AIDS-ONCOLOGY RESOURCES HANDBOOK

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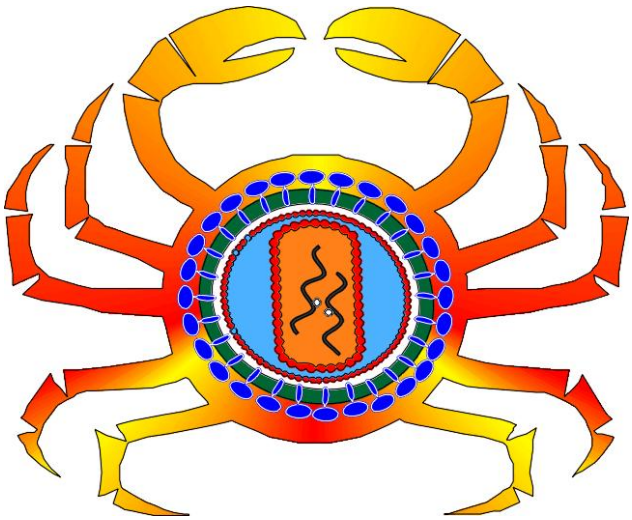

http://cancer.gov/dctd/aids/2004handbook.pdf

DIVISION OF CANCER TREATMENT AND DIAGNOSIS
NATIONAL CANCER INSTITUTE
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INTRODUCTION

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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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; Kaposi's sarcoma herpesvirus or human herpesvirus 8 ((KSHV/HHV8) 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 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 $a_3\beta_1$ integrin molecules on the surface of host cells. Initial attachment of gB to the $a_3\beta_1$ 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, PE-CAM and ICAM-1 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-1 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. Recent investigations have shown that the down-regulation of MHC I, PE-CAM and ICAM-1 occurs within 48 hours after de novo infection with KSHV/HHV8 in cultures of primary dermal microvascular endothelial cells in cells that express the latent cycle viral protein LANA.

- The latent KSHV/HHV8 protein LANA uses a signaling pathway (Wnt) to drive cell proliferation in infected cell lines through the accumulation of **$**-catenin. **$**-catenin enters the nucleus, where it associates with LEF/TCF nuclear proteins and induces specific genes involved in cell proliferation. A number of different tumor types without presently known viral etiologies have been described that use a similar strategy for **$**-catenin accumulation that leads to cell proliferation.

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Broad Research Questions Currently Under Study

All aspects of immunology and its relevance to cancer are included in the Cancer Immunology and Hematology Branch (CIHB). The CIHB supports three program areas: Cellular Immunology, Molecular Immunology and Hematopoiesis and Hematopoietic Malignancies. The Cellular Immunology program area supports research on the role of different cells in the immune response to tumors. These include T and B lymphocytes, regulatory T cells, macrophages, dendritic cells, natural killer cells, granulocytes the role of MHC class I proteins in antigen presentation and the integration of the innate and adaptive immune responses. The Molecular Immunology program area, supports research on genes and proteins of potential importance in regulating the immune response to transformed cells. This research program covers areas such as the B and T lymphocyte ontogeny, lymphocyte activation, the effect of somatic mutations on lymphomagenesis, immunodeficiencies, and the use of transgenic animals to address complex biological problems. All programs support research on the basic biology of AIDS and lymphomas.

Important Study Findings/Highlights

Although cell-mediated immune responses to HIV in peripheral blood have been extensively characterized, a recently initiated study will assess HIV specific CD8+ T lymphocyte immune responses in mucosal tissue in AIDS patients. Preliminary results demonstrate heterogeneity in expression of cytotoxic effector molecules among HIV+ CD8+ T lymphocytes from different anatomic compartments. Additional structural studies have led investigators to conclude that selective pressure from the host immune response generates variant gp120 envelope proteins that enable the virus to persist in the presence of vigorous immune response. Another program objective is to gain insight into pathogenic mechanisms involved in retrovirus-induced cell death in nervous and immune cells. Recent data using ts1 (a pathogenic retrovirus with similar tropism compared to HIV) indicate that cytotoxicity is triggered by ER stress, resulting in thiol deficiency and subsequent loss of protection against antioxidants (ie. ROS. Studies of B cell proliferation in HIV/AIDS patients indicate varying levels of antigen driven polyclonal B cell activation. 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.

A complicating factor in AIDS is non-Hodgkins lymphoma (AIDS-NHL) that becomes a significant risk when CD4 counts drop below 200mm/3. HIV/AIDS patients demonstrate polyclonal B cell activation/proliferation during their infection with 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.

Total Number of Grants and/or Contracts Funded Within CIB

FY2002: 140 with 79 (100% AIDS) and 61 (<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 herpesvirus 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, and 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

The debate over the effect of highly active antiretroviral therapy (HAART) on cervicovaginal HPV infection and cervical intraepithelial neoplasia in HIV-positive women is still ongoing. To date, the data suggest that the widespread use of HARRT has not led to a reduction in prevalence of genital HPV infections in HIV-positive women. However, data on the effect of HAART on the natural history of CIN are mixed. Some studies show no effect of HAART on CIN, whereas other studies show a modest positive impact. For example, after adjusting for HPV infection, CD4+ cell levels and cytologic status the women in the Women's Interagency HIV study were more likely to exhibit CIN regression and less likely to show progression. The difference in regression rates between women on and off HAART was small (36% vs. 30%). It is concluded that HIV positive women should continue to be actively screened and treated for CIN whether or not they are on HAART. *(Palefsky and colleagues, University o f California-San Francisco,CA*)

HPV type 16 is associated with approximately half of all cervical cancers. Although, HPV infection has an important role in the development of cervical neoplasm there may be other factors present for the infection to progress to a significant lesion. For this reason, a subcohort of 1392 women were selected from the on-going Ludwig-McGill HPV natural history epidemiologic study in Brazil to determine whether nutritional status may be an important cofactor, affecting both persistence of HPV infection and progression to CIN. This study offered the opportunity to conclude that increasing dietary intake of lutein/zeaxanthin, β-cryptoxanthin and vitamin C appear to be associated with reduced risks of persistence of type-specific HPV infection. In addition, consumption of papaya, a major source of dietary carotinoids was associated with reduced risk of persistent infection. *(Giuliano and colleagues, University of Arizona, AZ*)

It is unknown whether antibodies to HPV capsids, elicited by natural infection are protective. A definitive study on the protective role of naturally occurring anti-HPV virus like particle (VLP) antibodies to HPV types 16, 18 or 31 was proposed to address this question. The study evaluated 7046 women from Costa Rica who had follow-up HPV DNA results five to seven years after recruitment and had serostatus measured at baseline. No significant protection from natural infection was observed based on antibody detection. This study sets the stage for the introduction of the HPV VLP-based vaccinology. *(Burk and colleagues, Albert Einstein College of Medicine, NY*)

HPV is sexually transmitted and has been positively associated with the development of several epithelial anogenital cancers (cervical, anal, penile and vaginal cancers). Previous epidemiologic studies have also found positive associations between the risk of prostate cancer and sexual activity (i.e., age at first intercourse and numbers of sexual partners). To evaluate the potential relationship between HPV and prostate cancer, a population-based study was conducted in King County Washington on Caucasian and

African-American men that were between the age of 40 and 64. The data suggest that there was not an association between serologic evidence of HPV-16 (OR, 1.05; 95% CI, 0.71 – 1.57) or HPV-18 (OR, 1.36; 95% CI, 0.69 – 2.69) and the development of prostate cancer. Analysis of the clinical findings also revealed no relationship between HPV infection status and Gleason score, stage of disease, or disease aggressiveness. *(Stanford and colleagues, Fred Hutchinson Cancer Research Center, WA)*

It has been reported that the IL-10 promoter genotype is associated with slower progression to AIDS over five years. However, IL-10 is also a B cell stimulatory cytokine that can contribute to B cell hyperactivation and/or promote the growth of emerging lymphoma cells. Results of a cross-sectional study of IL-10 serum from HIV-infected MACS participants suggest that elevated serum IL-10 or the IL 10 promoter 592 C/C genotype is associated with the development of AIDS lymphoma. Detectable IL-10 was five times more likely to be seen in men who went on to develop AIDS-associated lymphoma compared to the CD4-matched controls with AIDS but with no malignancies. *(Martinez-Maza and colleagues, University of California-Los Angeles, CA*)

Total Number of Grants and/or Contracts Funded Within DCCPS
FY 2003: 47 (AIDS relevant) and 39 (<100% AIDS)

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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 the 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, natural history cohort studies of HIV+ men and women, and infrastructure at institutions that receive significant AIDS funding. In addition, the AMP is working to facilitate research on AIDS malignancies in the international arena.

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 other cancers. 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 repositories 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 (NIAID)-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 25 clinical trials with 5 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-α2β 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.
- Despite promising phase I and phase II studies, results of a randomized phase III study of IM862 using a 5 mg QOD dose failed to provide evidence for efficacy. However, HAART was associated with a substantial rate of sustained tumor response and this is the largest study to demonstrate the efficacy of HAART therapy alone. The unanticipated persistence of the HAART effect on AIDS-KS seen here may have contributed to prior estimates of IM862 response. Therapeutic trials for AIDS-KS must account for ongoing immune reconstitution in the setting of concurrent HAART that may confound estimates of therapeutic activity.
- 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.
- A phase 1 trial of the stressgen HPV E7-HSP vaccine for the treatment of high grade anal intraepithelial neoplasia recently completed accrual and analysis is underway.

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/.

Partners in AIDS Oncology Research

The AMP partners with other NIH Institutes and Centers to facilitate epidemiology and natural history studies, infrastructure, training, and international capacity building for research on cancer in HIV+ individuals.

The AMP supports studies of cancer epidemiology, natural history, and specimen repositories of the NIAID-sponsored Multicenter AIDS Cohort Study (MACS) (http://statepi.jhsph.edu/macs/macs.html) and the Women's Interagency HIV Study (WIHS) (https://statepiaps.jhsph.edu/wihs/). The MACS is an ongoing multicenter prospective study of the natural and treated histories of HIV-1 infection in homosexual and bisexual men, which began in 1984. Important study findings include:

- In addition to providing samples for the discovery of HHV8 by external investigators, the MACS investigators have conducted collaborative studies establishing the seropositivity of HHV8 infection with the development of KS (Gao SJ et al. Seroconversion to antibodies against Kaposi's sarcoma-associated herpesvirus-related latent nuclear antigens before the development of Kaposi's sarcoma. New England Journal of Medicine 1996;335:233-41).

- Research on immunopathogenesis of HHV8 infection has provided additional information on the role of cytotoxic cell responses in regulating HHV8 infection (Wang QJ et al. $CD8^+$ cytotoxic T lymphocyte responses to lytic proteins of human herpes virus 8 in human immunodeficiency virus type 1-infected and -uninfected individuals. Journal of Infectious Diseases 2000;182:928-32).
- Investigators have relied on MACS samples to investigate the clinical, immunological and virological parameters of primary HHV8 infection. Specifically, MACS investigators have shown that the development of KS is enhanced in men who acquire incident HHV8 infection after established HIV-1 infection (Jacobson LP et al. Interaction of human immunodeficiency virus type 1 and human herpesvirus type 8 infections on the incidence of Kaposi's sarcoma. Journal of Infectious Diseases 2000;181:1940-9).
- Recent studies have shown that DC-SIGN is a primary cell receptor for HHV8. Studies of the host genetics related to this viral receptor are being initiated using MACS specimens.
- With the use of highly active antiretroviral therapy, KS incidence has declined while that of non-Hodgkin's lymphoma did not show a clear decline (Jacobson LP et al. Impact of potent antiretroviral therapy on the incidence of Kaposi's sarcoma and non-Hodgkin's lymphomas among HIV-1-infected individuals. Multicenter AIDS Cohort Study. Journal of Acquired Immune Deficiency Syndrome 1999;21 Suppl 1:S34-S41).
- Elevated expression of immune system activation-associated molecules precedes the clinical appearance of AIDS-NHL (Martinez-Maza O et al. Elevated levels of serum sCD23, sCD27, and IgE precede the appearance of AIDS-associated non-Hodgkin's lymphoma. In: Guenounou M, ed. HIV and Cytokines Agents. Paris: Les Editoins INSERM, 1997:337-40).
- HIV+ individuals heterozygous for the CCR5- 32 mutation, or those who had a "low-expressor" IL10 promoter genotype, have a reduced risk for the development of AIDS-NHL **(**Breen EC. Pro- and anti-inflammatory cytokines in human immunodeficiency virus infection and acquired immunodeficiency syndrome. Pharmacol Ther 2002;95:295-304).
- The use of HAART prolonged overall survival among HIV-positive men diagnosed with KS and NHL even when initiated after the diagnosis of NHL and KS (Tam HK et al. Effect of highly active antiretroviral therapy on survival among HIV- infected men with Kaposi sarcoma or non-Hodgkin lymphoma. International Journal of Cancer 2002;98:916-22).
- A recent MACS study has shown an 18-fold increased risk of liver death in individuals coinfected with HIV and HBV compared to those only infected with HBV (Thio et al. The Lancet 2002).

The WIHS is a multicenter prospective study initiated in 1994 and designed to comprehensively investigate the natural history of HIV-1 infection in women in the United States. The WIHS has donated fresh-frozen tissue from lesional and non-lesions sites of the cervix, vaginal, and vulvar to the ACSR since 1999. Important study findings include:

- Based on results from the analysis of HPV test results from repeated studies in the WIHS and HIV Epidemiology Research Study (HERS), a weak association exists between HPV 16 detection and CD4 cell strata. HPV 16 may be better at avoiding the effects of immune surveillance, which could contribute to HPV 16's strong association with cervical cancer (Strickler et al. J Natl Cancer Inst 2003;95:1062-71).
- A recent study examining serum specimens from the WIHS and HERS showed that HPV 16 seropositivity was associated with the lifetime number of sex partners ($p<0.001$) among both HIV-infected and HIV-uninfected women. Approximately 50% of HIV-infected women had serological evidence of prior HPV-16 infection, but only approximately 5% had an HPV-16 cervical infection at baseline. Despite the higher prevalence of HPV infection in this group, most HIV-infected women are able to control HPV-16 replications at the cervix, and reactivation, if it occurs, is not very common (Viscidi et al. J Infect Dis 2003;187:194-205).
- The examination of cervical cancer incidence in WIHS revealed that invasive cervical cancer is uncommon in HIV-infected US women participating in a regular prevention program (Massad et al. AIDS 2004;18:109-113).

- A recent study of 503 HIV-infected WIHS participants over six years of follow-up revealed that recurrence and incidence of candidiasis are reduced by HAART, and that recurrence is reduced independently of CD4 and HIV-1 RNA (Greenspan et al. J Dent Res 2004; 83:145-150).
- HIV-infected WIHS women were found to have increased incidence rates for KS and non-Hodgkin's lymphoma, but not for invasive cervical cancer when compared to population-based expected rates (SEER data). In addition, both HIV-infected and uninfected women had increased incidence rates of lung cancer (Hessol et al. 7th International Conference on Malignancies in AIDS and Other Immunodeficiencies, Bethesda, MD, April 2003).

HIV-infected women tend to have lower than expected rates of breast cancer. Further research by WIHS investigators indicates that the apparent deficit of breast cancers among women in the WIHS can be explained by their overall lower risk, given the distributions in this population of a number of established breast cancer risk factors.

Centers for AIDS Research (CFARs)

The 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 21 sites across the US using the P30 mechanism. Additional information about the CFAR program is available at http://www.niaid.nih.gov/research/cfar/ .

International Research

Rates of HIV-associated malignancies have increased in developing countries as a result of the HIV epidemic, however international collaborations focusing on AIDS-associated malignancies involving US-based investigators and those in developing countries have been limited. This has hampered both the training of scientists in developing countries as well as research efforts in this area by US investigators. The NCI AMP is helping to strengthen the capacity for research in AIDS-related malignancies in these areas by partnering with the Fogarty International Center (FIC) in their AIDS International Training and Research Program (AITRP). The purpose of this program is to strengthen the capacity to conduct integrated prevention and care research across the full range of conditions and issues that relate to the care of adult and pediatric patients with HIV/AIDS, including cancer, in developing countries. The AITRP program will increase research training in AIDS associated cancers in those regions with significant HIV/AIDS incidence and prevalence and enhance US based research efforts by providing access to unique sources of patient populations, resources, and research collaborations. In FY 2003, the NCI co-funded four meritorious cancer relevant competitive supplement applications:

- The aim of Dr. Detels UCLA AITRP supplemental program is to develop the ability of trainees, primarily from Brazil and China, to perform advanced research in cellular and molecular epidemiology and pathogenesis relevant to AIDS malignancies associated topics. A secondary aim is to enhance the research goals of UCLA-based investigators with NIH-sponsored research projects in AIDS malignancies, by providing unique access to population, reagents, or expertise.
- Dr. Reingold was awarded supplemental funding to his very successful 16 year running AITRP program to develop a unique collaboration between the USCF CFAR and the University of California Berkeley AITRP to leverage the expertise of both programs for training and research capacity building in nine key regions with significant HIV/AIDS prevalence including Zimbabwe, Cambodia, Brazil, Uganda, India, Peru, Honduras, Thailand, and Ivory Coast. Members of the expanded team include the NCI funded investigators Jeff Martin, Ruth Greenblatt, and Joel Palefsky.
- Dr. Warren Johnson will conduct training in the Dominican Republic, where there is only a rudimentary understanding of the connection between HIV and HPV; no systematic HIV data collection or screening has been performed here to correlate HIV with abnormal cytology. The investigators plan to build upon the existing gynecologic services in place at Profamilia to determine if it's useful to use abnormal cytology as sentinel evidence to trigger HIV counseling and testing. Prospective follow-up will be implemented to evaluate the development of CIN and cancer.

- Dr. Woods proposes to expand training activities to China by collaborating with Nankai University in Tiajin. The critical increasing STD problem in China has been well documented, however, there is a gap in information about the impact of AIDS related malignancies on morbidity and the prevalence and incidence of HPV, and KSHV. The objectives of this expanded training program will facilitate acquisition of this information to better prepare Chinese health officials. To accomplish this goal, trainees will participate in the following coursework: 1) biomedical research rotations to investigate the role of HIV, herpesviruses, and papillomavirus in the development of AIDS associated malignancies, 2) rotations in clinical management of AIDS and AIDS related malignancies, 3) training in epidemiology, behavioral interventions, medical informatics and laboratory techniques, and 4) research ethics and regulatory documentation.

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 2004, the AMP sponsored the 8th International Conference on Malignancies in AIDS and Other Immunodeficiencies (ICMAOI).

Total Number of Funded AIDS-related Grants and/or Contracts in Office of the Director, DCTD
FY 2003: 76, 100% AIDS (R01, U01, R21, P01, P30, D43)

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Broad Research Questions Currently Under Study

- Database of HIV status of BMT recipients
- BMT as treatment for AIDS-related malignancies
- Treatment and prevention of viral and fungal infections
- Gene therapy to improve BMT outcome and treat HIV disease

HIV Status of Transplant Recipients

Dr. Mary Horowitz, principal investigator of a resource cooperative agreement (5U24CA76518-04, Medical College of Wisconsin) maintains a database on pretransplant HIV status of allogeneic and autologous blood or marrow transplant (BMT) patients and the clinical outcomes of HIV+ BMT patients. Eight-one patients out of 45,385 allograft recipients were HIV+. Thirty-three patients out of 22,549 autologous graft recipients were HIV+. Five HIV+ patients were transplanted for their HIV disease alone.

AIDS-related Malignancies

HIV+ patients are at increased risk for lymphoma and other malignant diseases. Standard treatment for lymphoma includes chemotherapy, monoclonal antibody therapy, and autologous blood or marrow transplant (BMT) for patients who relapse. Lymphomas in HIV+ patients tend to be more aggressive and thus more difficult to treat than those of HIV- patients. In addition, HIV+ patients may be more sensitive to the side effects of chemotherapy. Thus improved treatment for lymphoma in HIV- patients should benefit HIV+ patients with lymphoma, but additional improvements will be needed.

Investigators at the City of Hope Medical Center continue to follow 15 HIV-related lymphoma patients (median age, 43 years) who were treated with BMT between March 1998 and May 2001. All patients engrafted and the median follow-up for the surviving patients is 24 months. The two year probability of overall survival and disease-free survival (DFS) is 79%. This suggests that BMT is well-tolerated in patients with HIV-related lymphoma, with no apparent negative effects on the underlying HIV infection. It appears that transplant offers the possibility for durable remission and should be considered as an alternative to conventional salvage regimens for HIV+ lymphoma patients who relapse.

Immunotherapy using Rituxan, a monoclonal antibody against the CD 20 protein found on lymphoma cells, has emerged as an important new treatment for lymphoma. Investigators at the City of Hope Medical Center tested Zevalin (an antibody similar to Rituxan), but with a radioactive element attached which can kill lymphoma cells. Eighteen non-HIV-infected patients with follicular lymphoma, diffuse large-cell lymphoma or mantle cell lymphoma were treated with Zevalin plus hematopoietic cell transplantation (HCT) between May 2000 and December 2004. The treatment was well tolerated. Mucositis, neutropenic fever and skin rash were the most common acute toxicities. All patients engrafted; the median time to engraft white blood cells was 10 days and 18 days for platelets. One year estimated overall survival and disease-free survival are identical at 92%. These data suggest that high dose Zevalin can be given safely in combination with high dose chemotherapy and HCT. Zevalin plus BMT did not increase transplant related toxicity nor did it delay engraftment. The current Phase II study is continuing. A second trial of Zevalin combined with a chemotherapy regimen called BEAM for older patients with lymphoma has accrued twelve patents. There have been no transplanted related deaths, two relapses and the DFS at 11 months is 79%.

With funding from NCI's Quick Trial Program, investigators at Boston University have developed another potential new treatment regimen for lymphoma, and other tumors, which develop in Epstein-Barr virus (EBV)-infected cells. Unlike cytomegalovirus (CMV), which can be killed by treatment with ganciclovir, EBV does not routinely produce thymidine kinase (TK), the enzyme required for ganciclovir's activity. The study drug, butyrate, stimulates TK production. This enzyme then metabolizes ganciclovir into a toxic agent, which is incorporated into viral DNA and kills the tumor cells. The maximal tolerated dose of butyrate has been identified (1000 mg/kg/dose), with somnolence being

the dose-limiting side effect. So far, 11/16 patients had a decrease in tumor size after only one cycle of treatment. This response, in a patient population with refractory, bulky disease, suggests that this regimen may provide a promising new therapy for patients with a usually-fatal malignancy.

Stem Cell Gene Therapy for AIDS

The treatment of patients with HIV-related non-Hodgkin's lymphoma (NHL) and Hodgkin's disease (HD) is less successful than in the non-HIV setting, in part due to the aggressive character of these lymphomas but also due to the underlying HIV infection. High-dose therapy with stem cell transplantation has been used with success in the HIV-negative lymphoma setting for high-risk or relapsed disease. However, for patients with HIV-NHL and HIV-HD, ultimately the chance for long-term lymphoma-free survival also depends on successful control of the HIV infection. Gene therapy approaches may provide the opportunity for this long-term control by rendering the transplanted cells resistant to HIV infection.

Investigators at City of Hope Medical Center are studying BMT in conjunction with gene therapy for the treatment of HIV-associated lymphomas. Studies of gene-transduced human cord blood stem cells established parameters for proper vector design, demonstrated multi-lineage engraftment of transduced cells in mice, and showed that "true" stem cells had been targeted by their growing in secondary recipients. They also showed that cells transduced with vectors containing "HIV-resistance" genes avoided infection in culture. Studies in the mouse HIV-lymphoma model are underway to verify this finding in a whole-animal system. On a parallel track, AAV and Lenti viral vectors are being fitted with sequences designed to ensure site-specific insertion and other safety measures. Later this year, investigators plan to use AAV-transduced hematopoietic stem cells for transplant of HIV-lymphoma patients with anti-sense constructs that convey resistance to infection.

Anti-fungal Therapy

Several projects in Dr. Fred Appelbaum's program project (5P01CA18029-28, Fred Hutchinson Cancer Research Center) have AIDS relevancy. In project 5, Dr. Lawrence Corey is studying the T-cell response to aspergillus to better understand how to improve post transplant immune responses against this fungus. Development of a sensitive assay of T-cell immunity is expected to allow prediction of patients at greatest risk of infection.

The Blood and Marrow Transplant Clinical Trials Network, co-funded by the NCI and National Heart, Lung and Blood Institute (NHLBI), is conducting a phase III clinical trial entitled "A Randomized Double-blind Trial of Fluconazole vs. Voriconazole for the Prevention of Invasive Fungal Infections in Allogeneic Blood and Marrow Transplant Patients." 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 antifungal agents will also be assessed through the collection of adverse events and routine laboratory monitoring. In addition to determining which of the two drugs is more efficacious, this trial will also provide data regarding the predictive value of a clinical assay for Aspergillus (galactomannan assay) developed by BioRad Laboratories.

Anti-viral Therapy

Individuals infected with human cytomegalovirus (CMV) usually appear asymptomatic unless they receive immunosuppressive drugs (for example transplant recipients) or acquire an infection such as HIV, which allows reactivation of latent virus. Immunotherapeutic approaches to limit CMV morbidity and mortality in BMT, AIDS and other immunocompromised patients are currently under investigation as an alternative to antiviral drugs. A safe and effective vaccine to limit disease has been elusive.

Dr. Don Diamond and colleagues at the City of Hope Medical Center have a multi-faceted approach to develop and clinically deliver an efficacious and broadly-reactive vaccine. One strategy is to identify several parts of the virus which elicit a vigorous immune response and combine them in one vaccine.

These investigators have begun a series of clinical trials in BMT patients which will examine the advantages of donor immunization versus adoptive immunotherapy. Another approach uses gene therapy to induce neutralizing antibodies.

Investigators at the Fred Hutchinson Cancer Research Center are conducting clinical trials of anti-viral drug therapy in BMT patients. A randomized double-blind multicenter trial of valganciclovir vs. placebo for the prevention of late CMV infection is underway at seven centers (Fred Hutchinson Cancer Research Center, University of Florida, Mayo Clinic, Duke University, Memorial Sloan-Kettering Cancer Center, City of Hope National Medical Center, University of Michigan, M.D. Anderson Cancer Center). Eighty patients have been randomized with no major problems. Samples are being stored to measure CMV-specific immune responses and ganciclovir resistance. This randomized trial will demonstrate if valganciclovir is effective, describe any long-term toxicity, and provide data on the development of drug resistance and its impact on CMV-specific immune reconstitution.

A protocol for a randomized double-blind multicenter trial of valacyclovir vs. placebo has been developed to examine whether prevention of subclinical primary CMV with valacyclovir will reduce the post transplant development of life-threatening bacterial and fungal infections. 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.

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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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 preclinical 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 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. During 2002 GCOB transferred AIDS preclinical treatment-related R01 grants to the Office of the DCTD Director in an effort to consolidate the preclinical and clinical grants in this area in one office. The transfer involved all AIDS-related grants except those focused on chemistry and multi-disciplinary studies funded through cooperative agreements.

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 *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-engineered its drug discovery programs in both cancer and HIV/AIDS to emphasize molecular targets, especially those that represent particular vulnerabilities or are 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. In the future, more imaging agents or measurements of biomarkers are expected to be used in clinical trials to evaluate both "target" and "off-target" effects of new agents and to stratify patients. 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 that can be found at the DTP web site. The initiatives, which focus on potential targets for therapeutic intervention, use a variety of funding mechanisms. Once funding actions are complete, abstracts for each award are posted on the DTP web site at 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. Most new initiatives involve cooperation and collaboration with other areas of NIH. GCOB is cooperating with the NCI Office of Cancer Complementary and Alternative Medicine, National Center for Complementary and Alternative Medicine, National Institute of Nursing Research, and the National Institute of Dental and Craniofacial Research to invite research grant applications for basic and clinical complementary cancer research in PA-04-053: Developmental Projects in Complementary Approaches to Cancer Care. GCOB is representing NCI in a new initiative with the National Institute of Diabetes and Digestive and Kidney Diseases and the National Institute of Allergy and Infectious Diseases on PA-04-031: Development of Assays for High Throughput Drug Screening. The purpose of this PA is to encourage the development of high throughput small molecule screening for use in both research and drug discovery programs by funding the development of innovative assays that may be adapted for automated screening. The assays could be used to identify new tools for basic research and promising new avenues for therapeutics development.

Public-Private Partnerships are being encouraged by NIH in several Roadmap initiatives as a way to deal with complex issues such as speeding progress toward better treatments (http://www.nihroadmap.nih.gov/). Since 1983 GCOB has supported multidisciplinary or team science through the National Cooperative Drug Discovery Group (NCDDG) program, which involves partnerships between academia, industry and government to create new anticancer therapies. On January 16, 2004, GCOB issued a Request for Applications to continue the NCDDG program, and especially solicited applications for AIDS-related malignancies. Applications are due May 19, 2004 and awards are planned for 2005. During 2003 DTP competed the Academic Public Private Partnership Program (AP4), a new partnership arrangement that will require funding from both government and industrial sources. However, the principal investigator must be from an academic institution. Planning grants for this new endeavor are undergoing review, with award anticipated in 2004. GCOB also is co-funding several 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. New awards were made during 2003. 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.

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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) also offers screening and mechanistic follow-up as a service to the research community (http://www.niaid.nih.gov/daids/PDATguide/HIVThera.htm).

The NIH Inter-Institute Program for the Development of AIDS-Related Therapeutics (http://dtp.nci.nih.gov/docs/dart/dart.html) was introduced in January 2001 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. This Program 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. Details regarding approved projects from the first five cycles of review are available at the bottom of the webpage listed above. Among other IIP projects, 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, several 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 and may provide relevant drug development leads for the future. Progress has been marked in the development of a HTS assay for human herpesvirus 8 (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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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. Using a microplate-based HHV8 polymerase (Pol) and processivity factor (PPF) DNA synthesis assay (HHV8 Pol/PPF assay), established in collaboration with Dr. Robert Ricciardi of the University of Pennsylvania, we completed the screening of the NCI Diversity Set and identified 28 hit compounds with greater than 50% inhibition. The inhibitory activities of 23 of 26 hit compounds available for further investigation were confirmed by the recombinant enzyme-based assay with the IC50 concentrations ranging from 0.23 ± 0.04 (mean ± SD) to 15.78 ± 1.26 μM. Automation of HHV8 POL/PPF assay is currently being pursued to promote future HTS campaigns. We also uncovered anti-HHV8 activity of a new thymidine analog, n-methanocarbathymidine (N-MCT), using HHV8-infected BCBL-1 cell-based assay. We found that IC50 of N-MCT was up to 10-fold lower than cidofovir, one of the most potent HHV8 Pol inhibitors identified to date. Mechanistic characterization of N-MCT anti-HHV8 activity is ongoing in collaboration with Dr. Victor Marquez, NCI and Dr. Riad Agbaria, Ben-Gurion Univ. of the Negev. One of the newer HHV8 molecular targets pursued in our laboratory is latency-associated nuclear antigen (LANA). LANA tethers HHV8 DNA to host chromosomes, playing a key role in the maintenance of episomal HHV8 in host cells. LANA-binding element of viral DNA has been mapped to specific sites within the terminal repeat (TR) of HHV8 genome by Dr. Rolf Renne of the Case Western Reserve University. Small molecules that disrupt or interfere with the LANA-DNA binding may hinder or suppress the critical functions of LANA. In collaboration with Dr. Renne, we are currently trying to establish LANA-DNA binding inhibition assay using fluorescence polarization-based methodology toward the development of anti-LANA drug screening assay.

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 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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Lentiviral Vectors for Gene Therapy and Functional Genomics

While a source of great misery, lentiviruses can be modified and channeled toward usefulness as vectors for gene transfer in gene therapy, functional genomics, and animal model transgenesis. Because of their unique properties, they are ideally suited for gene transfer into non dividing cells, including stem cells. They are also vehicles of choice for siRNA delivery for gene function validation and for molecular target-directed therapy. The lentiviral vectors may well be the only means available to create transgenic animals of species other than the mouse, barring heroic efforts. For full exploitation of their translational potential, three inter related issues must be addressed - regulation, targeting, and safety.

Lentiviral vector regulation

To achieve the goal of acquiring the ability to regulate vector expression in vivo from outside and at will, we hypothesize that molecule(s) exist, or can be made to exist, that modulate all biological functions. As proof of principle, we have chosen to target the transcriptional apparatus of the lentiviral vectors, focusing on the viral LTR promoter, to search for such a drug-like molecule. In parallel, we are also targeting cellular hTERT promoter, as a control for the viral promoter. We have undertaken high throughput screening of chemical and natural products libraries in a cell-based assay. Genetically modified cell clones for the cell-based assay were created that carried a cassette of the promoter (viral LTR and cellular hTERT) linked to the indicator GFP gene for read out, and puromycin-resistance gene for cell selection. The clones met the following criteria: low basal activity of the promoters, responsiveness to known modulatory signals (Tat for LTR promoter and c-Myc for hTERT promoter), stable maintenance of the transgene cassette, and deficient MDR activity of the cell itself. Once screening of the 'diversity set' part of the chemical library is completed to validate the assay and set the parameters of the search, a campaign to screen thousands of compounds will be mounted. The current results suggest that fine mapping of the determinants and more defined exchanges will yield vectors with the desired characteristics.

Lentiviral vector safety

The major concern of safety is the generation of replication competent recombinants during vector production, or in patients who may be infected with HIV. We hypothesize that lentiviruses HIV-1 and HIV-2 are functionally homologous but are divergent enough in sequence that the chimera derived from them will have minimized the chance of recombination during vector production. Similar argument holds that the vectors derived mainly from HIV-2 will be safer for gene therapy of HIV-1 infected individuals and conversely, HIV-1 derived vectors better for HIV-2 infected individuals. The exercise here is to design chimeric vectors with enhanced safety but with no loss of efficiency of gene transfer. Utilizing the knowledge of the determinants of encapsidation and transduction, we have created chimeric vectors with judicious exchange of genes and regulatory elements between HIV-1 and HIV-2.

Vector validation

An integral part of the research effort is to validate vectors with model genes and promoters. We have chosen induction of cell death of a neoplastic cell as a model. With the idea that two hits are better than one, our objective is to promote apoptosis by delivering Bax transgene and suppress cell survival by delivering Bcl2 siRNA. The challenge of creating high titer lentiviral vectors with cytotoxic transgenes, such as Bax, was initially under appreciated. One solution to this problem was to use a regulated promoter, preferably a eukaryotic promoter with relevance to cancer. Ideally there will be a promoter that is, or can be, down regulated in the producer cell line and is, or can be, up regulated in the target cell. We are using cancer cell-selective hTERT promoter as a prototype. The search for drug-like molecules to up or down regulate hTERT promoter noted above is tied to this idea. Whether this will be sufficient to produce high titer Bax transgene and Bcl2 shRNA vectors remains to be determined.

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http://ccr.cancer.gov/labs/lab.asp?labid=38

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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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 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 addition, we have initiated a protocol to study amonoclonal anti-VEGF antibody (bevacizumab) in patients with 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 etopside, 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. Finally, we are currently planning a clinical trial to investigate the natural history and targeted virolytic therapy in multicentric Castleman's disease (MCD).

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, Drs. David A. Davis, Robert Yarchoan, and colleagues 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. Finally, we are attempting to design novel protease inhibitors that work by binding to the dimer interface.

We recently made the observation that hypoxia can activate lytic replication of Kaposi's sarcoma herpesvirus (KSHV), also known as human herpesvirus 8 (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 focusing additional efforts on analyzing the upregulation of ORF34 and related genes by hypoxia. We are also studying Epstein-Barr virus for a similar response to hypoxia.

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Pediatric HIV/AIDS Clinical Research

The pediatric HIV clinical research program in the HIV and AIDS Malignancy Branch (HAMB) 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. Studies of this agent, 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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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 interested in understanding how the host cell may be involved in the maintenance of HIV latent infections. In other projects, we are working to understand the viral replication and pathogenesis strategies of Kaposi's sarcoma-associated herpesvirus (KSHV). 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, and are now doing additional experiments to dissect the viral gene expression program using inhibitors that block viral replication at known points in the viral life cycle. We are making several cell lines that inducibly express KSHV genes to determine their effects on KSHV replication and the viral gene expression program. We expect that these studies will provide new insights into 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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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 high-risk 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. Based on this observation and using a special strategy to limit 16E6E7 RNA splicing, we have identified three nuclear localization signals (NLS) for the first time in HPV16 E6E7 that drive the protein to accumulate in the nucleus. When inserted into corresponding regions of low-risk HPV6 E6, the three NLSs sequences described for 16E6 were active in converting the normally cytoplasmic HPV6 E6 into a nuclear protein. We are also 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 does not 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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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 eight 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, and 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 RRL. The RRL includes the Vector Design and Replication Section, under the direction of Dr. Stephen H. Hughes, the Retrovirus Assembly Section, directed by Dr. Alan Rein, the Model Development Section, led by Dr. Vineet N. KewalRamani, and two sections that joined the Laboratory in 2003, the Virus-Cell Interaction Section, under the direction of Dr. Eric O. Freed, and the Retrovirus Gene Expression Section, directed by Dr. David Derse.

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.

The research efforts of Dr. Derse's lab are directed toward understanding the molecular genetics of HIV-1 and human T cell leukemia virus (HTLV-1). The latter is associated with adult T cell leukemia and degenerative neurologic diseases. Although HTLV-1 is only distantly related to HIV-1, both viruses evade the immune system and are slow to cause disease. The premise that virus-encoded regulatory factors and elements largely determine pathological consequences of infection, such as tissue tropism, disease spectrum, latency, and cytopathology, has motivated Dr. Derse and the members of his lab to identify and characterize the complex regulatory circuits that modulate virus gene expression and alter host cell metabolism. Analyses of HIV-1 and HTLV-1 genetics combined with comparative analyses of distant relatives of the human viruses have provided novel insights into the regulatory strategies and pathogenic mechanisms of these agents.

Collectively, the interests of the five 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 RT, its interaction with templates and substrates, and its role during replication in mutation and recombination. The RML consists of three sections: RT Biochemistry Section, under the direction of Dr. Stuart Le Grice, Viral Recombination Section, under Dr. Wei-Shau Hu and 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_{50}) 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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Regulation of Retrovirus RNA Splicing and Transport

Infectious transmission of retroviruses can be divided into two phases: 1) the assembly and release of virions from the infected cell and 2) virion entry and replication in the target cell. For human T-cell leukemia virus type 1 (HTLV-1), the molecular mechanisms underlying the individual steps in these processes are not well defined. Recent experimental evidence supports a model in which HTLV-1 can be transmitted directly between T lymphocytes that have formed a stable cell-cell synapse. The model suggests that infection may be coordinately regulated with formation of the cell-cell junction. However, the integration of cellular and viral pathways has not been defined. Our goal is to elucidate the components and pathways of HTLV-1 transmission and replication in the conceptual framework of this model. We are examining the mechanisms of HTLV-1 infection and replication with cell-free and cell-associated virus particles produced from viral vectors. We are characterizing HTLV-1 replication enzymes and analyzing reverse transcription in infected cells. We are also determining the mechanisms that HTLV-1 has evolved to evade cellular defense mechanisms that restrict the replication of other retroviruses. We are also studying the mechanisms and pathways of virion assembly and release. An important part of these studies are experiments aimed at defining the interactions between virus proteins and cellular membrane proteins that direct the virion to the site of cell-cell contact, where HTLV-1 is transmitted to the target cell. Although the basic mechanisms for transmission and replication are conserved among retroviruses, variations on common themes are likely to reveal important insights into the strategies that different viruses use for propagation and persistence in vivo. In this respect, it is useful to compare HTLV-1 and HIV-1; both are T-cell-tropic retroviruses whose distinct biological properties may be traced back to divergent strategies for escaping host-mediated immune and cellular defense mechanisms.

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Although not well-appreciated, pathogens are associated with a number of human malignancies. These include cancers with limited geographic distribution as well as common cancers with global impact: gastric cancer (helicobacter pylori); bladder cancer (Schistosoma haemtobium); adult T cell leukemia/lymphoma (Human T-cell leukemia virus (HTLV)-1); hairy cell leukemia (HTLV-2); hepatocellular carcinoma (Hepatitis B virus (HBV)); Kaposi's sarcoma [Human herpes virus-8, human immunodeficiency virus (HIV)-1]; nasal pharyngeal carcinoma and Burkitt's lymphoma [Epstein Barr virus (EBV)]; anogenital and cervical carcinoma (Human papilloma virus, HIV-1); and lymphomas (HIV-1). Pathogen infections are notable for the diversity of host responses to infection, rate of progression and disease outcome. Our laboratory has focused on differential responses to viral pathogens due, in part, to naturally occurring genetic variation among people. 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, the hepatitis B and C viruses (HBV and HCV) and EBV. We are also investigating the role of host genetic variation in the development of EBV persistant infection leading to nasal pharyngeal carcinoma in a cross-sectional case-control study of Han Chinese with chronic EBV infection and nasal pharyngeal carcinoma.

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 a viral pathogen 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 for HIV-1, HBV, HVC and in a nasopharyngeal carcinoma (NPC) case-control study. We have identified and characterized SNP organization and haplotype structure for: 1) The *APOBEC3G* (*CEM15*) gene, an RNA editing enzyme that introduces G->C hypermutation in retroviruses including HIV-1Δ*vif* (deleted for the vif accessory gene); we identified a codon changing variant that may be associated with accelerated progression to AIDS and 2) the *MCP-1 MCP-3 – Eotaxin* chemokine gene cluster. We have identified a single haplotype associated with resistance to infection by HIV-1. We have also confirmed that genetic variants in MIP1A are strongly associated with more rapid progression to AIDS. These studies provide proof of principle that chemokines and cytokines may be potential targets for drug development. To date more than 12 host genetic factors that are involved with viral cell entry, innate resistance or acquired immune response have been shown to effect HIV-1 infection or progression to AIDS.

In an effort to identify genes associated with NPC, one of the most common cancers in South China and Southeast Asia, we have organized case-control study of 366 NPC cases, 218 IgA antibodies positive to EBV capsid antigen (EBV/IgA/VCA) cases (IgA+), and 419 spouses with EBV/IgA/VCA negative (IgA-). EBV-associated NPC is caused by the interaction between genetic susceptibility and environmental factors. A recently reported genome-wide linkage analysis of familial NPC implicated a susceptibility locus on short arm of chromosome 4. We have completed genotyping 34 microsatellite markers in the region to confirm the family study and to identify NPC candidate genes for further analysis.

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Anti-HIV Activity of the Chemopreventive Drug, N-(4-hydroxyphenyl) Retinamide (4-HPR)

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

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Potent Cross-Reactive HIV-1 Neutralizing Human Monoclonal Antibodies

Major achievements include the identification and characterization of several potent broadly HIV-neutralizing human monoclonal antibodies that may have potential as therapeutics and help in the design of vaccines. Among those the scFv m9 exhibits the most potent and broad neutralizing activity especially for clade B and C isolates – it neutralized with high potency (on average IC50 of 1 ug/ml) almost all (90%) of tested 20 clade C isolates and 15 clade B isolates. Some of the results are described in the publications listed below

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We and others have identified a human homologue of *CD209*, which we termed *L-SIGN* (*CD209L1*), corresponding to a partial sequence described previously. Like CD209, CD209L1 captures HIV-1 through gp120 binding and enhances HIV-1 infection of T cells *in vitro*, but it is specifically expressed on liver sinusoidal cells and on vascular-associated cells in the lymph node rather than on DCs. Thus, we proposed that CD209L1 might facilitate interactions between liver sinusoidal endothelium and trafficking lymphocytes, as well as function in the pathogenesis of HIV-1 in a DC-independent manner. The identification of two *CD209* family homologues in humans raised the possibility that additional members of this gene family might exist in rhesus monkeys and other nonhuman primates. We found that all Old World monkeys (OWM) and apes tested have orthologues of human *CD209*, and although *CD209L1* is present in apes, it is missing in OWM. Further, we discovered a third family member in rhesus monkey, *CD209L2*, which is also present in OWM and all apes except for humans. Thus, the CD209 gene family has undergone recent evolutionary processes involving duplications and deletions, which may result in differential expression and function across primate species.

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Inhibitors of HIV Integrase as Potential AIDS Therapeutics

The reliance of current AIDS treatments on combination regimes directed at just two enzymes, HIV reverse transcriptase and HIV protease, has lead to a loss of therapeutic effectiveness through development of resistance. HIV integrase (IN) is a third enzyme required for HIV reproduction that represents an attractive target for new AIDS-directed antivirals. Accordingly, one focus of our research is the design and synthesis of IN inhibitors as potential anti-AIDS drugs. Currently, our efforts are directed toward two aspects of inhibitor development. In one project novel "azide" functionality, which we recently showed enhances cellular efficacy of "diketo-aryl" (DKA)-based IN inhibitors, is being examined for its potential utility in other IN-directed platforms. In a second project, the use of IN-directed "affinity acylators" is being undertaken to study the molecular basis IN•inhibitor interactions. This work seeks to develop small molecules that specifically bind and trans-acylate amino acid side chains at sites of inhibitor binding to the IN protein. Such covalent modification could effectively tag and identify the sites of inhibitor binding as well as potentially inactivate or modify enzyme activity.

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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 the 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 have solved the crystal structure of IL-19 and are studying the complexes 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. Another related enzyme, kumamolisin-As, is a thermostable collagenase. We solved crystal structures of its free and inhibited forms, as well as of an active-site mutant. We have also solved crystal structures of the catalytic and substrate-recognition domains of an *E. coli* protease Lon, finding that this is a unique enzyme among serine proteases. 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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The Viral Epidemiology Branch (VEB) has used a variety of approaches to define the nature and magnitude of malignancies associated with HIV-1 and other chronic infections, including analyses of population-based data, prospective cohort studies, and laboratory investigations. We are currently conducting a limited follow-up of 2,480 subjects with CDC-defined AIDS (i.e., <200 CD4+ T-cells/ul) recruited in 1998-99. Analyses of this cohort have identified alcohol drinking as a risk factor for antibody reactivity to human herpesvirus 8 (HHV-8), the Kaposi's sarcoma (KS)-associated herpesvirus. Among HHV-8 seropositives, alcohol was additionally associated with increased risk of KS. Similarly, cigarette smoking was protective both against HHV-8 antibody as well as against KS risk among HHV-8 seropositives. Alcohol and cigarettes each appear to have consistent effects at multiple stages of KS etiology, and further studies are planned to identify the underlying mechanisms. Nested case-control studies of incident cancers are planned, utilizing the detailed clinical and interview data and banked blood specimens (plasma and cryopreserved lymphocytes) collected at enrollment. We are currently planning an intensive laboratory investigation of non-Hodgkin's lymphoma (NHL)-associated mutations in these lymphocyte samples and their potential relationship to risk of NHL. Findings will be compared for HIV-related and unrelated NHL. Our two previous cohort studies of HIV-infected hemophilia patients have been incorporated into a larger cohort that includes HIV-uninfecteds, whose prospective followup for cancer has recently been initiated. These cohorts will be applied to studies of potential risk factors for AIDS-related malignancy, including genetic polymorphisms and/or altered baseline levels of cytokines and other immune factors, chromosomal translocations, EBV and HHV-8 viral loads, antibody titers, and cell mediated immunity, and environmental exposures and medications. We are continuing our studies of EBV infection and immune response as a causative factor in AIDS-related lymphoma, with determinations of viral load, cell-mediated immunity, antibody reactivity, and other immune system parameters. These studies also include examination of host genetic variation in B-cell-stimulatory cytokines. In collaboration with the Occupational Epidemiology Branch, we are continuing our investigations of cytokine polymorphisms as risk factors for gastric cancer. In a collaboration with the Veterans' Administration, we are investigating a cohort of liver disease patients originally recruited in the 1970s, for which we are assessing effects of baseline hepatitis C viremia and subsequent infection with HIV.

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October 1, 2002 to September 30, 2003	Other VEB/NCI Personnel: RJ Biggar, JJ Goedert, EA Engles
Title of Project: HIV and AIDS Cancers	Cooperating Units: Laboratory of Genetics, 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 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 nine 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 overexpressed 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.

Scientists at the NIDCR have recently developed an *in vivo* high throughput endothelial specific retroviral gene transfer system, and use it to specifically express candidate HHV-8 oncogenes in mouse endothelial cells. Among the many HHV-8 genes tested, only one gene, the HHV-8-GPCR, was able to promote the development of visible dermal and internal vascular tumors that strikingly resemble human KS lesions. Furthermore, they provided evidence that HHV-8-GPCR can initiate Kaposi's sarcomagenesis, and that cells expressing this gene can further promote the cancerous growth of cells expressing other HHV-8 genes, such as those expressed during the latent phase of HHV-8 infection. These findings further support a critical role for HHV-8-GPCR in initiating KS tumor development, and suggest that this receptor may represent a suitable target for anti-KS drug development. *Cancer Cell. 2003 Jan;3(1):23-36*

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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Extramural AIDS-related Malignancy Trials (Active)

PROTOCOL NUMBER	SOURCE	TITLE	PRINCIPAL INVESTIGATOR
AMC 020	AIDS Malignancy Consortium	Pilot Study of the Safety & Feasibility of Autologous Peripheral Blood Stem Cell Transplantation for Patients With Relapsed AIDS-related **Lymphoma**	D. Scadden scadden.david@mgh.harvard.edu
AMC 032	AIDS Malignancy Consortium	Phase II Infrared Coagulator for Treatment of High Grade Squamous **Intraepithelial Neoplasia of the Anal Canal** in HIV-infected Individuals	E. Stier stiere@mwkcc.org
AMC 033	AIDS Malignancy Consortium	Phase II Trial of Induction Therapy With EPOCH Chemotherapy & Maintenance Therapy With Combivir/Interferon for HTLV-1 Associated T-cell **Non-Hodgkin's Lymphoma**	L. Ratner Lratner@imgate.wustl.edu
AMC 034	AIDS Malignancy Consortium	Randomized Phase II Trial of EPOCH Chemotherapy & Rituximab for HIV-associated **Non-Hodgkin's Lymphoma**	J. Sparano sparano@jimmy.harvard.edu
AMC 035	AIDS Malignancy Consortium	Phase I Study of Stressgen 00101 for **High-grade Anal Intraepithelial Neoplasia**	J. Palefsky joelp@medicine.ucsf.edu
AMC 036	AIDS Malignancy Consortium	Phase II Trial of Halofuginone in Patients With HIV-related **Kaposi's Sarcoma**	S. Krown krowns@mskcc.org
AMC 037	AIDS Malignancy Consortium	Zevalin Radioimmunotherapy for Patients With **Post Transplant Lymphoproliferative Disease** Following Solid Organ Transplantation	D. Scadden scadden.david@mgh.harvard.edu
AMC 038	AIDS Malignancy Consortium	A Pilot Trial of Valproic Acid in Patients With **Kaposi's Sarcoma**	R. Ambinder rambinder@jhmi.edu
AMC 039	AIDS Malignancy Consortium	Ultra-sensitive Infra-red Thermographic Analysis of **Kaposi's Sarcoma** Skin Lesions	C. Martins crmartin@jhmi.edu
CWRU-029828J, NCI-G02-2126	Case Western University	Phase II Study of Lomustine/Etopside/Cyclophosphamide/ Procarbazine (CECP) in Patients With AIDS-related Stage IIB-IV **Hodgkin's Disease**	S. Remick scr@cwru.edu
BUMC-3756 NCI-V00-1609	Boston University	Phase I Study of Arginine Butyrate & Ganciclovir in Patients With **Epstein Barr Virus-Induced Malignancies or Lymphoproliferative Disorders**	D. Faller dfaller@bu.edu

Intramural AIDS-related Malignancy Trials (Active)			
PROTOCOL NUMBER	**SOURCE**	**TITLE**	**PRINCIPAL INVESTIGATOR**
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 **Kaposi's Sarcoma** in HIV-Infected Individuals	R. Yarchoan ry1n@nih.gov
01-C-0030, NCI-2890	MOCRU, CCR, NCI	Short-course EPOCH-Rituximab for Untreated CD-20+ HIV-associated **Lymphomas**	W. Wilson wilsonw@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, &/or **Cancer**	R. Yarchoan ry1n@nih.gov
01-C-0067	HAMB, CCR, NCI	A Phase II Study Liposomal Doxorubicin & IL-12 in AIDS-associated **Kaposi's Sarcoma** Followed by Chronic Administration of IL-12	R. Little rl48e@nih.gov
01-C-0158	HAMB, CCR, NCI	Protocol to Assess Vascularity in **Kaposi's Sarcoma** 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) & in AIDS-associated **Kaposi's Sarcoma**	R. Yarchoan ry1n@nih.gov

Intramural AIDS/HIV Trials (Non-malignancy, Active)			
PROTOCOL NUMBER	**SOURCE**	**TITLE**	**PRINCIPAL INVESTIGATOR**
OH99-C-NO18	HAMB, CCR, NCI	Collection of Samples for Studies Concerning the Pathogenesis & Immunology of Viral Diseases	S. Zeichner zeichnes@mail.nih.gov
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 & Reverse Transcriptase Inhibitors	S. Zeichner zeichnes@mail.nih.gov
01-C-0025	HAMB, CCR, NCI	A Phase I Study of Capravine (AG-1549), a Novel Non-nucleoside Reverse Transcriptase Inhibitor in Children with HIV-1 Infection	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, &/or Cancer	R. Yarchoan ry1n@nih.gov
02-C-0006	HAMB, CCR, NCI	A Phase I Study of Tenofir Disproxil 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 & 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 & Their Family Members	S. Zeichner zeichnes@mail.nih.gov
03-C-0169	HAMB, CCR, NCI	Psychological & Environmental Factors Associated With Medication Adherence in Children & Adolescents with HIV Infection	L. Wood woodl@mail.nih.gov
04-C-0106	HAMB, CCR, NCI	Proton Magnetic Resonance Spectroscopy & Neuropsychological Functioning in Children With HIV: A Pilot Study	S. Zeichner zeichnes@mail.nih.gov

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

Funding for AIDS Related Malignancies
Fiscal Year 1999 - 2003
(dollars in thousands)

	FY 2003 Actuals			
Mechanism	AIDS Related Malignancies	% of Total AIDS Related Malignancies	Total AIDS	% of Total AIDS
Grants……..	60,260	54.19%	114,457	44.99%
Contracts……..	7,775	6.99%	41,000	16.12%
Intramural……..	30,506	27.43%	92,485	36.35%
RMS……..	12,663	11.39%	15,500	6.09%
Total -	**111,204**	**100.00%**	**263,442**	**103.56%**

	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%
Total -	**124,086**	**100.00%**	**254,396**	**100.00%**

	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%
Total -	**120,794**	**100.00%**	**255,960**	**100.00%**

	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%
Total -	**98,660**	**100.00%**	**244,145**	**100.00%**

	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%
Total -	**92,737**	**100.00%**	**239,190**	**100.00%**