Combating HIV, One Tumor at a Time

Robert Yarchoan, M.D., vividly remembers the day in 1981 when, as a Clinical Fellow in the Metabolism Branch of NCI, he saw a young man who had developed a profound immunodeficiency. That patient, who had almost no T lymphocytes, represented a moment in medical history—the first NIH patient with what would come to be known as acquired immunodeficiency syndrome (AIDS). Since that time, Yarchoan, now Chief of the HIV and AIDS Malignancy Branch and Director of the newly formed NCI Office of HIV and AIDS Malignancy (OHAM), has seen firsthand how HIV/AIDS has risen as a global epidemic, how the development of highly active antiretroviral therapy (HAART) has revolutionized AIDS treatment, how malignancies associated with AIDS have tested both patients and doctors, and how the long-term effects of living with AIDS can bring with them a new host of challenges.

The discovery by Luc Montagnier and Robert C. Gallo of a novel human retrovirus, and Gallo's demonstration that this virus was the cause of AIDS, were the first major milestones in our efforts to understand, prevent, and treat the disease.

An estimated 35 million people suffer from HIV/AIDS worldwide, about one million of whom live in the United States. However, when the first patient with AIDS crossed the threshold of the NIH Clinical Center in 1981, we had no way of foreseeing the shape that the future AIDS epidemic would take or the global, cultural, and societal impacts that it would have. All we knew was that we were seeing something new. It was soon evident that clusters of cases of rare tumors like Kaposi's sarcoma were part of this same condition. And over several months, it became apparent that that this disease was widespread in the U.S., and that the more severe patients with AIDS we were seeing represented just the tip of the iceberg.

There was great confusion and distress in the medical community as AIDS first surfaced. Though heroic measures were often utilized to try to treat these patients, they generally died within several months. It was not known how many people had this disease, how it was transmitted, or if anything could be done to treat it effectively.

The NCI had many tools in place to address this new disease, including expertise in immunology, retrovirology, tumor biology, and drug development. I believe that the rapid development of advanced treatments for HIV, in particular highly active antiretroviral therapy (HAART), was the result of a keen and farsighted, though sometimes controversial, research focus at NCI on HIV itself.

To Treat a Virus

The discovery by Luc Montagnier and Robert C. Gallo of a novel human retrovirus, and Gallo's demonstration that this virus was the cause of AIDS, were the first major milestones in our efforts to understand, prevent, and treat the disease. Prior to these discoveries, most of the research on AIDS was descriptive.

Once the scientific community understood what was causing the immunodeficiency, it was possible to envision effective therapy directed at the root cause. Then-NCI Director Vincent DeVita, Jr., M.D., asked Samuel Broder, M.D., and his group, of which I was a member, to spearhead an effort to develop treatments for AIDS. Broder, Hiroaki Mitsuya, M.D., Ph.D., now Head of the Experimental Retrovirology Section of the HIV and AIDS Malignancy Branch, and I constructed the hypothesis that by blocking HIV replication, it might be possible to reverse the immunodeficiency caused by the virus. This idea was somewhat controversial at the time, as no such therapies existed for other progressive viral diseases, and it was

not clear that blocking HIV would lead to immunologic improvement. In a short time, our group in NCI developed the first therapies to effectively fight HIV infection, including zidovudine (AZT), didanosine (ddI), and zalcitabine (ddC). AZT was developed in collaboration with Burroughs Wellcome Co., while ddI and ddC were developed within NCI and then licensed to pharmaceutical companies. These drugs all blocked an essential and unique enzyme of HIV, reverse transcriptase. The initial clinical trials of these drugs, both singly and in combination, gave rise to the first effective anti-HIV treatment regimens.

A limitation of these initial regimens was the frequent development of viral resistance. Thus, about a decade later, the first drugs were developed that attacked a different HIV target, the viral protease. The creation of these so-called protease inhibitors, which block the active site of HIV protease, was in part based on structural studies of this enzyme conducted in the NCI-Frederick program. When combined with two nucleoside reverse transcriptase inhibitors (like AZT and ddI), protease inhibitors were able to suppress HIV replication dramatically, often to undetectable levels. This combination drug therapy, the backbone of HAART, revolutionized the treatment of HIV. Patients improved clinically and had marked improvement in their immune function. There was a dramatic drop in the death rate from AIDS. In addition, there was a marked decrease in the number of AIDS-associated tumors, and survival of patients with these tumors improved. For our contributions to the development of HAART, Mitsuya and I were honored in 2006 with the first NIH World AIDS Day Award (Figure 2).

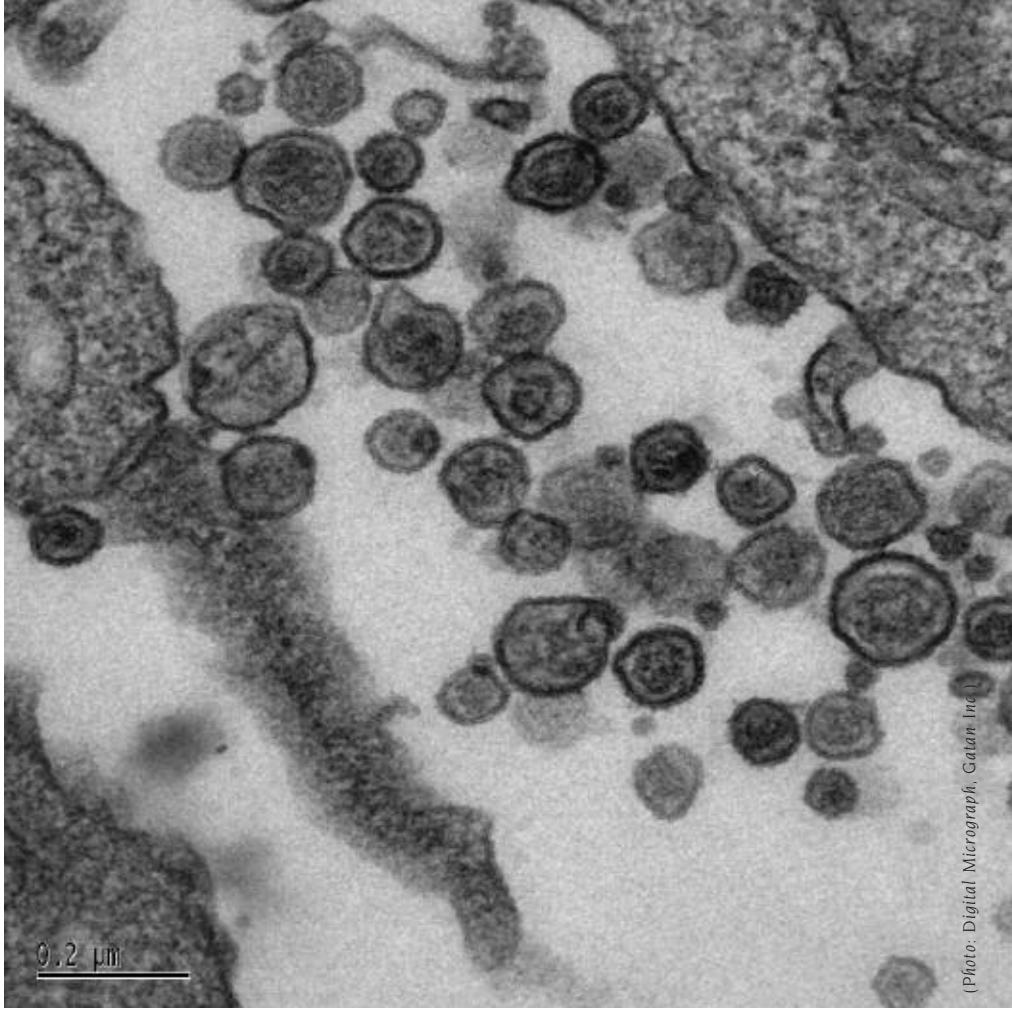


Figure 1: Tireless work on the part of researchers at NCI and other institutions has led to dramatic decreases in the mortality associated with HIV/AIDS and AIDS-related malignancies. But challenges still remain.

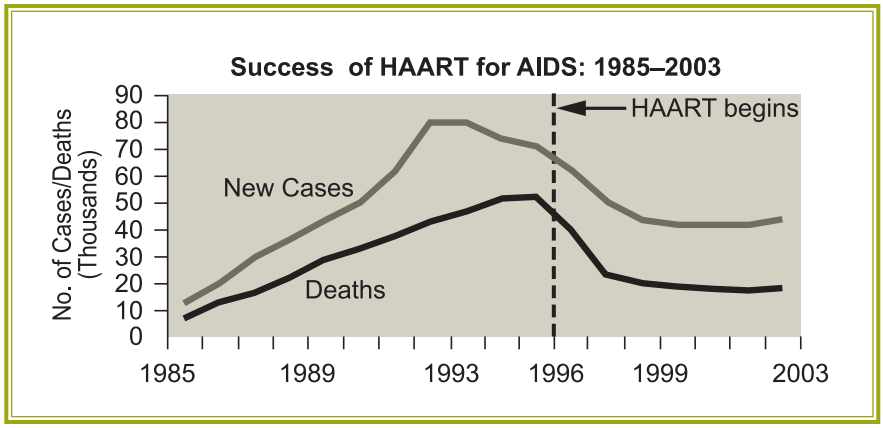
> This combination drug therapy, the backbone of HAART, revolutionized the treatment of HIV patients.

The first-generation protease inhibitors, while effective, had significant side effects and required high doses to be effective. Moreover, the development of resistance has been a vexing problem. Therefore, a number of scientists (including Mitsuya) and pharmaceutical companies have been looking into the development of improved, second-generation protease inhibitors. Darunavir, developed by Mitsuya and collaborators and approved by the U.S. Food and Drug Administration in 2006, is one such drug. Darunavir has the advantage of retaining its activity even in patients who have become resistant to other inhibitors.

For several years, my colleague David Davis, Ph.D., and I have been studying the possibility of inhibiting HIV protease by a new mechanism: by blocking the linkage of its two monomers into an active dimer. Working with the two of us, Mitsuya recently found that in addition to blocking the enzyme's active site, darunavir does just this, making the drug quite different from other approved HIV protease inhibitors. This observation was highlighted as one of CCR's major scientific advances of 2007 (see "Multiple Strategies for Attacking HIV").

The Tumors of AIDS

One of the hallmarks of HIV is a dramatic increase in the incidence of certain unusual cancers, especially Kaposi's sarcoma and certain aggressive B-cell lymphomas. Kaposi's sarcoma was frequently the cause of death for patients in the early days of the AIDS epidemic. This rare sarcoma—which, prior to the rise of HIV/AIDS, had primarily been known as a cancer of elderly men of Mediterranean or Eastern European Jewish descent—develops in the endothelial cell lining of blood vessels and often affects



(Graph: R. Yarchoan, CCR)

Figure 2: The development of highly active antiretroviral therapy (or HAART) has been the driving force behind a revolution in the treatment of HIV/AIDS.

the skin, though it may also involve the mouth, gastrointestinal tract, and lungs. The tumors appear as angry red and purple patches, often on the legs and feet. These lesions are a visible manifestation of HIV disease, often causing substantial psychological distress for patients in addition to severe pain and debilitation.

The cause of Kaposi's sarcoma remained a mystery until 1994, when Yuan Chang, M.D., and Patrick Moore, M.D., M.P.H. (now at the University of Pittsburgh), discovered a new gammaherpesvirus and showed it to be the cause of Kaposi's sarcoma. We now know that this virus, called Kaposi's sarcoma-associated herpesvirus (KSHV) or human herpesvirus-8 (HHV-8), is also the etiologic agent of two other tumors seen in AIDS patients—primary effusion lymphoma (PEL) and some cases of multicentric Castleman's disease (MCD).

After focusing initially on treatments for HIV, my group started turning its collective attention to AIDS-associated malignancies. In the early 1990s, we showed that paclitaxel (Taxol®) was active against Kaposi's sarcoma. Then, since Kaposi's sarcoma is a cancer of blood vessels, we began exploring anti-angiogenic approaches that would interfere with blood vessel growth. We subsequently showed that thalidomide, a strongly anti-angiogenic agent, was also active against the tumor.

More recently, my colleagues James Pluda, M.D., Giovanna Tosato, M.D., and Richard Little, M.D., and I have turned our attention to the cytokine interleukin-12 (IL-12), a powerful immune system regulator, as a possible treatment for Kaposi's sarcoma. We believed that there were at least three ways in which IL-12 could act against Kaposi's sarcoma:

- It could enhance the immune response against KSHV.
- It could act as an anti-angiogenic agent, shrinking the cancer's heavily vascularized tumors.
- It could work through another protein, a chemokine called inducible protein-10 (IP-10), which inhibits a virally encoded G-protein-coupled receptor that is important for the pathogenesis of Kaposi's sarcoma.

We carried out a clinical study, published in 2006, showing that IL-12 was active against Kaposi's sarcoma when used as a single agent. Subsequently, we decided to see if more rapid and complete responses could be attained by combining IL-12 with a cytotoxic agent, pegylated liposomal doxorubicin (or Doxil®), with known activity against Kaposi's sarcoma.

This research led to the launch of a Phase II clinical trial, spearheaded by Little, in 36 patients with advanced AIDS-associated Kaposi's sarcoma who were either on HAART or needed urgent therapy. All of the patients received IL-12 plus Doxil for 18 weeks, followed by IL-12 alone.

Thirty of the patients demonstrated significant responses, including nine complete remissions. Most of the patients maintained, or even improved, their condition while on the IL-12 only portion of the study, so we published the results in December 2007. While this regimen has yet to be tested in a controlled trial, the uncontrolled results are quite promising.

Coming to the Foot of the Matter

Working within CCR provides physician-scientists exceptional opportunities to conduct translational research—studies that can be viewed as a two-way street between the laboratory and the clinic. For some time, clinicians treating Kaposi's sarcoma had been struck by the tendency of this tumor to preferentially develop in certain parts of the body, particularly the feet. The feet are relatively poorly oxygenated, raising the possibility that hypoxia might have some connection to the growth and development of Kaposi's tumors. Later, in discussing this finding with colleagues, it dawned on us that PEL, another of the tumors caused by KSHV, also formed in a site with limited oxygenation (pleural effusions, accumulations of fluid within the chest cavity).

In thinking about these peculiarities with my colleagues, we considered the possibility that KSHV might somehow be responsive to hypoxia. Like other herpesviruses, KSHV can undergo either latent or lytic replication. My group, including Davis and Muzammel Haque, Ph.D., found that in PEL cell lines the virus was activated to lytic replication by hypoxia, and that certain specific genes of the virus could directly respond to hypoxia-inducible factors (HIF) produced by host cells. This was the first time that any virus had been shown to respond to hypoxia or HIF, and it gave great insight into the pathogenesis of KSHV-induced tumors (see "Why Do Kaposi's Sarcoma Lesions Most Often Develop on the Feet?").

This observation led us to consider a novel therapeutic approach for KSHV-induced associated tumors. When activated by hypoxia or other factors, KSHV expresses two lytic genes that encode enzymes capable of catalyzing AZT and another antiviral drug, ganciclovir, into forms that are toxic to human cells. We reasoned that this capability could be used to target tumors caused by KSHV, in particular PEL and MCD, a disease marked by serious, sepsis-like illness caused in part by the production of a form of the cytokine interleukin-6 encoded by KSHV

(*Photo: B. Branson*)

Robert Yarchoan, M.D.

itself. MCD is also unique among KSHV-related diseases because the virus's lytic genes are already activated.

Together with Little and Deirdre O'Mahony, M.D., we are investigating the use of these two drugs in patients with MCD in a unique clinical trial that is also helping us to further understand the disease's natural history. This trial plays to one of CCR's main strengths: its ability to recruit patients from around the nation and study very rare diseases. Thus far, about half of the MCD patients treated with high doses of AZT and valganciclovir (a prodrug of ganciclovir) have responded. The trial involves a collaboration of many groups within NCI and the NIH that we hope will lead to a better understanding of MCD's pathology and responses to therapy; in the future, we hope to explore this approach in patients with PEL as well. But this chain of events—from the clinical observation of Kaposi's anatomical distribution, to the discovery of KSHV's hypoxic elements, to a novel therapeutic option for MCD—is a prime example of the how clinical observation can lead to laboratory insights that then lead back to the clinic to inform the design of a new treatment.

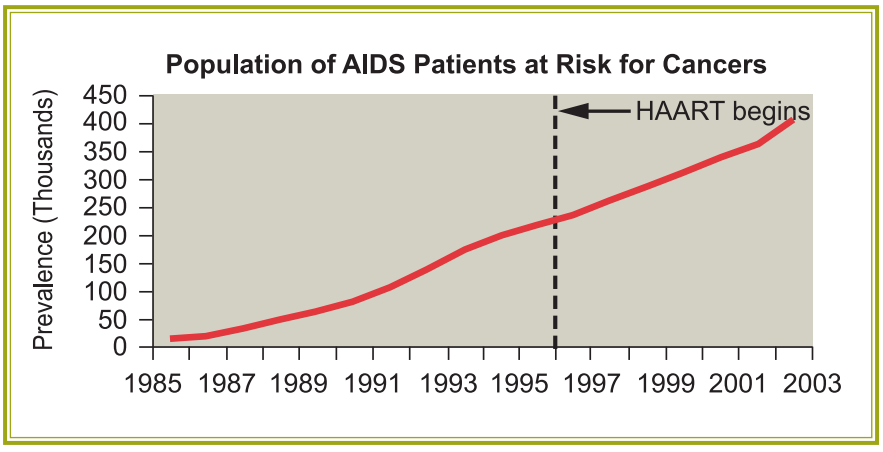
Long-Term Survival, Long-Term Challenges, and Long-Term Opportunities

The development of HAART has arguably been the greatest advance in the prevention and therapy of AIDS-related tumors. One of the major victories of HAART has been a reduction in the incidence of certain AIDS-associated malignancies, especially those that develop in patients with markedly reduced CD4 counts. Patients are living for years on HAART, compared to the weeks or months that they might have hoped for 20 years ago.

However, as we enter the second decade of HAART therapy, the epidemiology of AIDS-related tumors and diseases is changing (Figure 3). In some patients, Kaposi's sarcoma tumors that had been under control for years are beginning to grow again. Also, tumors other than those "classically" defined as AIDS-related, such as Hodgkin's disease, lung cancer, and anal carcinoma, are on the rise in the HIV-infected population. The number of deaths from opportunistic infections and advanced AIDS is dropping, while cancer is fast becoming the most common cause of death in AIDS patients. In the meantime, in Africa and other parts of the world where HAART is not commonly available, HIV-associated tumors continue to be major causes of morbidity and mortality.

These new trends, as well as the new opportunities afforded by recent scientific advances, will require a renewed research effort by the National Cancer Institute. To this end, the NCI Director has recently created a new Office of HIV and AIDS Malignancy (OHAM) that will coordinate AIDS and AIDS malignancy research throughout the institute. At the same time, CCR has launched a new Center of Excellence in HIV/AIDS and Cancer Virology, led by Stuart Le Grice, Ph.D. With the renewed interest in this area and the creation of these two groups, the NCI is poised to have a substantial impact on AIDS malignancy and AIDS research on a global scale.

> As we enter the second decade of HAART therapy, the epidemiology of AIDS-related tumors and diseases is changing.



(*Graph: R. Yarchoan, CCR*)

Figure 3: The epidemiology of AIDS-related tumors and illness is changing as the number of AIDS patients in the United States grows. This represents an increasing population at risk of developing malignancies.

Why Do Kaposi's Sarcoma Lesions Most Often Develop on the Feet?

(*Photo*: NCI)

Because of hypoxia-responsive genes encoded by the virus that causes Kaposi's sarcoma, the lesions of this tumor have a tendency to show up in relatively poorly oxygenated tissues, like the feet.

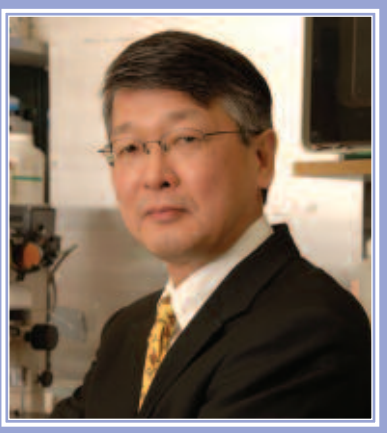
In his original description of Kaposi's sarcoma in 1872, Moritz Kaposi noted that the purple, brown, or black lesions of the condition tended to occur on his patients' feet. Over time, researchers and physicians alike have been struck by a predilection of Kaposi's to involve the feet and other areas of the body with a poor blood supply. It has been speculated that this tendency is because the legs and feet often have relatively low tissue-oxygen levels (hypoxia). Interestingly, another tumor caused by Kaposi's sarcoma-associated herpesvirus (KSHV), primary effusion lymphoma (PEL), arises in pleural effusions—accumulations of excess fluid in the chest with no direct blood supply and, consequently, poor oxygenation.

Generally, after initial infection, herpesviruses enter a dormant or resting phase, allowing them to remain within the host for years undetected only to arise when stimulated by various factors. This stimulation to lytic replication—which results in the rupture of the host cell—generally involves one or more switch genes, which in turn activate a cascade of viral genes.

In exploring KSHV-induced tumors' tendency to arise in hypoxic areas, Robert Yarchoan, M.D., and his colleagues found that lytic replication KSHV in PEL cell lines is activated in hypoxic cells. In addition, the team found that certain specific KSHV genes could be directly activated by hypoxia, including a cluster of lytic genes stretching from ORF34 to ORF37. Cells exposed to hypoxia express increased levels of so-called hypoxia-induced factors (HIFs). Yarchoan's group found that the ORF34–37 gene cluster could be directly stimulated by the binding of HIFs to a single viral hypoxia response element (HRE) located in the cluster's promoter region. Because some of these KSHV genes play important roles in the pathogenesis of KS or other tumors, their hypoxic activation could thus help explain why these tumors arise where they do.

His group is now exploring the possibility that the activation of certain KSHV genes by hypoxia could be used as a means to treat tumors caused by KSHV. Two hypoxia-induced viral genes, ORF21 and ORF36, can chemically modify the drugs ganciclovir and zidovudine (AZT) into forms toxic to cells. Yarchoan is now testing whether these two drugs can be used to specifically target KSHV-induced tumors in which ORF21 and ORF36 are activated by hypoxia or other means.

Multiple Strategies for Attacking HIV



(*Photo*: B. Branson)

Hiroaki Mitsuya, M.D., Ph.D.

While highly active antiretroviral therapy (HAART) is able to keep levels of HIV very low and deter the onset of AIDS, it does not provide a cure. Lingering viral reservoirs persist undectable in the blood and other tissues, ultimately enabling HIV to develop resistance to antiretroviral drugs and begin propagating despite HAART. The identification of new types of drugs that attack HIV in different ways might help keep these drug-resistant viruses in check.

A group led by Hiroaki Mitsuya, M.D., Ph.D., recently developed a new antiretroviral called darunavir, which was specifically designed to tightly bind and inhibit the HIV protease. Darunavir is more potent than most available first-generation protease inhibitors (PIs), and it can block replication of HIV strains resistant to multiple other PIs.

Mitsuya recently teamed up with his long-standing collaborator Robert Yarchoan, M.D., and other researchers to learn more about how darunavir inhibits HIV protease. The HIV protease is made up of two protein monomer subunits that must come together, or dimerize, to create the mature, active form of the enzyme. It is the mature protease that plays a critical role in HIV replication.

Mitsuya, Yarchoan, and colleagues found that darunavir can work just like other PIs that have been used for years—by preventing the mature protease from processing other HIV proteins. However, they discovered that darunavir has another trick up its sleeve—it is also able to prevent the two parts of the protease from dimerizing. This study marked the first time that a small molecule was found to disrupt protease dimerization. Because it inhibits HIV protease using a unique mechanism (i.e., inhibition of protein dimerization), darunavir—and possibly other molecules like it—may be useful for treating individuals infected with HIV that have developed resistance to more traditional PIs and other antiretroviral therapies.