chemotherapy and that the addition of high-dose Zevalin to high-dose VP-16/Cyclophosphamide and autologous stem cell support does not increase transplant-related toxicity nor does it delay engraftment. This regimen appears to be active in heavily pretreated patients with refractory B-cell non-Hodgkin's lymphoma. Fifteen HIV-related lymphoma patients at the median age of 43 years were treated by transplantation. All patients engrafted and the median follow-up for surviving patients is 24 months. The two-year probability of overall survival and event-free survival is 79% suggesting that transplant is well tolerated in patients with HIV lymphoma and has no apparent deleterious effect on the underlying HIV infection. With longer follow-up, the procedure appears to offer the possibility for durable remission and should be considered for patients who have relapsed disease as opposed to conventional salvage regimens. Dr. K.K. Wong, in Project 4 of the Forman P01, plans to utilize AAV to transduce the hematopoietic stem cells utilized for transplant of HIV lymphoma patients with anti-sense constructs that convey resistance to infection. The master cell bank for AAV has been obtained. Clinical grade virus vector will be produced for transduction in 2003.

**Total Number of Funded AIDS-related Grants and/or Contracts in CTEP**

FY 2002: 47 grants and cooperative agreements (R01, R21, R37, R42, P01, U01, U24) with 3, 100% AIDS relevancy.

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## **GRANTS AND CONTRACTS OPERATIONS BRANCH**

### **Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis**

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The [Grants and Contracts Operations Branch \(GCOB\)](#) is part of the [Developmental Therapeutics Program \(DTP\)](#), [Division of Cancer Treatment and Diagnosis](#). DTP's mission is to 1) promote research leading to novel discoveries and their translation into effective therapies for cancer and HIV/AIDS and 2) support a broad contract-based preclinical drug development program to facilitate the introduction of novel therapies into clinical trial. DTP contracts typically include collections of natural products worldwide, drug synthesis, production of laboratory- and clinical-grade drugs and biologics, formulation, and toxicology on new agents under consideration for clinical trials. GCOB's role is to maintain a large portfolio of grants and cooperative agreements covering all aspects of preclinical cancer drug discovery and development and to coordinate contract competitions that support the drug development activities.

#### **Contract-Based and Other Assistance to Extramural Investigators**

With advice from extramural advisory committees, DTP has created programs to assist the academic community with drug discovery activities. In brief, DTP has upgraded its web site at <http://dtp.nci.nih.gov/> to provide more information on available resources and services, such as access to synthetic and natural products repositories, reference reagents, chemical libraries, laboratory animals, cell lines, cytotoxicity data on diverse compounds in a 60-cell line panel of cancer cells, AIDS screening data in a cell-based assay, screening services in cancer, AIDS, and opportunistic infections, chemical structures, molecular target data, analysis tools, such as the COMPARE program, and most recently, *in vivo* antitumor data on many marketed and experimental compounds.

[Rapid Access to Intervention Development \(RAID\)](#), an assistance program, is helping extramural academic investigators develop promising agents for the treatment of cancer and AIDS-associated malignancies. RAID accepts applications from the extramural community twice per year. Applications are peer-reviewed by academic investigators, and DTP contract resources are used to complete tasks, such as large animal toxicology studies.

NCI and NIAID created a special assistance mechanism, [NIH Inter-Institute Program for the Development of AIDS-Related Therapeutics](#), to assist the HIV/AIDS research community by reducing barriers to the discovery and development of new products to clinical trial. In contrast to the RAID program, this program offers assistance to investigators from either academia or small businesses, and will support discovery as well as development activities. This program accepts applications from the extramural community twice each year. The applications are peer-reviewed, and successful applicants receive requested services that are supported by NCI and NIAID contracts. For example, production of clinical-grade material can be arranged through this mechanism at no charge to the applicant.

In addition to providing more research services, DTP has re-vitalized its drug discovery programs in both cancer and HIV/AIDS to emphasize molecular targets, especially those that take advantage of particular vulnerabilities, including targets important in disease pathogenesis. More information on these new directions in cancer drug discovery can be found in the [NCI ByPass Budget request to Congress](#). It is also expected that future clinical trials will include more imaging or biochemical studies to determine if intended targets are modulated. Increased knowledge about cancer and other diseases has contributed an abundance of new targets for drug action. This information, coupled with the use of new technologies, provides the underpinnings of a new era in drug discovery. To assist investigators in these endeavors, GCOB supports several funding opportunities in molecular target drug discovery for cancer, which can be found at the [DTP web site](#). The initiatives, which focus on the identification, characterization, and validation of potential targets for therapeutic intervention, use a variety of funding mechanisms, such as cooperative agreements (<http://grants.nih.gov/grants/guide/rfa-files/RFA-CA-00-002.html>), exploratory grants, small business grants, and competing supplements to existing grants. Program Announcements for exploratory grants and competing supplements were reissued in 2001 and awards were made in 2002. The last planned competition is ongoing for additional exploratory grants and small business grants with awards planned for late 2003. Once awards are made, abstracts for each award are posted on the DTP web site at [http://dtp.nci.nih.gov/branches/gcob/gcob\\_web9.html](http://dtp.nci.nih.gov/branches/gcob/gcob_web9.html) to foster collaborations and communication about potential drug targets, which may have application to more than one disease.

## **Grant Initiatives**

GCOB sponsors a number of [initiatives](#) that can be used effectively by investigators in the HIV/AIDS malignancy community as well as others. Because many of the most innovative ideas for new therapies are found in the small business community, GCOB reissued the [Flexible System to Advance Innovative Research for Cancer Drug Discovery by Small Businesses” \(FLAIR\)](#) Program Announcement on February 25, 2003 called with receipt dates of July 14 and November 14, 2003. GCOB reissued two program announcements in 2002 for innovative toxicology models that promote use of new technologies that will allow faster and cheaper data collection on organ toxicities and a decrease in animal use: [Innovative Toxicology Models for Drug Evaluation: Exploratory/Developmental Grants and Phased Innovation Award](#) and [Innovative Toxicology Models: SBIR/STTR](#) both with receipt dates of April 23 and December 23, 2003.

In addition to grant initiatives for single projects, GCOB also supports multidisciplinary or team science. During 2003, GCOB will reissue a Request for Applications to continue the National Cooperative Drug Discovery Group (NCDDG) program that involves partnerships between academia, industry and government to create new therapies. Recently, DTP received approval to compete the [Academic Public Private Partnership Program \(AP4\)](#), a new partnership arrangement will require funding from both government and industrial sources. However, the principal investigator must be from an academic institution. A competition for planning grants for this new endeavor will take place in 2003. GCOB also has contributed to the funding of six [International Cooperative Biodiversity Groups \(ICBGs\)](#) to address issues of biodiversity conservation, economic growth, and human health through discovery of therapeutic agents for cancer, infectious diseases including HIV/AIDS, mental disorders, and diseases of primary concern to developing countries, such as parasitic diseases and tuberculosis. The ICBG program is a unique interagency initiative led by the Fogarty International Center with support from several institutes of the NIH, including NCI, as well as the National Science Foundation and the United States Department of Agriculture. During 2003 this effort is undergoing competition with awards expected by the end of the year.

## **Grant Management Changes**

During 2002, GCOB worked closely with Dr. Jodi Black in the Office of the Director, DCTD, to transfer grants involving AIDS-associated malignancies, opportunistic infections, and HIV/AIDS from GCOB to the Office of the Director. This transfer, which involved all AIDS-related grants except for those focused exclusively on chemistry, consolidated the DCTD research portfolio in one office for better coordination and “one-stop” shopping for the grantee community. Dr. Black’s office maintains close ties with the National Institute of Allergy and Infectious Diseases (NIAID) in the coordination of all AIDS-related activities. Based on traditional National Institutes of Health (NIH) grant referral guidelines, her office focuses primarily on the discovery of new therapies and approaches to treat AIDS-associated malignancies while NIAID specializes in research on HIV/AIDS and opportunistic infections. Dr. Black also assumed responsibility for two grant initiatives formerly managed by GCOB. The first included [Models for HIV Disease and AIDS-Related Malignancies](#) which was released on May 16, 2001 in collaboration with the NCI Division of Cancer Biology to encourage development of models that will foster the evaluation of different paradigms of disease onset and progression and the discovery of new treatments. The PA was revised from earlier versions to allow R01 applications for more mature projects and R21 applications for exploratory/developmental studies that lack sufficient preliminary data to be competitive as R01s. The second, [Therapeutics Research on AIDS-Associated Opportunistic Infections and Malignancies](#), was issued jointly by NIAID, NCI, and the National Institute of Dental and Craniofacial Research on June 28, 2001. This solicitation encourages the use of state-of-the-art technologies and molecular targeted approaches for the discovery of new therapies.

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## SCREENING TECHNOLOGIES BRANCH

### Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis

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Research sponsored by the Branch is directed towards the discovery and development of new agents with the potential for treatment of patients with cancer, AIDS, and opportunistic infections. Various drug screening models are employed to identify and characterize novel therapeutic agents from synthetic chemical and natural product libraries.

In recent years, AIDS-related efforts have focused primarily on the identification of anti-HIV-1 agents using a cell-based primary screen. Current anti-HIV-1 research is directed towards the characterization and exploitation of molecular targets unique to this virus. New initiatives in the areas of AIDS associated malignancies and opportunistic infections are currently being formulated. The rapidly expanding research literature regarding viral involvement in AIDS-associated malignancies suggests novel molecular targets for drug discovery that may be addressed through screening or molecular modeling and drug design.

#### Important Study Findings/Highlights

Many structural classes of anti-HIV compounds previously unrecognized as antiviral agents have been discovered using the cell-based screen. Detailed preclinical studies addressing formulation, pharmacokinetics, and toxicology have been pursued for promising compounds. The cell-based screen was instrumental in the discovery of two Food and Drug Administration approved drugs, 3TC and the carbovir prodrug Ziagen. Based on recommendations of external Scientific Review Committees, the NCI has discontinued use of the cell-based model for large-scale drug screening. The National Institute of Allergy and Infectious Diseases (NIAID) currently offers screening and mechanistic follow-up as a service to the research community (<http://www.niaid.nih.gov/d aids/PDATguide/HIVThera.htm>).

In January 2001 a new program designed to make the drug discovery and development resources of the NCI and NIAID available to the extramural research community in a coordinated way was introduced. The [NIH Inter-Institute Pilot Program for AIDS-Related Therapeutics](#) receives applications twice per year that are peer-reviewed. Successful applicants receive access to NIH resources intended to support investigator development of therapeutic concepts towards clinical trial. The Branch has concluded a research project in natural product drug discovery for cancer and AIDS which was enabled by a successful application to the Inter-Institute Program. More recently, two peer-approved projects involving drug screening/discovery are in the early stages of implementation, and in one case may add valuable mechanistic information concerning historical NCI AIDS screening data.

Ongoing efforts include: development of high throughput screening (HTS) methods which would allow rapid screening of chemical libraries, the development of multidrug resistant HIV which is compatible with HTS which may provide relevant drug development leads for the future. Progress has been marked in the development of a HTS assay for HHV-8 polymerase (Dorjsuren et al. Protein Expr Purif 29(1): 42-50 (2003). Preliminary work of screening the NCI diversity set chemical library using *in vitro* translated HHV-8 polymerase in collaboration with Dr. R. Ricciardi (U. Penn) has been completed. Future plans involve the screening of larger chemical libraries. A CRADA with Phytobiotech Inc. has been implemented in which the company will provide novel plant-derived natural products for screening of new drug leads for cancer and AIDS related screens.

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**LABORATORY OF ANTIVIRAL DRUG MECHANISMS**  
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**Treatment and Diagnosis**

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The [Laboratory of Antiviral Drug Mechanisms \(LADM\)](#) concentrates on three areas of research: 1) identification of novel molecular targets, 2) development of high-throughput screening (HTS) assays, and 3) discovery of novel therapeutics, which are relevant to AIDS and AIDS-associated malignancies. One of our main focuses has been to facilitate the discovery of novel inhibitors of human herpesvirus 8 (HHV8), a causative agent for Kaposi's sarcoma and a subset of AIDS-associated lymphoma, through molecular target-based screening and secondary characterization assays. We recently established a microplate-based HHV8 polymerase (Pol) and processivity factor (PF) DNA synthesis assay (HHV8 Pol/PF assay) in collaboration with Dr. Robert Ricciardi of the University of Pennsylvania. We screened the NCI Diversity Set and identified several chemotypes of compounds that significantly blocked DNA synthesis activity of HHV8 Pol/PF. Secondary screening of these active compounds is currently underway in a cell-based assay system for lead optimization. In addition, automation of the HHV8 Pol/PF assay is currently being explored for future HTS campaigns. We have also uncovered anti-HHV8 activity of a new thymidine analog, *n*-methanocarbothymidine (N-MCT), using HHV8-infected BCBL-1 cell-based assay. Mechanistic characterization of N-MCT activity is ongoing in collaboration with Dr. Victor Marquez, NCI. Other HHV8-targeted projects include establishing HTS assays for the identification of inhibitors of other viral molecular targets, such as HHV8 helicase/primase and latency-associated nuclear antigen.

The LADM also contributes to many facets of anti-HIV therapeutics development. The lab screens compounds submitted by intramural and extramural investigators for anti-HIV activities, using conventional cell-based assays. Selected compounds are further evaluated for their ability to inhibit replication of various HIV isolates in primary and laboratory-established cell line-based assays, specifically designed to elucidate the distinct molecular targeting sites: virus attachment, fusion, reverse transcriptase, integrase, protease (PR), nucleocapsid protein, virus assembly, and budding. Information obtained from the mechanistic characterization assays determines if given compounds are to be pursued for further development. Another ongoing effort is the development of a cell-based HTS assay, using specific HIV-1 strains known to be resistant to many conventional inhibitors of reverse transcriptase (RT) and PR (anti-MDR-HIV screen), in order to find anti-HIV agents that act through novel mechanisms other than inhibition of viral RT or PR. We have identified suitable MDR strains that are highly resistant to multiple RT or PR inhibitors while maintaining high degree of replication fitness. Optimization of anti-MDR-HIV for HTS is actively pursued at this time. In summary, the LADM conducts an array of virological studies with a major focus on the discovery of novel molecular targets and development of new therapeutic agents for AIDS and AIDS-associated malignancies.

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**AIDS VACCINE PROGRAM**  
**Center for Cancer Research (Contract)**

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The AIDS Vaccine Program (AVP) pursues an integrated, multidisciplinary program of basic and applied studies aimed at the development of an effective vaccine for the prevention of HIV infection and AIDS. Researchers in the AVP have identified the Zn<sup>2+</sup> finger motifs of retroviral nucleocapsid (NC) proteins as conserved sequence elements, and defined key roles played by NC at multiple different steps of the viral replication cycle, including packaging of viral genomic RNA, various stages of reverse transcription, and integration. AVP scientists have exploited these observations to develop a novel approach for chemical inactivation of retroviruses, by selectively targeting free sulfhydryl groups on the cysteines of NC and other internal virion proteins, leading to non-infectious whole inactivated virion vaccine immunogens with immunologically intact and functional envelope glycoproteins. Such inactivated virions appear to have numerous desirable properties for inducing both antibody and cellular immune responses and are being evaluated in animal models, including challenge studies in non-human primates. The inactivated virions are also proving to be a valuable reagent for in vitro studies. AVP scientists are actively exploring approaches to modify the quantitative and qualitative characteristics of the envelope glycoproteins on inactivated virions, to produce improved vaccine candidates based on this inactivation strategy. Other AVP scientists have pioneered approaches for the analysis of cellular proteins associated with retroviruses and factors influencing their incorporation into virions, and are contributing importantly to the emerging understanding of the role of proteasome processing in retroviral assembly and budding. Additional efforts involve the observational and interventional studies of virus/host dynamics in non-human models of AIDS, to better understand the factors leading to sustained effective control of AIDS virus infection.

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### **Laboratory of Retroviral Pathogenesis**

The Laboratory of Retroviral Pathogenesis studies viral and host determinants involved in the pathogenesis of primate lentiviral infection and conducts evaluations of experimental therapies and candidate vaccines for the treatment and prevention of primate lentiviral infection and AIDS. Both prophylactic and therapeutic vaccination approaches are studied. Much of the work in the laboratory emphasizes the use of non-human primate models, with cutting edge quantitative analysis of virologic and immunologic parameters. Whole inactivated HIV-1 and SIV virions with conformationally and functionally intact surface proteins, prepared by a novel approach, are being evaluated as a vaccine immunogen and in a variety of in vitro assay systems.

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### **Lentiviral Vectors for Gene Transfer in Gene Therapy AND Functional Genomics**

With rapid progress in human genomics and the ability of the lentiviral vectors to transfer genes into dividing and non-dividing cells, lentiviral vectors will play an important role in gene therapy and gene function elucidation. These vectors will be particularly useful for stem cell genetic modification. We have designed novel HIV-2 vectors in parallel with HIV-1 vectors. The HIV-2 vectors may have certain advantages over previously designed HIV-1 vectors, including their attenuated pathogenicity. For pre-clinical validation, we have designed vectors with a number of model genes representing different disease entities, target cell specificities, and biological processes. These include aromatic amino decarboxylase acid (AADC) gene for neurotransmitter deficiency in Parkinson's disease, alpha-glycosidase A gene in the inborn error of metabolism in Fabry disease, and BAX gene for apoptosis in neoplasia. The AADC and AGA vectors successfully transduced relevant target cells *in vitro*. For example, AADC vectors not only transduced primary neuronal cells in culture but also corrected functional defects in the rat model of Parkinson's disease. These results encourage the idea that gene therapy approaches may also be useful in AIDS-associated malignancies, by themselves or in combination with HAART. For example, with a clearer understanding of the role of KSHV genes in angiogenesis and Kaposi's sarcoma, gene therapy approaches will become feasible.

The attractiveness of the lentiviral vectors will be compelling if the issues of regulation and safety can be satisfactorily addressed. For regulation, and to acquire the ability to modulate vectors from outside, it will be useful to obtain small "drug-like" molecules that affect vector expression. We have designed "response vectors" that can be used in cell-based assays to screen in high throughput formats chemical and natural products libraries to obtain "lead" compounds that regulate HIV-1 and HIV-2 vector expression. As a proof of principle, we will screen libraries for molecules that mimic Tat function, that is, activate LTR-directed transgene expression. The "response vectors" carry the indicator GFP gene under the control of the viral LTR and they also carry the IRES-puroR cassette for cell selection. A number of cell lines have been screened for cell-based assay to meet the following criteria: stable maintenance of the transgene cassettes, low basal LTR activity, tat responsiveness, and deficient MDR function. As a control to account for generic transcription factor modulation, vectors with human TERT promoter for parallel screening have been created. Any compounds modulating the activity of this promoter will be useful in negative regulators for cancer, including AIDS-associated malignancies, and positive regulators for aging.

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### **Genetic Immunization in AIDS**

Prime-boost strategy holds promise for immunization against HIV infection, AIDS, and associated diseases. We are working on the premise that it is advantageous to present as many of the HIV antigens as possible as a packet to constitute a constellation of epitopes. Thus, we have created replication-defective HIV-1s and SIVs which can be administered as DNA or as *ex vivo* packaged particles. The former includes packaging signal-defective proviruses and the later include integrase-negative particles. *Ex vivo* transduction of mouse and rhesus dendritic cells with the replication-defective, integrase-minus HIV-1 and SHIV molecular clones apparently induce HIV-1-specific CTL response. In monkeys, subcutaneous injected genetically modified dendritic cells migrate into the draining lymph nodes, express viral DNA, and present viral epitopes to induce vigorous HIV-specific effector CTLs, which later develop into a memory CTL response. Interestingly, antibodies did not appear in these animals, suggesting that the genetically modified cells may induce a pure Th1 response. Multiplying disabled HIV, containing multiple genes and thus potentially able to present numerous epitopes, may be useful to enhance the breadth and strength of the immune response.

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## **The SIV Macaque Model for AIDS Vaccine Development and Pathogenesis**

### **Background**

The infection of macaques by simian immunodeficiency virus (SIV) results in many of the characteristics of HIV infection and progression to AIDS in humans, including loss of CD4<sup>+</sup> T cells and susceptibility to opportunistic infections. In this animal model, individuals can be inoculated at a specific time, by defined routes, and with known amounts of molecularly characterized virus isolates. Frequent specimens can also be obtained, providing a good model for evaluating the role of viral and host factors in the progression to disease. This model has therefore been extensively used in attempts to develop an effective vaccine to prevent AIDS in macaques as well as to test antiviral compounds for their ability to prevent infection or decrease the viral load in infected primates, as a model to prevent and control HIV infection in humans.

The virus we have used was isolated from a pig-tailed macaque (*Macaca nemestrina*) that died with lymphoblastic lymphoma in 1982, and is named SIV/Mne. The original isolate, obtained on HuT 78 cells, is moderately pathogenic in three species of macaques (rhesus (*M. mulatta*), cynomolgus macaques (*M. fascicularis*), and pig-tailed macaques). When inoculated intravenously or mucosally, this isolate causes CD4<sup>+</sup> cell depletion in approximately 8 to 26 months, depending on species of macaque. A single cell clone of infected HuT 78 cells derived from the original culture, CL E11S, and a molecular clone (CL 8, obtained from CL E11S) have also been used for a variety of pathogenesis and vaccine studies. SIV/Mne CL E11S is less rapidly pathogenic than the uncloned virus. SIV/Mne and its single cell and molecular clones have been used for vaccine, antiviral and pathogenesis studies by various investigators at the Washington National Primate Research Center (Drs. Julie Overbaugh, Shiu-Lok Hu, Che-Chung Tsai, William Morton), by the AIDS Vaccine Program at NCI-Frederick (Drs. Jeffrey Lifson, Larry Arthur, Louis Henderson, Robert Gorelick, Elena Chertova), at NIH (Dr. G. Franchini), at the Henry Jackson Foundation (Dr. Mark Lewis), and by Dr. Norman Letvin (Beth Israel Hospital, Boston, MA) and Dr. Nancy Haigwood (Seattle Biomedical Research Institute, Seattle, WA).

Our principal vaccine approaches have focused on the use of recombinant vaccinia virus expressing different regions of SIV/Mne, DNA vaccines, and inactivated whole virus particle vaccines.

### **Vaccinia Virus Recombinant AIDS Vaccines**

Our infectious and pathogenic molecular clone of SIV/Mne has been used to construct various recombinant vaccinia viruses that express different regions of the virus. We previously reported that envelope (gp160)-based vaccines, when used in a recombinant vaccinia virus priming and subunit protein boosting regimen, protected almost all macaques from infection by the cloned homologous virus. The breadth of this immunity, however, appears to be limited, since approximately half of the immunized animals were infected after challenge by the uncloned virus SIV/Mne. In order to identify the regions of the virus needed for increased protection against a heterologous challenge, we designed a study in which various regions of SIV were systematically added, as recombinant vaccinia constructs, to the envelope-based vaccine regimen.

These studies indicate that the inclusion of the transmembrane protein in the envelope-based vaccine is essential in order to obtain protection. In addition, the presence of both envelope and core antigens were found to now protect against a heterologous virus challenge, indicating that responses to core antigens contributed to the broadening of protective immunity. Our results argue for the inclusion of multiple antigenic targets in the design of recombinant vaccines against AIDS, and should be directly applicable to designing vaccines to protect humans against HIV.

### **Inactivated SIV with Functionally Intact Virion Surface Proteins as Vaccines**

SIV was inactivated by incubation with aldrithio-2 (AT-2), which covalently modifies the nucleocapsid protein (p8<sup>NC</sup>). The resulting virus is non-infectious and maintains conformationally and functionally intact virion surface proteins. Pig-tailed macaques were immunized with AT-2 inactivated CL E11S, leading to both cellular and humoral immune responses, and then challenged with CL E11S virus grown in pig-tailed macaque peripheral blood lymphocytes (PBMC). All control macaques became infected; the majority of the immunized macaques did not show measurable SIV RNA in plasma, and virus isolation and detection of viral sequences by PCR was only intermittent, compared to controls. These encouraging results suggest that immunization contained the pathogenic challenge virus.

Many of the vaccine studies in the SIV/Mne model system have been performed with different challenge stocks and in different species of macaques, which are known to vary in their susceptibility to the pathogenic effects of this virus. Studies are underway, in pig-tailed macaques, directly comparing the AT-2 inactivated SIV vaccine with the vaccinia recombinants expressing the Gag/Pol/Env proteins of SIV/Mne. All macaques will be challenged with SIV/Mne, propagated in pig-tailed macaque PBMC, which has been characterized molecularly for the divergence of its envelope viral sequences. Such direct comparisons of vaccine approaches should systematically identify the most effective vaccine constructs and provide guidelines for similar vaccines needed to control the human pandemic.

### **Recombinant Vaccines against Primate Type D Virus (SRV) Infection**

We have previously shown that recombinant vaccinia, expressing the envelope genes of SRV, completely protected macaques against a high dose intravenous challenge with the homologous virus. This was the first report of a subunit vaccine effective against a primate retrovirus infection. These studies have now been extended to protection in a natural situation, with transmission of the SRV occurring between infected and control animals housed together. Future studies are planned to test this vaccine against the other SRV serotypes known to be present in macaque colonies and in feral populations.

### **Molecular Determinants of SIV/Mne Control in Infected Macaques**

One of the necessary components of a vaccine experiment is the availability of well-characterized stocks of virus. We have isolated single-cell clones from the original culture of SIV/Mne that are being analyzed for sequence variation in a variable region of the envelope surface protein (gp120). This information is valuable for the selection of viral stocks that differ from the homologous SIV/Mne molecular clone and can be used to challenge vaccinated macaques. In addition, with the collaboration of Drs. Bruce Crise, Elena Chertova and Louis Henderson of the AIDS Vaccine Program at NCI/SAIC-Frederick, we are also examining the relative content of the surface protein gp120 in these clones, and constructing cloned viruses that contain either truncated or full-length envelope transmembrane proteins (gp32/41). These constructs will be used for pathogenesis studies, and to examine viral loads and rates of clearance of SIV after challenge.

The vaccine and molecular studies outlined here systematically address the question of how to design an effective vaccine that protects macaques from infection by SIV. These studies should provide a scientific basis for the design of vaccines to prevent or control HIV infection.

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## **HUMAN RETROVIRUS GENE REGULATION**

### **Molecular Biology of HIV**

The human immunodeficiency virus type 1 (HIV-1) transactivator Tat protein is essential for efficient viral gene expression and virus replication. The goal of the following studies was to gain insight into the molecular mechanism of Tat transactivation.

#### Flavopiridol Inhibits The Tat/TAR-Dependent P-TEFb Kinase Activity To Phosphorylate RNAP II CTD and Transcription Elongation Factor SPT5 and Tat-SF1 During HIV-1 Transcription Elongation

The human immunodeficiency virus type 1 (HIV-1) Tat protein activates transcription elongation by recruitment of P-TEFb (CDK9/cyclin T1) to the TAR RNA. The TAR-bound CDK9 induces phosphorylation of RNAP II CTD and other RNAP II-associated proteins leading to a transition from nonprocessive to processive transcription. In addition to P-TEFb, the transcription elongation factors SPT5 and Tat-SF1 are also required for Tat transactivation. Using an immobilized template assay that permits isolation of the transcription complexes, we have found that Tat-SF1 and SPT5 are recruited into the transcription complexes during early-stage of elongation shortly after initiation. Interestingly, P-TEFb induces phosphorylation of SPT5 and Tat-SF1 during HIV-1 transcription elongation in a Tat/TAR-dependent manner in parallel with the hyperphosphorylation of RNAP II CTD. Flavopiridol, a cyclin-dependent kinase inhibitor, has been shown to inhibit P-TEFb and specifically block HIV-1 transcription elongation through the inhibition of P-TEFb. The selective inhibition of Tat transactivation by flavopiridol is due to the sensitivity of the Tat/TAR-dependent kinase activity of P-TEFb on several transcription factors including RNAP II, SPT5, and Tat-SF1 during HIV-1 transcription elongation.

### **Molecular Biology of HTLV-I**

Human T-cell lymphotropic virus type 1 (HTLV-I) is the etiologic agent for adult T-cell leukemia. HTLV-I transforms lymphocytes, and there is increasing evidence that the viral encoded protein, Tax, plays a primary role in viral transformation. We have previously shown that Tax inhibits p53 activity. The present studies were initiated to analyze the mechanism of p53 inhibition and the molecular activities of the Tax protein.

#### Transcription Profile of Cells Infected With Human T-Cell Leukemia Virus Type I Compared With Activated Lymphocytes

Human T-cell leukemia virus type I (HTLV-I) is the etiologic agent for adult T-cell leukemia and the neurological disorder tropical spastic paraparesis/HTLV-associated myelopathy. CD4<sup>+</sup> T lymphocytes, the primary hosts for HTLV-I, undergo a series of changes that lead to T-cell activation, immortalization, and transformation. To gain insight into the genetic differences between activated and HTLV-I-infected lymphocytes, we performed Affymetrix GeneChip analysis of activated and HTLV-I-infected cells. Using the Hu6800 GeneChip, we identified approximately 763 genes that had differentially regulated expression in at least three of five HTLV-I cell lines. Classification of these genes into functional groups—including cellular receptors, kinases, phosphatases, cytokines, signal proteins, and transcription factors—provides insight into genes and pathways that are differentially regulated during HTLV-I transformation (Cancer Res 62:3562–3571, 2002).

#### Acetylation of Nucleosomal Histones By P300 Facilitates Transcription From Tax-Responsive HTLV-I Chromatin Template

Expression of the human T-cell leukemia virus type I (HTLV-I) is regulated by the viral transcriptional activator Tax. Tax activates viral transcription through interaction with the cellular transcription factor CREB and the co-activators CBP/p300. One key property of the coactivators is the presence of histone acetyltransferase (HAT) activity, which enables p300/CBP to modify nucleosome structure. Our data demonstrate that full-length p300 and CBP facilitate transcription of a reconstituted chromatin template in the presence of Tax and CREB. The ability of p300 and CBP to activate transcription from the chromatin template is dependent upon HAT activity. Moreover, coactivator HAT activity must be tethered to the

template by Tax and CREB, since a p300 mutant that fails to interact with Tax did not facilitate transcription or acetylate histones, p300 acetylates histones H3 and H4 within nucleosomes located in the promoter and 5' proximal regions of the template. Nucleosome acetylation is accompanied by an increase in the level of binding of TFIID and RNA polymerase II to the promoter. Interestingly, we found distinct transcriptional activity between CBP and p300. Unlike p300, CBP possesses a N-terminal activation domain that directly activates Tax-mediated HTLV-I transcription from a naked DNA template. Finally, using the chromatin immunoprecipitation assay, we provided the first direct experimental evidence that p300 and CBP are associated with the HTLV-I LTR *in vivo* (Mol Cell Biol 22:4450–4462, 2002).

#### p53 Facilitates Degradation of A HTLV-I Tax Binding Protein Through A Proteasome-Dependent Pathway

Human T-cell leukemia virus type 1 (HTLV-1), the etiologic agent of adult T-cell leukemia (ATL) and tropical spastic paraparesis (HAM/TSP), transforms human T-cells *in vivo* and *in vitro*. The Tax protein of HTLV-1 is essential for cellular transformation as well as viral and cellular gene transactivation. The interaction of Tax with cellular proteins is critical for these functions. We previously isolated and characterized a novel Tax binding protein, TRX, by screening a Jurkat T-cell cDNA library. In the present study, we present evidence that the tumor suppressor p53 targets the TRX protein for proteasome degradation. Pulse-chase experiments revealed that p53 enhanced the degradation of TRX protein and reduced the half-life from 2.0 to 0.25 hr. Both HTLV-1 Tax and the proteasome-specific inhibitor MG132 inhibited p53-mediated TRX protein degradation. In contrast, neither the cysteine protease inhibitor E64 nor the calpain 1 protease inhibitor ALLN, inhibited p53-dependent degradation. These results suggest that TRX degradation is mediated through activation of the proteasome protein degradation pathway (J Gen Virol, in press, 2003).

#### Cross-Reactivity Between Immunodominant Human T Lymphotropic Virus Type I Tax And Neurons: Implications For Molecular Mimicry

HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is associated with immunoreactivity to HTLV-I Tax. Antibodies isolated from patients with HAM/TSP and monoclonal antibodies (MAbs) to HTLV-I Tax stained neurons. In neuronal extracts, HAM/TSP immunoglobulin G identified heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) as the autoantigen. Importantly, Tax MAbs reacted with hnRNP A1. To identify the epitope recognized by the Tax MAbs, the fine epitope specificity of the antibodies was determined using overlapping peptides. This analysis identified an epitope at the C-terminus (tax<sup>346–353</sup>), which overlaps a human immunodominant domain. Preincubation of this peptide with the Tax MAbs inhibited antibody binding to Tax, hnRNP A1, and neurons. This indicates that a cross-reactive immune response between HTLV-I Tax and neuronal hnRNP A1 is contained within the immunodominant epitope of Tax and suggests that molecular mimicry plays a role in the pathogenesis of HAM/TSP (J Infect Dis 186:1514–1517, 2002).

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### **Regulation of Retrovirus RNA Splicing and Transport**

Retrovirus gene expression is dependent upon alternative splicing of the viral genome-length pre-mRNA and on diverse mechanisms to export intron-containing mRNAs from the nucleus to the cytoplasm. The genetically complex retroviruses such as human immunodeficiency virus type 1 (HIV-1), human T-cell leukemia virus type 1 (HTLV-1) and the non-human lentivirus equine infectious anemia virus (EIAV) encode post-transcriptional regulatory proteins termed Rev or Rex. These regulatory proteins are essential for virus gene expression and virus propagation. Rev/Rex proteins simultaneously bind to regulatory elements in viral mRNA and interact with cellular protein cofactors to mediate the nuclear export of intron-containing mRNAs. EIAV Rev also induces alternative splicing of virus pre-mRNA by binding to its cis-acting RNA response element, RRE. The EIAV RRE sequence overlaps a consensus exonic splicing enhancer element (ESE) that binds to the cellular splicing factor SF2/ASF and to EIAV Rev in vitro. Analyses of EIAV RNA splicing in vivo and in vitro revealed that the RRE/ESE is a key mediator of constitutive and Rev-mediated alternative splicing. We have also studied the alternative splicing of HTLV-1 mRNA that results in the controlled expression of regulatory proteins encoded in the 3' end of the viral genome. The various pX mRNAs encode five different open reading frames. We have established a real-time RT-PCR method to quantify the levels of each of these mRNAs and have begun to address how the relative amounts of each one are controlled. The relative amounts of the various mRNAs vary over a 10,000-fold range in chronically infected cell lines. The overall splicing pattern was not significantly affected by the viral regulatory proteins Tax and Rex, but was influenced by over-expression of cellular splicing factors SF2/ASF and hnRNP A1. We are currently exploring the viral and cellular cis- and trans-acting elements that regulate alternative splicing of the pX mRNAs.

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### **HTLV-1 Molecular Biology and Pathobiology**

We previously constructed an infectious molecular clone of human T-cell leukemia virus type 1 (HTLV-1) and developed cell culture systems to examine virus entry, replication and particle maturation. Recombinant HTLV-1 vectors have now been made to study individual steps in the virus infectious cycle. These vectors have been used to determine the basis for the very low infectivity of HTLV-1 compared to other retroviruses. We found that a major block to efficient HTLV-1 infection occurs at a post-entry step. We are currently asking whether the mode of transmission of the virus (as cell-free virus particles versus cell-to-cell contacts) influences infection efficiency. The recombinant HTLV-1 vector system also provides the first rapid and sensitive means to examine HTLV-1 susceptibility to antiviral agents. We found that HTLV-1 replication was inhibited by the reverse transcriptase (RT) inhibitors AZT, 3TC, d4T, tenofovir and abacavir. We have begun to define the catalytically active form of HTLV-1 RT that is produced from a polyprotein precursor by the viral protease. This work will establish the subunit composition of the active enzyme, prerequisite to expressing the recombinant protein for structural and functional analyses. We are also examining viral and cellular proteins that cooperate in the process of virus assembly and release. We have shown that a peptide motif in the matrix protein of the virus core particle interacts with components of the cellular endosomal sorting pathway. The interaction of the viral late assembly domain with Nedd4 protein was found to be essential for virus budding from the plasma membrane. The diseases associated with HTLV-1 infection are often accompanied by immunological abnormalities that could be related to the activation of specific genes in the HTLV-1 infected T cell. In the course of examining cytokine gene expression in T cells, we found that the Th2 cytokine, IL-13, was over-expressed in HTLV-1 chronically infected cell lines. Moreover, examination of CD4+ T cells from HTLV-1 infected patients revealed a correlation between the levels of expression of IL-13 and viral Tax protein. We are currently examining the mechanism of activation of the human IL-13 promoter by the viral trans-regulatory protein, Tax.

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**An EBV-like Virus, HV<sub>MNE</sub>, With Tropism for T-cells**

The oncogenicity of HV<sub>MNE</sub>, a novel Epstein-Barr (EBV)-like virus isolated from a simian T-cell leukemia/lymphoma virus (STLV) type I/II-negative *Macaca nemestrina* with a CD8+ T-cell mycosis fungoides/cutaneous T-cell lymphoma, was demonstrated in rabbits. All rabbits that seroconverted to viral capsid antigen (VCA) developed malignant lymphoma within months from inoculation, whereas four control rabbits, inoculated with heat-inactivated culture supernatants from the same cell line, failed to seroconvert to VCA and did not develop disease. Disseminated lymphoma cells of mixed origin were detected in most vital organs, including the spleen, liver, lungs, kidneys, and heart of the affected rabbits. Neoplastic infiltrates were also observed in lymph nodes, skin, and thymus. HV<sub>MNE</sub> DNA and EBV-like RNA (EBER) expression was demonstrated in the affected organs. Two transformed cell lines established from the blood and lymph nodes of two lymphomatous rabbits were of the same T-cell lineage as the original monkey CD8+ T-cell line (J94356<sub>PBMC</sub>). In addition we have recently demonstrated that HV<sub>MNE</sub> also transforms Common Tamarin CD4+ T-cells. Maintenance of HV<sub>MNE</sub> DNA and LMP1 expression by FACs analysis was demonstrated in all cell lines. Most of these cell lines became IL-2 independent and manifested a constitutive activation of the Jak/STAT pathway.

The demonstration of HV<sub>MNE</sub>'s high oncogenicity in New Zealand white rabbits provides a small-animal model for human T-cell lymphoma whereby genetic determinants for T-cell transformation by this EBV-like animal virus can be studied. Furthermore, the demonstration of Common Tamarin cells' susceptibility to HV<sub>MNE</sub> oncogenicity suggests the possibility for the development of a non-human primate animal model for human T-cell malignancy.

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The Viral Pathogenesis Section in the [Dermatology Branch, CCR](#) focuses on defining interactions between HIV, herpes viruses, and skin. Specifically, much past and current work has centered on characterizing in detail the cellular and molecular events that occur during sexual transmission of HIV. Langerhans cells, which are dendritic cells found within skin and mucosal epithelial surfaces, are likely initial targets for HIV following exposure to the virus. [Dr. Blauvelt](#) has studied how HIV infects Langerhans cells and has tested a variety of compounds that can inhibit this infection. These compounds are being explored as possible microbicides to prevent sexual transmission of HIV. The laboratory also studies the pathogenesis of Kaposi's sarcoma (KS), specifically cellular and molecular mechanisms involved in latent-to-lytic switching of Kaposi's sarcoma herpes virus (KSHV), the virus that causes KS, and the *in vitro* and *in vivo* function of LANA, a KSHV-encoded nuclear protein expressed in all KS tumor spindle cells. He recently succeeded in generating *k-cyclin* transgenic mice that demonstrate lymphatic dysfunction. Collectively, these studies are aimed at developing novel therapeutic strategies based on increased knowledge of how KSHV causes KS.

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### **Viral and Cellular Cytokines in AIDS-related Malignancies**

Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus 8, is a herpesvirus linked to the development of Kaposi's sarcoma (KS), primary effusion lymphoma, and a proportion of Castleman's disease. One of the features of this virus is a number of potentially useful cellular genes acquired through molecular piracy. One such gene is viral interleukin-6 (vIL-6), which is structurally homologous to human and murine IL-6.

To gain insight into the biology of vIL-6, we expressed vIL-6 in murine fibroblasts and injected stable vIL-6-expressing cells into athymic mice. Compared with the controls, vIL-6-expressing mice displayed increased hematopoiesis in the myeloid, erythroid, and megakaryocytic lineages, plasmacytosis in spleen and lymph nodes, hepatosplenomegaly, and polyclonal hypergammaglobulinemia. Fibroblasts expressing vIL-6 were significantly more tumorigenic than control clones, and the tumors produced by vIL-6-expressing fibroblasts were significantly more vascularized than the controls. This was attributed to vIL-6 stimulation of Vascular Endothelial Growth Factor (VEGF) that was detected in the spleen, lymph nodes, and tumor tissues from mice bearing vIL-6-expressing tumors.

Since vIL-6 displays many biological functions of hIL-6, it was proposed that vIL-6 utilized the same receptor and signaling cascade as hIL-6. However, compared to hIL-6, vIL-6 was found to require approximately 1000-fold higher amounts of protein to achieve similar biological effects, suggesting that the two cytokines differ in their receptor engagement. The hIL-6 receptor is composed of a binding subunit, the  $\alpha$ -chain, and a signal transducing protein, gp130. Human IL-6 binds to the IL-6 receptor  $\alpha$ -chain (IL-6R), and the complex then associates with gp130, allowing the transducing chain to dimerize. Dimerization of gp130 leads to downstream signaling. Using plasmon surface resonance, we found that vIL-6 binds to soluble gp130 with a dissociation constant of 2.5  $\mu$ M, corresponding to 1000-fold lower affinity than that of hIL-6/IL-6R complex for the transducing gp130 chain. Soluble IL-6 receptor neither bound to vIL-6 nor affected the binding of vIL-6 to gp130. These results document that vIL-6 binds directly to gp130 but does not bind IL-6R.

We have investigated the potential role of vIL-6 in the pathogenesis of KSHV-associated malignancies. Using a newly developed vIL-6 ELISA, we measured vIL-6 levels in blood and body fluids. Serum vIL-6 was detected in 38.2% of HIV+ patients with KS, in 85.7% of patients with primary effusion lymphoma (PEL) and/or Multicentric Castleman's Disease (MCD), and in 60% HIV+ mostly homosexual individuals without KS, PEL, or MCD. Only 23.1% of patients with classic KS and 2.5% of blood donors from the US had measurable vIL-6 in the circulation. Overall, circulating vIL-6 was associated with HIV+ status ( $P < .0001$ ), but not with the occurrence of KSHV-associated malignancies ( $P = .43$ ). Studies of body cavity effusions revealed the presence of vIL-6 in six of eight PEL effusions but in none of the control effusions. All PEL effusions also contained hIL-6, and seven of eight also contained hIL-10. Since vIL-6 can induce the expression of VEGF, which promotes vascular permeability, these results suggest that vIL-6 can contribute to the pathogenesis of PEL.

Despite some exciting new leads, PEL remains a fatal malignancy. We sought to target two of the molecules that function as autocrine growth factors for PEL, IL-10 and vIL-6, as a potential therapeutic approach to PEL treatment. It is noteworthy that IL-10, vIL-6, and VEGF (not an autocrine growth factor for PEL, but nonetheless a contributor to PEL pathogenesis) induce phosphorylation of STAT3 (signal transducer and activator of transcription-3) for intracellular signaling. We found that inhibition of STAT3 signaling leads to cell death by apoptosis of PEL cells *in vitro*. STAT-3 is constitutively activated in the PEL cell lines BC-1, BCBL-1, and VG-1, presumably due to autocrine growth stimulation. Transduction of a dominant negative STAT3 and pharmacologic STAT3 inhibition by Tyrphostin AG490 caused caspase-dependent cell-death. STAT3 inhibition in PEL cells was associated with transcriptional repression of Survivin, an inhibitor of apoptosis, but did not change the expression of Bcl-2 family proteins. In addition, forced over-expression of surviving rescued PEL cells from apoptosis was induced

by STAT3 inhibition. These results suggest that STAT3 signaling contributes to PEL progression, acting through the pro-survival protein survivin, and identifies STAT3 inhibitors as potential therapeutic drugs to be tested in PEL.

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### **Development of Novel Therapies for HIV Infection and Related Malignancies**

The [HIV and AIDS Malignancy Branch \(HAMB\)](#) was established in 1996 to conduct translational clinical and laboratory research aimed at the development of novel therapies for HIV infection and AIDS-related malignancies. HAMB also conducts laboratory research focused on an understanding of these diseases. More detailed information about clinical trials can be found at <http://ccr.cancer.gov/trials/>. Also, the research of other investigators in the Branch can be found separately in this Handbook.

#### **Research of Dr. Robert Yarchoan**

During the past year, Drs. [Richard Little](#) and [Robert Yarchoan](#) have been conducting several clinical trials to evaluate novel therapies for Kaposi's sarcoma (KS). We have recently obtained results indicating that thalidomide, an anti-angiogenesis agent, is active in patients with KS. We are also studying the cytokine and anti-angiogenesis agent IL-12, and preliminary results indicate that it has activity. We are following this observation up with a study of liposomal doxorubicin plus IL-12 in patients with advanced KS. In collaboration with [Dr. Wyndham Wilson](#) of the Medicine Branch in the [Center for Cancer Research](#), we have been exploring the possible role of infusional therapy with etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin (EPOCH) followed by IL-12 in AIDS-associated lymphomas. We are now extending this to study the use of short-course EPOCH plus rituximab.

We are exploring the area of therapeutic vaccination for patients with HIV infection and are planning a therapeutic trial of a peptide vaccine directed at reverse transcriptase. The long-term virological and immunological effects of protease-containing anti-HIV therapy in children with HIV infection is also being studied. Preliminary results suggest that some children have long-term increases in their naïve CD4 cells in spite of a transient or minimal anti-HIV response.

In the laboratory, we have investigated the role of conserved cysteines at positions 67 and 95 on the activity of HIV protease. We have found that glutathiolation of Cys 95 abolishes HIV protease activity, while glutathiolation of Cys 67 can enhance activity. In addition, we have found that a cellular protein called thioltransferase can deglutathiolate cysteines of HIV-1 protease and that this protein is taken up in secreted virions. HIV virions with mutations of Cys 95 and Cys 67 have been generated, and we are studying the effects that these mutations have on the fitness of HIV under various conditions. We are studying the regulatory activity of similar cysteines in the protease of other retroviruses. We are also studying mutations at position 95 that can occur in patients on long-term protease inhibitor therapy and several novel inhibitors of HIV protease that act at the dimer interface.

We recently made the observation that hypoxia can activate lytic replication of KSHV/HHV-8 in latently infected cell lines. We are exploring the mechanism for this effect and in particular are trying to dissect the molecular mechanisms by which KSHV responds to hypoxia. We have preliminary results showing that the virus contains several hypoxia response elements (HRE) that can respond to hypoxia inducible factor (HIF). We are also studying Epstein-Barr virus for a similar response to hypoxia.

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**HIV and Herpesvirus Molecular Virology and Pathogenesis**

Our group is principally concerned with the molecular biology of HIV and herpesvirus pathogenesis, particularly the pathogenesis of pediatric HIV disease. The course of HIV disease in pediatric patients differs substantially from the course of disease in adults. We are therefore particularly interested in trying to understand the involvement of host cell factors in HIV replication and the effect of HIV infection on the host cell. In order to better understand the involvement of the host cell in the processes of viral replication we are characterizing the cellular genes differentially expressed during HIV replication using microarray technology and are attempting to assign responsibility for the differential expression of the cellular genes to particular phases of the viral replication cycle and to individual HIV viral gene products. We are also working to understand the viral replication and pathogenesis strategies of Kaposi's sarcoma-associated herpesvirus (KSHV), and other herpesviruses. KSHV is the agent that causes Kaposi's sarcoma, certain primary effusion lymphomas, and some types of multicentric Castleman's disease. To better understand replication cycle of KSHV and the pathogenesis of the cancers caused by the virus we have developed microarrays containing the complete genome of KSHV. We have used these materials to produce a comprehensive description of the viral transcription program that is seen during KSHV replication. We are also using this approach to examine how the expression of particular KSHV genes controls the viral lytic replication cycle and helps maintain viral latency, and how the expression of certain KSHV genes leads to malignant transformation and influences the pathogenesis of the cancers caused by KSHV. Additional studies explore the viral gene expression patterns in the KSHV-related cancers and try to link the biological features of the tumors with particular patterns of viral gene expression. About 90% of the work is AIDS-related. (About 30% of the work is also related to cancer.)

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### **Pediatric HIV/AIDS Clinical Research**

The [HIV and AIDS Malignancy Branch \(HAMB\)](#) pediatric HIV clinical research program is a multidisciplinary effort that takes a comprehensive approach to investigating HIV treatment and its infectious, malignant, neurologic, and psychosocial complications. Antiretroviral and immunologic approaches are being investigated in both children and adolescents. Current protocols are aimed at several scientific goals: 1) The examination of the long term effects of highly active antiviral therapy (HAART), particularly HAART regimens containing a protease inhibitor, and the ability of such regimens to produce long term immune reconstitution in pediatric patients, 2) The development of new therapies for pediatric HIV patients, particularly therapies for patients who have failed prior antiretroviral therapies and are in need of “salvage” regimens. One current protocol uses a new antiretroviral agent, tenofovir and, in the course of the studies, will not only obtain the pharmacokinetic and toxicity/tolerability data needed to use the agent safely and effectively in children, but will also use the drug to obtain information about viral evolution, the potential for immune reconstitution in the heavily treated pediatric HIV patient, models that may help predict the response to therapy, and how to treat pediatric HIV patients who have failed several prior therapeutic regimens and are in need of “salvage therapy”, and 3) Studies aimed at optimizing currently available treatment, including studies that combine viral resistance testing with pharmacokinetic measures and antiretroviral drug dose adjustments in a “therapeutic drug monitoring” strategy that aims to customize antiretroviral drug doses to the resistance pattern of an individual patient’s virus and the pharmacokinetic characteristics of that individual patient.

Longitudinal studies of neurocognitive function, brain imaging, and immune measures in children receiving antiretroviral therapy are being conducted in addition to investigation of the pathophysiology of HIV encephalopathy. Psychosocial studies include investigation of issues surrounding disclosure of the diagnosis, the prevalence of psychiatric disorders in long-term survivors, and rates of adherence to treatment and factors which influence adherence among pediatric patients with HIV infection. HIV/AIDS-related work accounts for 100% of this project.

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## **RNA Splicing Regulation of Tumorigenic DNA Viruses**

Pre-mRNA splicing is one of the most important steps in the control of gene expression. This essential step involves intron removal from a primary transcript and exon ligation to form a real message. In many cases, the mechanisms that regulate RNA splicing remain poorly understood. Infection with DNA tumor viruses such as cervical cancer-associated human papillomaviruses (HPVs) and Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus 8, is common in AIDS patients. HPVs and KSHV have several critical genes undergoing regulation by RNA splicing at the post-transcriptional level. Our research focus is to understand the mechanisms that control viral RNA splicing and to look for new tools and molecular targets for antiviral and anticancer therapy at the RNA level. Present studies in our laboratory focus on 1) identification of viral *cis* elements that are involved in the regulation of the RNA splicing of viral structural and nonstructural genes including viral oncogenes in high- and low-risk HPVs and KSHV, 2) characterization of cellular splicing factors and viral proteins involved in processing of RNA splicing, and 3) development of RNA interference as a new tool for antiviral and anticancer therapy.

### **Regulation of Papillomavirus RNA Splicing**

We have been utilizing HPV 16 and 18 E6 and E7 RNA transcripts as a first step to approach our goal. The E6 and E7 genes of HPV 16 and 18 are two major viral oncogenes and are expressed in almost every cancer cell of cervical carcinoma. E6 and E7 proteins inactivate cellular tumor suppressor proteins p53 and pRb, respectively, and play key roles in the induction of human cervical cancer. However, expression of E6 and E7 is complicated by their transcription as a bi-cistronic mRNA and alternative splicing of their primary transcripts from which a large portion of E6 has been removed through using two alternative 3' splice sites within E6 coding regions. These alternatively spliced RNA species are termed E6\*I and E6\*II and form the majority of early viral transcripts both in cervical tumors and in tumor-derived cell lines. Ironically, transcripts for unspliced, full-length E6 are in extremely low abundance and sometimes difficult to detect in many tumors or tumor-derived cell lines. We have demonstrated that splicing of HPV 16 E6-E7 pre-mRNA is cap dependent and can be restrained by distance of the cap-proximal intron from RNA 5' cap. Other works are focusing on the characterization of cellular splicing factors and viral proteins involved in the E6-E7 RNA splicing.

Another approach in our laboratory is to determine how cellular splicing factors are involved in viral RNA splicing. We are using a bovine papillomavirus type 1 (BPV-1, a prototype virus in the papillomavirus family) late pre-mRNA as our model to address this question because this pre-mRNA has two alternative 3' splice sites (3'ss). Switching from one 3'ss to another in the splicing of this transcript relates to keratinocyte differentiation and involves viral *cis* elements interacting with cellular splicing factors. We have established a series of cell lines with stable transfection of BPV-1 late genes. These cell lines have a cellular splicing factor ASF/SF2 under the control of a tetracycline (tet)-repressible promoter. Using these cell lines, we have demonstrated that the splicing factor ASF/SF2 is required for usage of the proximal 3'ss, depletion of ASF/SF2 from the cells leads to use of the distal 3'ss. Activation of the ASF/SF2-depleted cells doesn't restore ASF/SF2 expression, but restores selection of the proximal 3'ss through activation of other splicing factor expression and PI3K/Akt pathway. Currently, we are focusing on the characterization of transcription and polyadenylation coupling with this feature of the splicing and what cellular splicing factor devotes to regulation of the distal 3'ss.

### **Regulation of KSHV RNA Transcription and Splicing**

KSHV is a newly identified human gamma herpes virus strongly associated with the development of Kaposi's sarcoma, body cavity-based B-cell lymphoma, and Castleman's disease. Currently, our lab is focusing on the KSHV K8 and K8.1 which are two juxtaposed, but unrelated genes positioned from nt 74850 to nt 76695 of the virus genome. However, both genes share a single poly (A) site at nt 76714. The

K8 gene consists of four exons and three introns, which are alternatively spliced during the viral gene expression. The K8.1, although sharing the exon 4 with K8, utilizes the intron 3 of K8 as its own coding region, which is also alternatively spliced to the exon 4. It has been proposed that a K8.1 pre-mRNA uses the K8 intron 3 as its coding region exon 1 and the K8 exon 4 as its exon 2, although neither the K8.1 promoter nor the 5' ends of their primary transcripts have been definitely identified. Alternative splicing of KSHV K8 and K8.1 pre-mRNAs each produces three different isoforms (" , \$ , and () of the mRNAs. We have mapped the 5' end of the K8.1 RNA in butyrate-induced KSHV-positive JSC-1 cells to nt 75901 in KSHV genome, 14 nts upstream of the first AUG of the K8.1 at nt 75915. A K8.1 late promoter has been identified immediately upstream of the K8.1 transcription start site, and its activity has been associated with viral DNA replication. Our current focus is to understand how viral DNA replication controls late promoter activity and its contributions to K8.1 transcription initiation and K8.1 RNA processing in the KSHV life cycle and B cell differentiation. In addition, works are in progress on the characterization of a KSHV protein that affects both viral and cellular RNA shuttling through cell nuclear membranes.

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## HIV DRUG RESISTANCE PROGRAM

### Center for Cancer Research

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The recent application of therapies using combinations of antiviral drugs has shown that virus growth in HIV infected people can be brought to a halt and, in many individuals, provide considerable and long-lasting improvement in their condition. These therapies have helped large numbers of people live relatively normal lives despite their HIV infection. Most importantly, they prove the concept that antiviral drugs can give long-term relief to patients with HIV infection, but fall far short of providing a long-term solution.

The problem facing all the strategies is the development of resistance in the virus due to the appearance of specific mutations. In an effort to avoid resistance, drugs have to be given at high—somewhat toxic—doses, in expensive combinations, and on exacting and difficult-to-follow schedules. Even then, the therapy often fails, and resistant virus appears. There is, therefore, a desperate need to understand how the virus develops resistance to drugs, and to use this understanding to develop more effective strategies for treating HIV infection.

The goal of the [HIV Drug Resistance Program \(DRP\)](#) is to establish a focused basic science research effort that addresses this need and builds on the existing strength of HIV and retrovirus research within the NCI. Specific goals include the following: (1) to extend our understanding of the structure and biochemistry of known and potential drug targets in HIV replication, as well as their mechanism of resistance to antiviral drugs; (2) to use this information to develop new strategies of inhibition of virus replication; (3) to improve our knowledge of the interaction of HIV with its human host, both in the single cell and the whole organism; (4) to understand the mechanisms by which virus variants arise during single infections and propagate in the infected patient; (5) to develop and test novel therapeutic strategies based on the improved knowledge obtained; and (6) to promote the exchange of information relevant to the problem of drug-resistant virus among researchers in all relevant disciplines.

The DRP includes seven principal investigators in two laboratories, as well as a patient-based research unit in the Clinical Center and a Virology Core Facility.

The Retroviral Replication Laboratory (RRL) focuses on obtaining a detailed understanding of important events in the virus life cycle, from initial interactions between virus and host cell, through reverse transcription, to mechanisms of virus assembly and release. Whole-organism studies, including the development of important animal models and the use of retroviral vectors, are included in the work of the Laboratory. At present, the RRL consists of three sections: the Vector Design and Replication Section, under the direction of [Dr. Stephen H. Hughes](#); the Retrovirus Assembly Section, under the direction of [Dr. Alan Rein](#); and the Model Development Section, under the direction of [Dr. Vineet N. KewalRamani](#). A fourth section, the Virus-Cell Interaction Section, under the direction of [Dr. Eric O. Freed](#), will join the RRL in the summer of 2003.

Dr. Hughes and the members of his section are studying several aspects of retroviruses. Their efforts are intended to provide a better understanding of retroviral replication. There are two major projects. In the first project, the goal is to design improved retroviral vectors, which are modified viruses that can be used to deliver foreign genes. The second project is intended to better understand the structure and function of the reverse transcriptase (RT) of HIV-1. RT is a major target for anti-HIV drugs; mutations in RT can lead to drug resistance. A better understanding of HIV-1 RT and of the mechanisms that underlie drug resistance could lead to the development of better anti-HIV drugs and drug therapies.

Research in the Retrovirus Assembly Section is directed toward a fundamental understanding of the retroviral life cycle, with a special emphasis on the molecular mechanisms involved in retrovirus particle assembly and maturation. The current research of Dr. Rein's lab involves protein-protein interactions, protein-nucleic acid interactions, and nucleic acid-nucleic acid interactions. Their experiments combine the power of molecular analysis of defined systems *in vitro* with the careful study of retroviral replication in living cells. It is hoped that the insights gained from their basic research will result in the development of new antiviral strategies.

The research focus of Dr. KewalRamani's lab begins with obtaining an understanding of the specific interactions between HIV viral components and human proteins. This process will define which host factors are required for a productive infection. An immediate objective of these studies is to provide new targets for the development of novel drug therapies. Using what is learned from these molecular studies, they are attempting to develop small animal models to study the interaction of HIV with the host's immune system.

Dr. Freed's research program focuses on a variety of key aspects of HIV-1 assembly and release. Of particular interest are the interplay between viral and host factors in the targeting of assembly to the plasma membrane, the role of lipid rafts in membrane association and virus assembly, and the mechanism by which the viral envelope glycoproteins are incorporated into virions. Recent studies have been aimed at defining the cellular pathways and host factors involved in the budding of retrovirus particles from the plasma membrane and identifying inhibitors of virus budding.

Collectively, the interests of the four sections of the RRL are relatively broad. We believe that having a broad range of research interests is beneficial; if intellectual or experimental issues arise in one of the sections that are outside the expertise of that section, other members of the laboratory usually can help provide the necessary expertise. We also believe that sharing ideas within the laboratory makes the intellectual environment of the laboratory better for everyone and, in particular, that this approach provides better training for the postdoctoral fellows. All of them should have the opportunity to understand retroviruses in a broad sense, and be exposed to a wide range of experimental approaches so that they will be able to take the most effective experimental approach when confronted with a problem.

The Resistance Mechanisms Laboratory (RML) focuses principally on the biochemistry and biology of reverse transcriptase (RT), its interaction with templates and substrates, and its role during replication in mutation and recombination. The RML consists of three sections: 1) RT Biochemistry Section, under the direction of [Dr. Stuart Le Grice](#), 2) Viral Recombination Section, under [Dr. Wei-Shau Hu](#) and 3) Viral Mutation Section, under [Dr. Vinay Pathak](#).

Despite a constantly expanding spectrum of highly potent and selective antiviral agents, the rapid acquisition of drug resistance continually confounds therapeutic strategies designed to combat HIV infection. Two properties of the virus-coded RT can be considered central to this problem—namely, an elevated rate at which inappropriate nucleotides are incorporated into the growing DNA chain, and the lack of an efficient proofreading mechanism. The capacity of the replication machinery to exploit information from both RNA genomes packaged into the virus, i.e., recombination, has the further consequence of increasing genetic diversity by an assortment of mutations. Therefore, multiple drug resistance can result from recombination between strains originally resistant to a single drug. The RML combines the disciplines of biochemistry with molecular, cellular, and structural biology to better understand these events at the molecular level, with the ultimate goal of applying the knowledge gained to future antiviral strategies.

Both the DNA polymerase and RNase H functions of RT from HIV-1 and related lentiviruses are under investigation as therapeutic targets in the RT Biochemistry Section. Despite an absolute requirement for virus-coded RNase H for replication, there have been few reports on agents targeted to this function. The development of model systems accurately mimicking specialized RNase H-mediated events (e.g., polypurine tract selection and excision from nascent DNA and tRNA primer release prior to second-strand transfer) and mutants specifically impaired in these steps provide important mechanistic information on this C-terminal RT domain. This knowledge is currently being applied to develop high-throughput "smart" screening strategies. Novel mutations in the primer grip of the DNA polymerase domain that lead to increased fidelity have been identified and are under investigation.

The projects of the RT Biochemistry Section are complemented by those of the Viral Recombination Section, which investigates the molecular mechanisms of recombination, RNA packaging and virus assembly, and interactions between distinct retroviruses. Evidence shows that recombinant viruses can be generated through packaging of heterologous viral RNA genomes, i.e., of avian and murine origin, into a single virion. The implications and limitations of these events are under investigation. Packaging of the viral genome is dependent on interactions between the Gag polyprotein with a packaging signal in the viral RNA. Experiments are underway to define the cis- and trans-acting elements involved in the specificity of viral RNA packaging.

The theme of recombination is extended to the Viral Mutation Section, which makes use of *in vivo* systems with recombinant retroviruses to understand the mechanisms that generate variation in retroviral populations. *In vitro* assays have identified structural determinants important for fidelity and RT template switching, which include the active-site YXDD motif, the dNTP-binding site and thumb subdomain of the DNA polymerase catalytic center, and, surprisingly, the C-terminal RNase H domain. The latter observations indicate important “communication” between the DNA polymerase and RNase H catalytic centers of RT. The *in vivo* approach of the Viral Mutation Section provides an excellent complement to the structure/function studies conducted *in vitro* by the RT Biochemistry Section.

The clinical arm of the DRP is the Host-Virus Interaction Unit (HVIU). The HVIU has the goal of using patient-based studies to elucidate mechanisms underlying the evolution of resistance *in vivo*, the dynamics of infection under treatment, the role of resistance mutations in subsequent treatment efficacy and failure, and the development of novel strategies to counter evolution of resistance in patients. Currently, the HVIU consists of the *In Vivo* Biology research group, under the direction of [Dr. Frank Maldarelli](#), and the Virology Core, under the direction of [Dr. Sarah Palmer](#). [Dr. John Coffin](#) serves as acting leader of the HVIU.

The primary research focus of the *In Vivo* Biology group is to study the population genetics, evolution, and dynamics of HIV infection in patients, particularly the development of and possible ways to overcome resistance. In collaboration with the NIAID/CCMD AIDS clinic and the NCI HIV AIDS Malignancy Branch, Dr. Maldarelli develops and secures IRB approval for clinical trials, and implements them using the support of these groups. At present, there are three active protocols, corresponding to the three ongoing projects: [HIV Expression in Patients with Viral Loads Suppressed on HAART \(Protocol 02-I-0232\)](#), [Genetic Analysis of HIV Prior to Initiation of Highly Active Antiretroviral Therapy \(Protocol 00-I-0110\)](#), and [An Assessment of the Relationship between Antiretroviral Drug Genotype/Phenotype \(IC<sub>50</sub>\) and Antiretroviral Activity in HIV-Infected, Drug-Experienced Patients with Suboptimal Suppression of Plasma Viral Load \(Protocol 01-I-0004\)](#).

The Virology Core conducts analyses of HIV samples from clinical and basic studies on HIV, which will provide new and important information on viral dynamics, genetics, and evolution both *in vitro* and *in vivo*. Dr. Palmer and the Virology Core staff have been working closely with Dr. Maldarelli, in consultation with Dr. John Mellors of the University of Pittsburgh, to develop methods for obtaining very large numbers of sequences from virus circulating in plasma and for measuring virus load with much greater sensitivity than can be reliably attained with currently available tests. As part of this work, the Virology Core is conducting *in vitro* drug resistance selection assays, genotypic and phenotypic analyses, and a range of other techniques such as polymerase chain reaction (PCR), site-directed mutagenesis, viral load monitoring, and viral enzyme analysis.

The DRP has established numerous collaborations with other NIH and extramural researchers to develop new screens for novel targets (such as RNase H and integrase) and to analyze compounds for resistance profiles. An important part of these projects will be to develop an understanding of how these new targets behave under selection pressure, to estimate fitness decreases due to resistance mutations, and to predict what the effect of drug therapy might be when specific antiviral compounds are administered to patients.

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**HUMAN GENETICS SECTION**  
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Viral infections in humans are notable for the diversity of host responses to infection, rate of progression and disease outcome. Differential responses to viral pathogens is due, in part, to viral strain differences, but there is also a host component that may be due to allelic differences at genes involved in response to infection. We are using a combination of epidemiology, molecular genetics, and population genetics to identify and characterize host genes that may be operative in resistance or increased susceptibility to infection by HIV-1, and the hepatitis B and C viruses (HBV and HCV). I am so investigating the interaction between HCV and HIV-1 in coinfecting persons on HIV-1 progression to AIDS, HCV clearance, and liver disease.

In animal models, genes have been identified which influence susceptibility or resistance to infection and disease progression following exposure to retroviruses. Although twin and family studies and epidemiological evidence in humans support the hypothesis that there is a strong genetic component to infectious disease susceptibilities, it is very difficult to identify and map these genes by conventional family studies. Our group is utilizing the extensive human genome map in combination with the methods of population genetic theory to identify, map, and characterize five classes of genetic loci: (1) those that restrict infection by the virus following exposure; (2) those that modulate or control the immune response; (3) those that influence outcome to infection; (4) those that influence the temporal course of infection (fast versus slow progression); and (5) those that contribute to resistance to drug therapies.

Considerable progress has been made on this project. The laboratory has developed cell lines for renewable DNA for over 10,000 persons enrolled in prospective cohorts infected with HIV-1, HBV, or HVC. We have identified and characterized: 1) an intronic regulatory element polymorphism that potently downregulates RANTES transcription and is associated with accelerated AIDS progression in African Americans; and 2) a TNF- $\alpha$  inducible promoter variant of the IFN-gamma gene that accelerates AIDS progression and may be associated with common immune disorders disproportionately affecting African Americans. My group has also identified single nucleotide polymorphisms (SNPs) and determined the haplotype structure of the *MCP-1 MCP-3 – Eotaxin* chemokine gene cluster. Using high-throughput genotyping methods, we identified a single haplotype strongly associated with resistance to infection by HIV-1. These studies suggest that chemokines and cytokines may be potential targets for drug development.

In collaborative studies, we have also shown that HIV-1 progression to AIDS is strongly influenced by coinfection by HCV. In a study of hemophiliacs infected in childhood by HCV, we were able to show individuals coinfecting with HIV-1 were more likely to be persistently infected by HCV and had more evidence of liver disease. We also showed that coinfecting individuals with high median RNA levels of HCV or HIV-1 progressed to AIDS at the same rate as those who had high median RNA levels for both viruses. These studies emphasize the need for further research regarding the use of HIV-1- and HCV-specific therapy in coinfecting persons. We are now continuing our investigation of HIV-1/HCV coinfection by examining the role of specific CTL response in controlling HCV and HIV-1 replication and the role of immune and genetic factors that may modulate CTL response.

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### **Mechanisms of HIV-1 Envelope Glycoprotein-Mediated Membrane Fusion**

The interactions between the HIV-1 envelope glycoprotein gp120-gp41 and the receptor molecule CD4 and co-receptors that lead to membrane fusion were studied using a variety of virological, cell biological, and biophysical techniques. The HIV-1 *env*-mediated fusion reaction is initiated by binding of the envelope gp120 to the CD4 molecule leading to conformational changes in gp120, which results in engagement of the chemokine receptors and conversion of the gp41 into its fusion active form. This process requires massive co-clustering of CD4 and co-receptors in glycosphingolipid-enriched membrane microdomains (“rafts”) (1). The fusion reaction proceeds along a series of steps before the final event occurs which results in the delivery of the viral genome into the cell. Using the photosensitized labeling techniques we have for the first time measured directly the kinetics of HIV and SIV virus-cell fusion and find that the fusion reaches maximum with a  $t_{1/2}$  of 18 min (2). This indicates that the CD4 and co-receptor-induced triggering events leading to HIV/SIV Env-mediated fusion are stochastic. The lack of synchrony in the activation of HIV/SIV Envs therefore provides an opportunity for the C terminal peptide inhibitors to bind to the pre-hairpin grooves which become transiently exposed following CD4 and co-receptor-induced triggering of HIV-1 Env (3). We have further investigated the nature of the transitional changes using CD4-coreceptor complexes attached to beads and demonstrated that these may result in destabilization of the viral membrane (4). These findings may lead to the development of vaccines targeted to fusion-competent portions of envelope glycoproteins.

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### **HIV-1 Entry and Cell Dynamics**

The major long-term research goals are elucidation of mechanisms of virus entry and cell dynamics, by using a combination of quantitative experimental and computational approaches, and development of tools for the prevention and treatment of infectious diseases and cancer. Two current major foci are the identification and characterization of human monoclonal antibodies selected from phage libraries and the development of novel immunogens and inhibitors with an emphasis on HIV. Other areas of interest are the dissection of the biochemical networks controlling cell differentiation, with an emphasis on telomerase regulation, and identification and/or selection of molecular targets for cancer treatment. We also wish to understand how humans respond to treatment with biologically active substances.

Major achievements include the identification of several broadly HIV-neutralizing human monoclonal antibodies that may have potential as therapeutics and help in the design of vaccines, as well as the development of tethered HIV envelope glycoproteins where gp120 and gp41 are joined by flexible linkers that could have potential as immunogens, in collaboration with Dennis Burton and Chris Broder, respectively. We are also analyzing clinical data from HIV-infected patients, including patients treated with IL-2 (in collaboration with Cliff Lane and Joe Kovacs) that suggest an important role of dynamically distinct cell subpopulations. Several other findings of relevance for HIV are described in the publications listed below.

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Recently, major advances have been made in understanding the role of natural killer (NK) cells in the innate immune response. The killer immunoglobulin-like receptors (KIR) on NK cells regulate inhibition and activation of NK cell responses through recognition of HLA class I molecules on target cells. We have begun to study the *KIR* genes and, given their receptor-ligand relationship, we have hypothesized that they may be involved in many of the diseases for which an *HLA* influence has been identified. In this regard, we have performed *KIR* genotyping in the AIDS cohorts. The data indicate that one activating *KIR* allele, *KIR3DS1*, in combination with its HLA ligand *HLA-Bw4* results in delayed progression to AIDS. In the absence of *KIR3DS1*, *HLA-Bw4* was not associated with any of the AIDS outcomes measured. Conversely, in the absence of *HLA-Bw4*, *KIR3DS1* is significantly associated with more rapid progression to AIDS. Thus, an additive effect of the two loci would not explain the observed protection in individuals who have both variants, and only a model involving a synergistic interaction between the two loci appropriately fits the observations. The effect was most apparent on progression to CD4 T cell depletion, suggesting that a protective response of NK cells involving *KIR3DS1* and its HLA class I ligands begins early after HIV-1 infection. These data provide a deeper understanding of the mechanisms involved in susceptibility to HIV-1 disease and may be useful in the development of effective therapeutics and vaccines against HIV.

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### **Inhibitors of HIV Integrase as Potential AIDS Therapeutics**

Anti-HIV based treatment of AIDS with combination drug therapies directed against multiple targets within the HIV life cycle are being studied. HIV integrase is an enzyme whose inhibition may be valuable in a multi-drug approach. Inhibitors of HIV integrase are being developed as potential anti-AIDS drugs in collaboration with the Laboratory of Molecular Pharmacology and the HIV Drug Resistance Program, CCR, NCI. Lead inhibitor structures have initially been derived from several sources, including a three-dimensional pharmacophore search of more than 250,000 compounds contained within the NCI's chemical repository. Promising compounds have been systematically explored through chemical synthesis of analogues to determine structure-activity relationships responsible for integrase inhibition. Information generated in this fashion has been applied to the design and preparation of new analogues having higher potency, reduced collateral cytotoxicity, and greater antiviral protective effects in HIV infected cells. Studies are underway to examine HIV integrase inhibition in whole cell systems and to obtain X-ray structures of inhibitors bound to the HIV integrase enzyme. Information obtained from such X-ray structures may provide a starting point for the computer-assisted design of potent new inhibitors. Current work focuses on beta-diketopropionic acids as a structural basis for integrase inhibitor design.

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## **Protein Structure Section**

We are using high-resolution X-ray diffraction to study the relationship between protein structure and function. Over the past years, our work has focused on four distinct areas:

### **I. Enzymes with Anticancer Properties**

We have been investigating crystal structures of several members of the family of L-asparaginases, some of which are used clinically as drugs directed against childhood lymphoblastic leukemia. While the mechanism of anticancer activity of these enzymes is not yet clear, we have concentrated on the studies of their enzymatic properties. We investigated a number of mutants of *Escherichia coli* L-asparaginase and many complexes of the *Erwinia chrysanthemi* enzyme with substrates and products, leading to the elucidation of the enzymatic mechanism. The structure of the latter enzyme, solved at 1 Å resolution, represents the largest protein investigated at atomic level. Another enzyme with potential therapeutic properties is Onconase, a cytotoxic ribonuclease isolated from frog eggs. We have been involved in reengineering this enzyme in order to make it applicable to human cancer therapy and to restore its activity in the absence of posttranslational modifications. The structure of the eosinophil-derived neurotoxin, another related antitumor ribonuclease, has also been evaluated by us at atomic resolution.

### **II. Cytokines and Cytokine Receptors**

Our section has been investigating the crystal structures of several cytokines and has made progress in preparing their receptor complexes. We have established that a helical cytokine, interleukin-10 (IL-10), is a domain-swapped dimer in which each compact half is composed of fragments of two identical molecules. The structure of a related cytokine encoded in the genome of Epstein-Barr virus has now been determined, providing the first glimpse of the molecular architecture of an agent used by the virus to control the host's immune system. We are studying complexes of IL-19 with its receptors.

### **III. Retroviral Enzymes**

Enzymes encoded by retroviruses such as HIV are prime targets for designing effective drug therapies. We have been studying the structure of native and drug-resistant HIV-1 protease (PR) complexed with inhibitors, with the aim of tracing the molecular basis of the resistance phenomenon. We have also determined the structures of related enzymes from feline immunodeficiency virus (FIV) and equine infectious anemia virus (EIAV). The latter PRs are poorly inhibited by most inhibitors of HIV-1 PR, including those in clinical use, although they are capable of cleaving HIV-1-derived sequences. To study the mechanism of drug resistance, we solved the structures of HIV-1, FIV, and EIAV PRs complexed with an identical inhibitor, while the studies of an inactive mutant of FIV PR with a substrate helped in delineating the catalytic mechanism. Another retroviral enzyme under investigation in our laboratory is integrase. We have solved the structure of the catalytic domain of avian sarcoma virus integrase in the presence and absence of divalent cations to atomic resolution and are attempting cocrystallization of complexes with different substrates and with monoclonal antibodies.

### **IV. Proteases and RNA-Processing Enzymes**

Our Section has been investigating the structures of a number of different proteases. In particular, we have discovered that the *Pseudomonas* serine-carboxyl protease (sedolisin) is a novel serine protease with a unique catalytic triad. The structures of a number of complexes of this enzyme, many of them solved at atomic resolution, have been helpful in the analysis of the mechanism of action of this family. We have also solved crystal structures of the plant aspartic protease phytepsin, as well as of inhibitor complexes of yeast proteinase A. Among the RNA-processing enzymes, we have solved the structure of RNA cyclase and of native and semi-reduced cyclic nucleotide phosphodiesterase from *Arabidopsis thaliana*.

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## **DIVISION OF CANCER EPIDEMIOLOGY AND GENETICS**

The [Viral Epidemiology Branch \(VEB\)](#) has used a variety of approaches to define the nature and magnitude of HIV-1-associated malignancies, including analyses of population-based data, prospective cohort studies, and laboratory investigations. Our current work has focused on Kaposi's sarcoma (KS) and its associated herpesvirus, HHV-8. We studied HHV-8 seroprevalence in the elderly since they are at highest risk of KS, and found 9% were seropositive by either K8.1 ELISA or immunofluorescence assays. We found evidence of sexual transmission in a study in Nigeria, based on higher prevalence of HHV-8 seropositivity among commercial sex workers and STD patients (31% in each) than in the general population (19%). We studied classical KS patients and HHV-8 seropositive controls in Italy, and unexpectedly found that KS risk for classical KS was approximately fourfold lower in cigarette smokers. We have continued our studies of HIV-infected hemophilia patients; among children with these conditions, we found that higher levels and rates of increase of HIV were each associated with increased mortality ( $P = .006$  and  $P = .03$ , respectively). We also verified that causes of death and prognostic factors for current HIV-uninfected hemophilia patients are similar to those noted before the HIV epidemic. We used T cell receptor excision circles and HIV-1 2-LTR episomal DNA to predict AIDS, and found PBMCs that have high levels of HIV-1 replication and low levels of recent thymic emigrants are associated with a substantially increased risk. In a study of twin pairs infected with HIV at birth, identical twins were not always infected by the same quasi-species and subsequent viral divergence appeared to depend on quasi-species stability, suggesting that factors intrinsic to HIV-1 are more important than host genetics in viral evolution. To clarify the effects of chemokine and chemokine receptor gene alleles on HIV-1 disease progression, we organized an international meta-analysis that confirmed strong protective effects of CCR-5 and CCR-2 variants. However, we found no consistent effect of CXCL12 (SDF-1) polymorphism. In a collaboration with the NCI Laboratory of Genomic Diversity, we found that epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. In a cohort of 6,570 injection drug users, we found HTLV-II infection was not significantly associated with mortality from any cause, suggesting that it is not a significant human pathogen, even when present with HIV infection. In an analysis of population-based cancer registration data through 1998, we found declines in KS and NHL incidence that may reflect the impact of highly active anti-retroviral therapy. Through our AIDS-cancer match registry, we determined that risk for hepatocellular carcinoma was higher in subgroups with high prevalence of hepatitis C virus (HCV) infection than in subgroups with low prevalence of HCV infection. However, we did not find an association of HCV viral load or genotype with liver disease risk in HIV co-infected hemophilia patients. Although HCV infection has been linked with NHL risk in some previous studies, we did not find an association in a prospective cohort study from northern California.

Z01CP05781-03 VEB

Period Covered: October 1, 2001 to September 30, 2002

Title of Project: HIV and AIDS Cancers

Principal Investigator: Charles S. Rabkin, M.D., M.Sc.

Other VEB/NCI Personnel: R.J. Biggar, J.J. Goedert, E.A. Engles, T.R. O'Brien

Cooperating Units: Laboratories of Genetics and Genomic Diversity, CCR, NCI

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**Broad Research Questions Currently Under Study**

Oral cancer remains a major cause of cancer mortality, with a five-year survival rate of less than 50% in patients diagnosed with oral cancer. Oral tumors, both benign and malignant, are early complications of the immunodeficiency caused by HIV infection/AIDS. A [National Institute of Dental and Craniofacial Research \(NIDCR\)](#) goal is to support outstanding research that will lead to the early detection, prevention, and treatment of oral cancers in HIV infected/AIDS patients. To this end, NIDCR is currently supporting extramural as well as intramural research on this topic.

Current studies are developing non-human primate models for Kaposi's sarcoma (KS) in oral cavity, and are using both animal, human, and *in vitro* models to study the oral biology of herpesviruses (e.g., HHV-8) associated with KS, oral immune responses to HHV-8, viral lysis of KS, as well as the natural history and transmission of HHV-8 in the oral cavity.

Epstein Barr Virus (EBV) and human papillomaviruses (HPV) persistently infect the oral cavity and are shed into surrounding tissues/saliva as a result of the immunosuppression in AIDS patients. Several projects are examining these viruses with regard to the host immune responses, reactivation and oral cancer. Other studies are focusing on viral genetic stability and host genetic susceptibility to these viral infections.

Oral hairy leukoplakia (OHL) is a benign epithelial hyperplasia of the lingual squamous epithelium in AIDS patients. The lesions are associated with reactivation of latent EBV infection. Studies are now underway to better understand the pathogenesis, detection, and treatment of OHL in these patients.

Finally, the prevalence of oral tumors is changing in the context of new therapies for AIDS. Studies are underway to evaluate the biological changes associated with therapy in order to further improve treatments and reduce treatment failures. Similarly, research is being supported to reduce oral cancer by strengthening the specific host immune responses.

**Important Study Findings/Highlights**

- In patients with AIDS, women have a lower prevalence of OHL and oral KS than men.
- The incidence of AIDS-related non-Hodgkin's Lymphomas was not affected by the introduction of highly active antiretroviral therapy (HAART). Among those, Plasmablastic lymphoma is a relatively new entity that is considered to be a diffuse, large B-cell lymphoma with a unique immunophenotype and a predilection for the oral cavity. The lymphoma cells stain strongly with plasma-cell-reacting antibodies VS38c/B-B4 and lack the CD20 and CD45 surface markers. The lymphoma appears to be associated with EBV and not HHV-8 infection, as most tumor cells express EBV-encoded RNA. Recognition of plasmablastic lymphoma is important because it represents an HIV-associated malignancy that predominantly involves the oral cavity, may mimic KS, and has a poor prognosis.
- The changes in the pattern of oral disease associated with HAART was assessed over the past 9 years in a San Francisco clinic. The data show a significant decrease in oral candidiasis, OHL, and KS over time, but no change in the occurrence of aphthous ulcers. There was an increase in salivary-gland disease and a striking increase in warts: three-fold for patients on antiretroviral therapy and six-fold for those on HAART ( $p = .01$ ). This pattern of oral disease in a referral clinic suggests that an increase in oral warts could be occurring as a complication of HAART.
- An intimate association between Kaposi's sarcoma-associated herpesvirus (HHV-8) and KS exists. It has been shown that HHV-8 encodes and expresses a large number of proteins with oncogenic potential. Such proteins include the latency-associated nuclear antigen (LANA), which can interfere with transcription of p53, and viral G protein-coupled receptor (GPCR), which induces angioproliferation. Intramural researchers at NIDCR have shown that HHV-8-GPCR, when over expressed in tissue culture cells, enhanced the expression and secretion of vascular endothelial growth factor through regulation of different intracellular signaling pathways. The results provide further evidence of the signal transduction pathways activated by HHV-8-GPCR and support the key role these receptors play in promoting the survival of viral-infected cells. Moreover, the findings also emphasize the importance of this G protein-coupled receptor in the development of HHV-8-related neoplasias.

- Investigators at the University of Michigan Dental School report that vascular endothelial growth factor, a potent mediator of angiogenesis that functions as a survival factor for endothelial cells, up-regulates Bcl-2 gene expression. Using a mouse model of tumor vascularized with human blood vessels, they showed that up-regulation of IL-8 levels and Bcl-2 expression in endothelial cells in the tumor microvessels enhances intratumoral microvascular survival and density and accelerates growth of oral squamous cell carcinoma or KS.
- A study of health-care providers found that, compared with dentists, medical clinicians fail to recognize 25%–60% of the HIV-related oral abnormalities, including tumors, and often describe the lesions inaccurately. Good teamwork between health care providers and more awareness of oral complications of AIDS-related lesions is warranted.
- HIV-infected adolescents develop the same types of oral lesions, including tumors, as are seen in HIV-infected adults. The prevalence of these lesions, however, was lower in teens than in adults.
- HIV-infected subjects with OHL displayed significantly higher levels of salivary IL-1 alpha and IFN-gamma compared with the HIV-infected individuals with no oral disease, and a higher level of IFN-gamma than from the healthy control subjects. These findings may be diagnostic as well as indicative of pathogenesis.
- The prevalence of oral lesions was assessed in a five-center subset of the Women's Interagency HIV Study (WIHS) and correlated with other features of HIV disease. Oral candidiasis and OHL were confirmed as being common features of HIV infection in women and appear to be associated with HIV viral load, immunosuppression, and various other behaviorally determined variables.
- Researchers at the University of North Carolina found a novel state of EBV infection with concurrent expression of replicative and transforming proteins. It is probable that both replicative and latent proteins contribute to OHL development and induce many of the histologic features of OHL, such as acanthosis and hyperproliferation. In contrast to other permissive herpesvirus infections, expression of EBV-transforming proteins within the permissively infected OHL tissue enables epithelial cell survival and may enhance viral replication.
- OHL is characterized by high-level replication of EBV and multiple EBV strains. Although multiple EBV strains were found in both the OHL and peripheral blood specimens, 13 of 16 (81%) patients showed evidence of strain identity for at least one strain and analysis of two patients suggested that EBV strains from OHL could infect the blood leukocytes. These data are consistent with active trafficking of EBV between the oral and blood compartments.

### **Total Number of Funded AIDS-Related Grants and/or Contracts**

FY2002: 36 projects funded on this topic.

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## Active Extramural AIDS-related Malignancy Trials (Active)

PROTOCOL NUMBER	SOURCE	TITLE	PRINCIPAL INVESTIGATOR
AMC 020	AIDS Malignancy Consortium	Pilot Study of the Safety and Feasibility of Autologous Peripheral Blood Stem Cell Transplantation for Patients With Relapsed AIDS-related <b>Lymphoma</b>	D. Scadden scadden.david@mgh.harvard.edu
AMC 031	AIDS Malignancy Consortium	Phase I Pilot Study of an Antigen-defined, GM-CSF Secreting Vaccine for HIV-related <b>Hodgkin's Disease</b>	R. Ambinder rambind@jhmi.edu
AMC 032	AIDS Malignancy Consortium	Phase II Infrared Coagulator for Treatment of High Grade Squamous <b>Intraepithelial Neoplasia of the Anal Canal</b> in HIV-infected Individuals	E. Stier stiere@mwkcc.org
AMC 033	AIDS Malignancy Consortium	Phase II Trial of Induction Therapy With EPOCH Chemotherapy and Maintenance Therapy with Combivir/Interferon for HTLV-1 Associated T-cell <b>non-Hodgkin's Lymphoma</b>	L. Ratner Lratner@imgate.wustl.edu
AMC 034	AIDS Malignancy Consortium	Randomized Phase II Trial of EPOCH chemotherapy and Rituximab for HIV-Associated <b>Non-Hodgkin's Lymphoma</b>	J. Sparano sparano@jimmy.harvard.edu
AMC 035	AIDS Malignancy Consortium	Phase I Study of Stressgen 00101 for <b>High-grade Anal Intraepithelial Neoplasia</b>	J. Palefsky joelp@medicine.ucsf.edu
AMC 036	AIDS Malignancy Consortium	Phase II Trial of Halofuginone in Patients With HIV-related <b>Kaposi's Sarcoma</b>	S. Krown krowns@mskcc.org
AMC 037	AIDS Malignancy Consortium	Zevalin Radioimmunotherapy for Patients With <b>Post Transplant Lymphoproliferative Disease</b> Following Solid Organ Transplantation	D. Scadden scadden.david@mgh.harvard.edu
AMC 039	AIDS Malignancy Consortium	Ultra-sensitive Infra-red Thermographic Analysis of <b>Kaposi's Sarcoma</b> Skin Lesions	C. Martins crmartin@jhmi.edu
AMC 041	AIDS Malignancy Consortium	Registration of HIV-infected Patients With Incident <b>Non-AIDS-defining Cancers</b> : A Pilot Study (prospective, observational study)	
AMC 030	AIDS Malignancy Consortium	Circulating Endothelial Precursors in <b>Kaposi's Sarcoma</b> During Active Treatment Observations: a Companion Study to AMC Trials	P. Browning philip.browning@email.vanderbilt.edu
RPCI-RP-9911	Roswell Park  Ohio State University	Phase I Study of Low-Dose Interleukin-2 and Stem Cell Factor in Patients With <b>AIDS-Related Malignancies</b>	Z. Bernstein zale.bernstein@roswellpark.org
CWRU-029828J, NCI-G02-2126	Case Western University	Phase II Study of Lomustine/Etoposide/Cyclophosphamide/Procarbazine (CECP) in Patients With AIDS-related Stage IIB-IV <b>Hodgkin's Disease</b>	S. Rermick scr@cwru.edu
BUMC-3756 NCI-V00-1609	Boston University	Phase I Study of Arginine Butyrate and Ganciclovir in Patients With <b>Epstein Barr Virus-Induced Malignancies or Lymphoproliferative Disorders</b>	D. Faller dfaller@bu.edu

<b>Intramural AIDS-related Malignancy Trials (Active)</b>			
<b>PROTOCOL NUMBER</b>	<b>SOURCE</b>	<b>TITLE</b>	<b>PRINCIPAL INVESTIGATOR</b>
00-C-0193	HAMB, CCR, NCI	A Study of the Effects of Potent Anti-HIV Therapy on Parameters Hypothesized to be Related to the Pathogenesis of <b>Kaposi's Sarcoma</b> in HIV-Infected Individuals	R. Yarchoan ry1n@nih.gov
01-C-0038	HAMB, CCR, NCI	Collection of Blood, Bone Marrow, Tumor or Tissue Samples From Patients with HIV Infection, KSHV Infection, Viral-related Pre-malignant Lesions, and/or <b>Cancer</b>	R. Yarchoan ry1n@nih.gov
01-C-0067	HAMB, CCR, NCI	A Phase II Study Liposomal Doxorubicin and IL-12 in AIDS-associated <b>Kaposi's Sarcoma</b> Followed by Chronic Administration of IL-12	R. Little rl48e@nih.gov
01-C-0030, NCI-2890	HAMB, CCR, NCI	Short-course EPOCH-Rituximab for Untreated CD-20+ HIV-associated <b>Lymphomas</b>	W. Wilson wilsonw@mail.nih.gov
01-C-0158	HAMB, CCR, NCI	Protocol to Assess Vascularity in <b>Kaposi's Sarcoma</b> Lesions Utilizing Non-invasive Imaging Techniques	R. Yarchoan ry1n@nih.gov
03-C-0110	HAMB, CCR, NCI	Phase II Study of Intravenous Recombinant Humanized Anti-Vascular Endothelial Cell Growth Factor Antibody (Bevacizumab) in Classical (HIV-negative) and in AIDS-associated <b>Kaposi's Sarcoma</b>	R. Yarchoan ry1n@nih.gov

<b>Intramural AIDS/HIV Trials (Non-malignancy, Active)</b>			
<b>PROTOCOL NUMBER</b>	<b>SOURCE</b>	<b>TITLE</b>	<b>PRINCIPAL INVESTIGATOR</b>
99-C-0134	HAMB, CCR, NCI	A Long-term Observational Study of Immunologic Reconstitution in HIV-1 Infected Children Who Are Receiving Combination Protease Inhibitor and Reverse Transcriptase Inhibitors	S. Zeichner zeichnes@mail.nih.gov
01-C-0038	HAMB, CCR, NCI	Collection of Blood, Bone Marrow, Tumor or Tissue Samples From Patients with HIV Infection, KSHV Infection, Viral-related Pre-malignant Lesions, and/or Cancer	R. Yarchoan ry1n@nih.gov
02-C-0006	HAMB, CCR, NCI	A Phase I Study of Tenofovir Disoproxil Fumarate (PMPA Prodrug), a Novel Nucleotide Analog Reverse Transcriptase Inhibitor, in Children with HIV Infection	S. Zeichner zeichnes@mail.nih.gov
02-C-0150	HAMB, CCR, NCI	Therapeutic Drug Monitoring and Viral Resistance Testing in the Treatment of HIV-infected Children	S. Zeichner zeichnes@mail.nih.gov
03-C-0084	HAMB, CCR, NCI	Collection of Blood Samples from Pediatric Patients HIV Infection and Their Family Members	S. Zeichner zeichnes@mail.nih.gov
03-C-0169	HAMB, CCR, NCI	Psychological and Environmental Factors Associated With Medication Adherence in Children and Adolescents with HIV Infection	L. Wood woodl@mail.nih.gov
OH99-C-NO18	HAMB, CCR, NCI	Collection of Samples for Studies Concerning the Pathogenesis and Immunology of Viral Diseases	S. Zeichner zeichnes@mail.nih.gov
Pending	HAMB, CCR, NCI	A Phase I Study of DPC 083, a Novel Non-nucleoside Reverse Transcriptase Inhibitor in Children with HIV-1 Infection	S. Zeichner zeichnes@mail.nih.gov
Pending	HAMB, CCR, NCI	Pilot Study of Postmenarcheal HIV-infected Adolescent Females: Cervical Cytopathology, HPV and HIV-1 Status, Mucosal Immunity, and Psychological Correlates of Pelvic Examination	L. Wood woodl@mail.nih.gov

Further information on NCI Clinical Trials can be obtained at <http://cancer.gov/clinicaltrials/>

**National Cancer Institute  
Funding for AIDS Related Malignancies  
(dollars in thousands)**

FY 2002 Actuals				
Mechanism	AIDS Related Malignancies	% of Total AIDS Related Malignancies	Total AIDS	% of Total AIDS
Grants	75,431	60.79%	106,904	42.02%
Contracts	8,213	6.62%	40,878	16.07%
Intramural	26,517	21.37%	91,288	35.88%
RMS	13,925	11.22%	15,326	6.02%
<b>Total</b>	<b>124,086</b>	<b>100.00%</b>	<b>254,396</b>	<b>100.00%</b>

FY 2001 Actuals				
Mechanism	AIDS Related Malignancies	% of Total AIDS Related Malignancies	Total AIDS	% of Total AIDS
Grants	84,457	69.92%	106,172	41.48%
Contracts	5,995	4.96%	56,807	22.19%
Intramural	19,865	16.45%	80,031	31.27%
RMS	10,477	8.67%	12,950	5.06%
<b>Total</b>	<b>120,794</b>	<b>100.00%</b>	<b>255,960</b>	<b>100.00%</b>

FY 2000 Actuals				
Mechanism	AIDS Related Malignancies	% of Total AIDS Related Malignancies	Total AIDS	% of Total AIDS
Grants	61,774	62.61%	100,415	41.13%
Contracts	9,002	9.12%	49,958	20.46%
Intramural	18,089	18.33%	82,072	33.62%
RMS	9,795	9.93%	11,700	4.79%
<b>Total</b>	<b>98,660</b>	<b>100.00%</b>	<b>244,145</b>	<b>100.00%</b>

FY 1999 Actuals				
Mechanism	AIDS Related Malignancies	% of Total AIDS Related Malignancies	Total AIDS	% of Total AIDS
Grants	64,972	70.06%	122,858	51.36%
Contracts	10,072	10.86%	36,191	15.13%
Intramural	11,210	12.09%	68,591	28.68%
RMS	6,483	6.99%	11,550	4.83%
<b>Total</b>	<b>92,737</b>	<b>100.00%</b>	<b>239,190</b>	<b>100.00%</b>