The Plot Thickens: KSHV and Molecular Piracy

Yuan Chang, MD
Professor of Pathology | College of Physicians & Surgeons
Columbia University | New York, New York

Summary by Tim Horn
Edited by Patrick Moore, MD, MPH, and Jeffrey Roth, MD, PhD

Reprinted from *The PRN Notebook*,® March 2002.
Dr. James F. Braun, Editor-in-Chief. Tim Horn, Executive Editor.
Published in New York City by the Physicians' Research Network, Inc.,®
John Graham Brown, Executive Director. For further information and other articles available online, visit **http://www.PRN.org**

Perhaps the greatest advance in the area of AIDS-related malignancies has been the identification of human herpesvirus-8 (HHV-8), also known as Kaposi's sarcoma-associated herpesvirus (KSHV). Since its discovery by Drs. Yuan Chang and Patrick Moore and their colleagues almost eight years ago, KSHV has been identified in virtually all AIDS- and non-AIDS-related KS lesions. At the same time, several research teams have identified the virus in a subset of other less common pathologic conditions, including primary effusion lymphomas (PEL) and multicentric Castleman's disease (MCD). But while a definitive link exists between KSHV and these specific malignancies, the precise role that it plays in their development is just now coming into focus.

This article is based on a PRN lecture delivered by Dr. Chang in November, 2001. In essence, it is a more focused update of an extensive review that originally appeared in the December 1999 issue of *The PRN Notebook* discussing KSHV, KS, and MCD, which can be easily accessed through the PRN web site (http://www.prn.org). Information related to the approved and experimental treatments for KS, as highlighted by Drs. Susan Krown of Memorial Sloan-Kettering Cancer Center and Patrick Hennessey of New York University Medical Center, while not discussed in this article, can be found in the previously published summary.

Backgrounder: KS and HIV

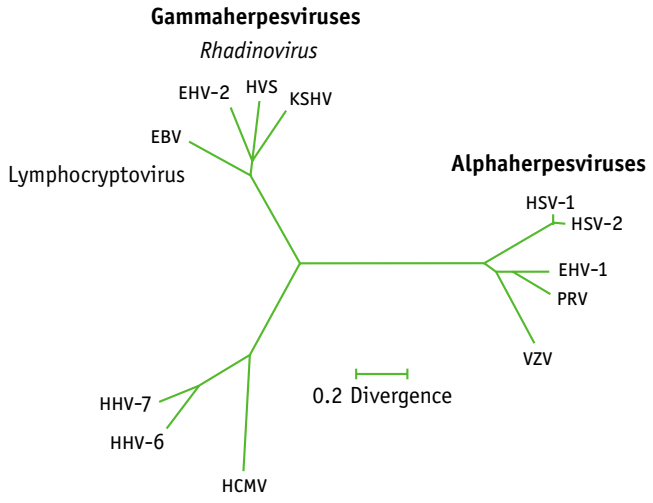
Kaposi's sarcoma (KS) was first described in 1872 by the Austro-Hungarian dermatologist Moritz Kaposi. He described five new cases of "idiopathic multiple pigmented [skin] sarcomas" of the lower extremities in elderly men (Kaposi, 1872). One patient died of gastrointestinal bleeding 15 months after the initial appearance of the skin lesions, and an autopsy showed visceral lesions in the lungs and the gastrointestinal tract. Subsequently, other investigators described four clinical variants of KS that had identical histologic features but developed in specific populations and had different sites of involvement and rates of progression.

Classic KS is characterized by indolent, purple-blue or reddish-brown skin lesions of the extremities seen in elderly white males of Ashkenazi or Mediterranean descent. While rarely fatal and slow in its progression—it can take years or decades for lesions to spread centrally from the hands and feet—approximately 10% of individuals with classic KS can go on to experience mucosal or systemic involvement.

Endemic KS, first described in 1934, typically involves African children and adults—usually males. There are two clinical types: cutaneous and lymphoproliferative. While most cases of endemic KS involve the cutaneous variety, tumors are more aggressive than the classic variety and are often associated with a poor prognosis. This is especially true of the lymphoproliferative type, with death usually occurring secondary to disease within two to three years.

There have also been numerous reports of KS occurring in patients receiving

FIGURE 1. Phylogenetic Tree of Known Human Herpesviruses as Well as Several Other Herpesviruses That Have Nonhuman Hosts



The tree was derived by comparing the amino acid sequences of the major capsid protein gene. Kaposi's sarcoma–associated herpesvirus/human herpesvirus 8 (KSHV; HHV-8) is a member of the subfamily of gammaherpesviruses, genus *rhadinovirus*. EBV denotes Epstein–Barr virus, EHV-2 equine herpesvirus type 2, HVS herpesvirus saimiri, HSV-1 herpes simplex virus type 1, HSV-2 herpes simplex virus type 2, EHV-1 equine herpesvirus type 1, PRV pseudorabies virus, VZV varicella–zoster virus, HCMV human cytomegalovirus, HHV-6 human herpesvirus 6, and HHV-7 human herpesvirus 7.

Source: Antman K and Chang Y. **Kaposi's sarcoma**. *N Engl J Med* 342(14):1027-38, 2000. Reprinted with permission of the *New England Journal of Medicine* and the Massachusetts Medical Society.

TABLE 1. Patterns of Infection with KSHV and KS Among Persons without HIV

Regions	Incidence of KS	Prevalence of KSHV	Route of Transmission	Groups at Risk
North America, northern Europe, Asia	Low	0% to 5%	Sexual, iatrogenic	Men who have sex with men (MSM), those with STDs, transplant recipients
Mediterranean, Middle East, Caribbean	Intermediate	5% to 20%	Sexual, iatrogenic, nonsexual?	MSM, those with STDs, transplant recipients, older adults
Africa, parts of Amazon basin	High	>50%	Nonsexual, sexual	Children, older adults, persons of low socioeconomic status

Source: Antman K and Chang Y. **Kaposi's sarcoma.** *N Engl J Med* 342(14):1027-38, 2000. Reprinted with permission of the *New England Journal of Medicine* and the Massachusetts Medical Society.

immunosuppressive drugs, mostly in the setting of organ transplantation to prevent rejection. This type of KS tends to be aggressive, involving lymph nodes, mucosa, and visceral organs in about half of patients, sometimes in the absence of skin lesions. Upon lowering or withdrawal of immunosuppressive therapy, however, lesions typically resolve, often completely.

Epidemic, or AIDS-associated, KS was first documented by Dr. Alvin Friedman-Kien—a longtime PRN member—and colleagues in what has come to be known as the world's first official report of what would soon be called AIDS (Friedman-Kien, 1981). Additional reports established that AIDS-associated KS affected gay and bisexual men with HIV-related immune-suppression 20 times as frequently as it did male patients with hemophilia and AIDS. Although the incidence of KS, at least in the United States, has decreased dramatically in recent years, it remains the most common of all the AIDS-related cancers.

KSHV and KS

A NUMBER OF MICROORGANISMS HAVE BEEN indicted over the years as the causative agent of KS. These include cytomegalovirus (which was proposed as the pathogen responsible for endemic KS in Africa); hepatitis B virus; human herpesvirus 6 (HHV-6); human papillomavirus 16 (HPV-16); *Mycoplasma penetrans*; HIV itself (most notably the HIV transactivation gene [tat]); and a furiously debated suspect, amyl nitrates (poppers). Countless studies attempted to isolate these pathogens in KS lesions, most of which were unpersuasive at best.

In 1994, the tide turned with the discovery of sequences of two small fragments of DNA in an AIDS KS lesion, which was reported in a landmark issue of *Science* by Dr. Chang and her dedicated team (Chang, 1994). The nucleotide sequence of these two fragments shared homology with genes of the minor capsid and tegument proteins of two known gamma-herpesviruses, the Epstein-Barr virus (EBV) and herpesvirus saimiri (see Figure 1). This finding led to the identification of a new human herpesvirus, which has now come to be known, descriptively, as KSHV, and formally, as HHV-8.

In 1995, two additional studies published in the *New England Journal of Medicine* and *Nature Medicine* went on to confirm the presence of KSHV in virtually all AIDS-related KS tissues and, in most cases, non-AIDS-related KS specimens, including those collected from African individuals (Moore, 1995; Schalling, 1995).

Serologic assays for HHV-8 antibodies are now used to test for the presence of the virus, bypassing the need for contamination-prone PCR-based techniques. The assays depend on cell lines derived from body cavity-based primary effusion lymphomas, which are infected with up to 100 copies of the virus and are a rich source of a viral latency-associated nuclear antigen (LANA). Studies using LANA seroassays have found that KSHV-specific antibodies are detectable in 70% to 90% of all patients with KS and almost 100% of immunocompetent patients with the disease. "In AIDS-related KS," Dr. Chang explained, "there is a lower LANA positivity rate as measured by existing seroassays, probably due to decreased antibody production in patients with late-stage HIV disease."

Epidemiology of KSHV

RESULTS OF SEROLOGIC STUDIES SHOW THAT, unlike other human herpesviruses, KSHV is not ubiquitous. Instead, the infection rate appears to correlate with the risk of KS within different populations, including those within different geographical regions of the world (see Table 1). The rates of KSHV infection in the general population of Mediterranean countries (e.g., Italy, Greece, Israel, Saudi Arabia) are typically between 5% and 20%, where classic KS is seen with relatively high frequency. Lower rates of KSHV seropositivity are seen among general populations of the United States, northern Europe, and throughout Asia. In Japan, for example, the seroprevalence of KSHV among blood donors is approximately 0.2%, where KS is rare (Fuji, 1999). In contrast, high KSHV seroprevalence—in excess of 50%—has been reported in various African cohorts, including those in Uganda (Gao, 1996), Zambia (Olsen, 1998), and South Africa (Sitas, 1999).

Irrespective of geography, there are certain at-risk populations with particularly high KSHV seroprevalence rates (see Table 2). In HIV-negative blood donors in the United States, KSHV seropositivity rates have been estimated to be between 1% and 2%, compared to prevalence rates in excess of 30% in gay and bisexual men, regardless of their HIV serostatus (Martin, 1998). The KSHV seropositivity rate among HIV-positive hemophiliacs or transfusion recipients, on the other hand, has been reported to be only 2% to 3%. Similarly, only 3% to 4% of HIV-positive heterosexual women participating in the Women's Interagency HIV Study were KSHV-positive (Kedes, 1997).

The concordance of positive KSHV

TABLE 2. Patterns of Infection with KSHV Among Persons with and without HIV: Risk-Factor Populations

San Francisco Men's Health Study (Martin, 1998)	
Gay and bisexual	38%
Heterosexual (exclusively)	0%
Women's Interagency HIV Study (Kedes, 1997)	
HIV-positive or at high risk	3%
HIV-positive	4%
HIV-negative	1%
Other Populations (Kedes, 1996)	
HIV-negative blood donors	1%
HIV-positive heterosexual hemophiliacs	2%
HIV-negative blood donors with syphilis	8%
HIV-negative, gay and bisexual blood donors	13%
HIV-positive, gay and bisexual blood donors	35%

Source: University of California San Francisco AIDS Research Institute. **KSHV: The Mysterious Virus Behind Kaposi's Sarcoma.** *Science to Community* (Clinical #2), June 2000.

serologies with KS prevalence implies that clinically healthy HIV-positive men who are KSHV-positive have a high risk of KS once immunodeficiency supervenes. In a 1996 report, researchers found that 21 HIV-positive patients with KS had started testing positive for KSHV antibodies six to 75 (median of 33 months) months before the tumors' appearance (Gao, 1996). Similarly, the San Francisco Men's Health Study has found that after ten years, KS had developed in 50% of those entering the study with positive tests for both HIV and KSHV (Martin, 1998).

Transmission of KSHV

THE HIGH RATES OF KS AND KSHV SEROPOSITIVITY among gay and bisexual men—particularly those with a high number of sexual partners (Martin, 1998)—do lend credence to the hypothesis that KSHV is sexually transmitted, but to categorize it exclusively as an STD would be an erroneous oversimplification. In endemic areas, for example, KSHV infection can occur in early childhood, or in infants infected perinatally—two observations that speak to the likelihood of nonsexual transmission (Olsen, 1998; Sitas, 1999; Mayama, 1998).

Interestingly, KSHV is uncommon in semen, but is found more frequently and in higher titers in saliva (Koelle, 1997; Vieira, 1997). To help make sense of this, Dr. Chang discussed a study conducted by Dr. John Pauk and his colleagues of the University of Washington in Seattle, which was designed to determine the mode of transmission and to identify risk factors in two groups of gay and bisexual men without KS (Pauk, 2000).

In the first group, Dr. Pauk's team collected mucosal, PBMC, seminal, anal, and prostatic fluid samples and tested them for KSHV using quantitative PCR and *in situ* assays. His team detected KSHV in mucosal samples from 30/50 (60%) gay and bisexual men with mixed HIV serostatuses who were seropositive for KSHV. KSHV shedding was found in 30% of oropharyngeal samples, but in just 1% of anal and genital samples ($P<0.001$). Levels of KSHV were 2.5 times greater in secretions of the oral cavity than in all other sites. The buccal mucosa had the highest titers in the mouth.

To identify risk factors for KSHV infection, Dr. Pauk's team analyzed a second group of 92 men, 26 of whom were HHV-8 seropositive; only HIV-negative men were included in this analysis, as HIV-related immunosuppression may confound risk factors for the acquisition of HIV-8. Risk factors included older age; a greater number of sexual partners, particularly partners who were HIV-positive; a history of sexually transmitted diseases, especially herpes simplex virus type 2; deep kissing; and the use of poppers.

While these data suggest that KSHV may be acquired by oral-oral contact and, quite possibly, the use of saliva as a sexual lubricant, important questions regarding the transmission of KSHV still remain. Why, for example, is KSHV infection in North America mainly restricted to gay and bisexual men? The observation that KSHV is not ubiquitous among all populations suggests that it may not be easily transmitted and that acquisition may depend on the degree of exposure to infected persons, especially to those who are immunocompromised. Alternatively, there may be unidentified cofactors that greatly increase either the shedding of infectious virus in KSHV–positive persons or the risk of infection in uninfected persons. "Until there is a better understanding of the mechanisms of KSHV transmission," states Dr. Pauk's team in its *New England Journal of Medicine* article, "it will be difficult to define the most effective approach to prevention."

The Etiology and Pathogenesis of KSHV

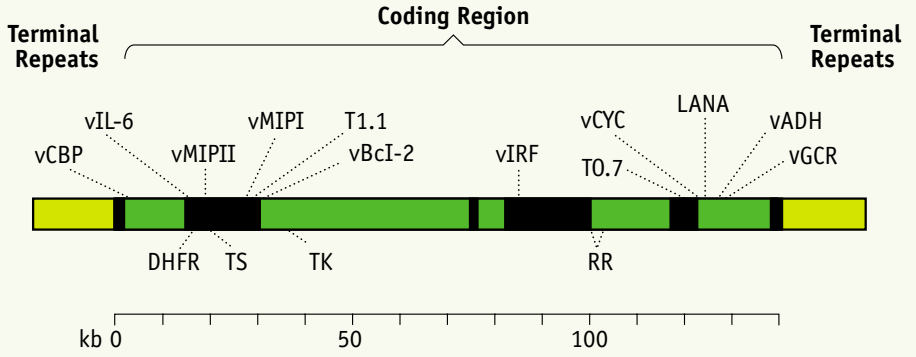
THERE IS STILL A GREAT DEAL TO BE LEARNED about the mechanism(s) by which KSHV leads to the development of KS lesions. Essentially, two schools of thought have come to dominate the field. The first hypothesis postulates that KSHV plays a crucial role in creating the necessary microenvironment for lesion development through autocrine and paracrine mechanisms. The second hypothesis holds that KSHV is more directly involved, particularly in the transformation of normal cells into malignant ones.

It is likely that KS begins as a hyperplastic proliferation mediated by growth factors and angiogenic factors, which, under certain conditions, becomes a true malignancy—a highly vascularized cell mass—as the disease progresses. Therefore, it is likely that KS progenitor cells, thought to be of endothelial cell lineage, become activated following exposure to inflammatory cytokines and angiogenic factors.

But at what point does KSHV establish infection and exert its pathogenic effect? As suggested by Dr. Robert Gallo in a 1998 review article, endothelial cells may be prone to KSHV infection only after they have been transformed into KS progenitor cells (Gallo, 1998). Once infection is established, Dr. Gallo argues, KSHV expresses viral proteins and/or yields a unique combination of host cellular factors that promote the growth and survival of both infected and uninfected KS cells.

As told by Dr. Chang, however, recent data suggest that this earlier theory underestimates the pathogenic potential of KSHV and that the virus plays a pivotal role in the initial development of KS progenitor cells. For starters, recent immunohistochemistry and monoclonality studies conducted at the Institut Pasteur in Paris indicate that some gross tumors can arise from solitary KSHV-infected cells through

FIGURE 2. The KSHV Genome



The entire coding region is flanked by terminal-repeat sequences (hatched boxes). The gene encodes numerous proteins that are homologous to cell-signaling and regulatory-pathway proteins found in human cells (solid boxes) and that are unique to KSHV and related rhadinoviruses. The proteins encoded include viral complement-binding protein (vCBP), viral interleukin-6 (vIL-6), viral macrophage inflammatory protein type 1 (vMIPI) and type II (vMIPII), viral Bcl-2 (vBcl-2), viral interferon regulatory factor (vIRF), viral cyclin (vCYC), latency-associated nuclear antigen (LANA), viral adhesin (vADH), G-protein–coupled receptor (vGCR), dihydrofolate reductase (DHFR), thymidylate synthase (TS), thymidine kinase (TK), and ribonucleotide reductase (RR). Stippled boxes indicate regions that are homologous to those of other herpesviruses. T1.1 and TO.7 denote nonhomologous open reading frames with undetermined activities.

Source: Antman K and Chang Y. **Kaposi's sarcoma**. *N Engl J Med* 342(14):1027-38, 2000. Reprinted with permission of the *New England Journal of Medicine* and the Massachusetts Medical Society.

monoclonal expansion. Other studies indicate that only a small proportion (10%) of the spindle cells in early lesions appear to be infected with KSHV but, as the disease progresses, virtually all KS cells become infected (Dupin, 1999). While this second study might serve as evidence supporting Dr. Gallo's hypothesis—that KSHV infection is not necessary (or at least is secondary) for the development of KS spindle cells—Dr. Chang and her colleagues have a different appreciation of the data: that, in early KS lesions, the cells infected with KSHV induce tumor growth through paracrine mechanisms—not that KS lesions exist prior to infection.

Of particular interest to Dr. Chang and many of her colleagues has been the striking correspondence between genes encoded by KSHV and human genes involved in the control of cell growth (the entire KSHV genome is illustrated in Figure 2). For example, the KSHV gene ORF72 encodes the viral protein vCYC, which is homologous to a key cellular counterpart, cyclin D. Cyclin D inactivates the retinoblastoma protein, which plays a key roll in controlling progression through the G1 cell-cycle checkpoint. Similarly, KSHV's ORF73 gene encodes for LANA, which has been shown to interact with and inactivate p53, a potent transcriptional regulator of cell growth. More simply put, KSHV—like other DNA tumor viruses (e.g., human papillomavirus)—encodes two proteins that independently inhibit the activity of two key tumor suppressor proteins and, as a result, might help the virally infected cells circumvent normal cell-cycle checkpoints (see Figure 3).

There are also the ORF16 and K13 genes, which encode for the KSHV proteins vBcl-2 and vFLIP, respectively. The protein vBcl-2 is a homolog of cellular Bcl-2, whereas vFLIP is homologous to FLICE (Fas-associated death domain-like interleukin-1β–converting enzyme)—two inhibitors of apoptosis.

Other regulatory KSHV proteins include a G-protein–coupled receptor, which can stimulate signaling pathways linked to cellular proliferation and can act as an oncogene *in vitro*. It can also stimulate the release of vascular endothelial growth factor (VEGF), which is associated with angiogenesis.

Some KSHV proteins are homologs of the complement receptor 2 (CR2/CD21), an adhesion molecule and a T-cell surface molecule with putative immunomodulatory properties.

Also reported has been an interferon regulatory factor (IRF) homolog, vIRF. This viral gene and its protein can modulate the effects of interferon-alpha. The protein vIRF has been found to interact with the transcriptional coactivator protein p300 and CBP to alter gene expression.

KSHV contains an interleukin-6 homolog, which is a functional B-cell growth factor and a negative regulator of apoptosis. This protein is likely responsible for the B-cell proliferation seen in MCD, through a paracrine mechanism. However, PEL tumors are also autocrine-dependent on vIL-6—an extremely unusual situation where a human cell is dependent on the presence of a virus-provided cytokine.

Three viral macrophage-inflammatory proteins (vMIP-I, II, and III) with homology to human chemokines have also been identified. These three viral chemokines have been shown to be angiogenic *in vitro* and may inhibit the Th1-type immune response.

While these various genes and proteins clearly have the potential for both the initiation and progression of KS by altering normal cellular functions, it is now up to research to determine whether they are expressed in KSHV-related tumors and how their expression is regulated. Similarly, it will be important to determine if there are differences in KSHV gene expression in KS, PEL, and MCD. As discussed in a recent paper by Dr. Carlo Parravicini, the KSHV homolog of interleukin-6 is expressed at low levels in PEL cell lines and has been detected in PEL biopsies and MCD, but is not present in KS lesions. Thus, the viral protein might play a unique role in the pathogenesis of the two former malignancies, but not the latter (Parravicini, 2000). What's more, at least one study has demonstrated that a majority of KS cells are latently infected with KSHV, and that only a small portion of tumor cells are undergoing lytic cycle replication (Staskus, 1997). Therefore, viral proteins expressed during either the latent or lytic cycle might be important in the paracrine stimulation of the cells within the tumor, whereas only proteins produced during latency are likely to be responsible for cellular transformation.

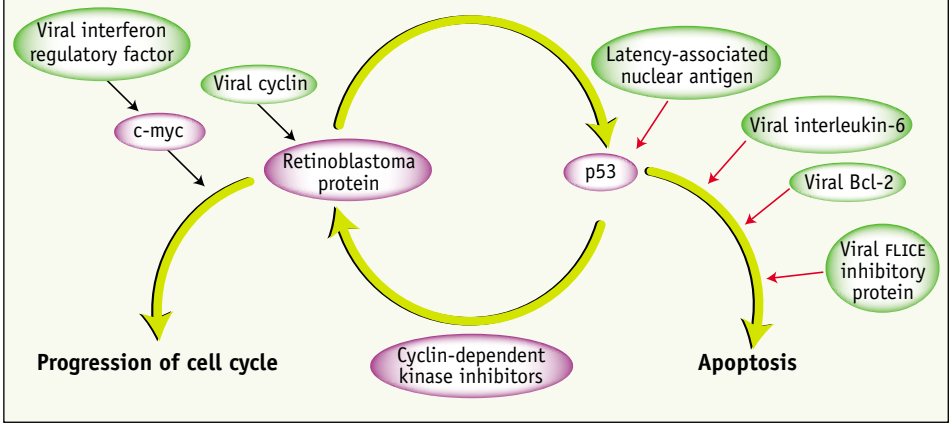
There is also the question of what role HIV infection plays in the pathogenesis of KSHV. According to one study published in 1997, HIV Tat appeared to induce lytic cycle replication in PBMCs and PEL cell lines (Harrington, 1997). Beyond these preliminary data, however, there has been little information regarding the interplay between KSHV, HIV, and host cells in terms of either

oncogenic gene expression or the autocrine/paracrine mechanisms responsible for tumor formation and proliferation. At the present time, it is generally believed that immune suppression—whether it's tied to HIV infection (e.g., epidemic KS), the use of immunosuppressive therapy (e.g., KS in transplant recipients), aging (e.g., classic KS), or poverty (e.g., endemic KS)—is the necessary cofactor for KSHV to unleash its opportunistic streak.

Conclusion

ALTHOUGH SEVERAL OUTSTANDING QUESTIONS regarding the natural history and pathogenesis of KSHV have yet to be answered, a great deal has been learned about the role of this wily virus in the development of KS, PEL, and MCD. Now that some of the key pieces of the puzzle have been found and put into place, it is hoped that research into the treatment and prevention—including a possible vaccine—of KSHV-related malignancies will get under way. "KS is obviously less of a problem in the United States than it was before HAART became available," Dr. Chang said. "But we still have a significant problem in other parts of the world and it's not clear what we'll be seeing here in the future." It is precisely because of these uncertainties that the pathogenesis and medical management of KS must remain a research priority.

FIGURE 3. KSHV Proteins That Interact with the Tumor-Suppressor Pathways Governed by Retinoblastoma Protein and p53



The viral interferon regulatory factor prevents interferon from repressing c-myc. FLICE denotes Fas-associated death domain–like interleukin-1β–converting enzyme. Red arrows indicate an inhibitory effect.

Source: Antman K and Chang Y. **Kaposi's sarcoma.** *N Engl J Med* 342(14):1027-38, 2000. Reprinted with permission of the *New England Journal of Medicine* and the Massachusetts Medical Society.

References

Diamond C, Huang ML, Kedes DH, et al. **Absence of detectable human herpesvirus 8 in the semen of human immunodeficiency virus-infected men without Kaposi's sarcoma.** *J Infect Dis* 176:775-7, 1997.

Dupin N, Fisher C, Kellam P, et al. **Distribution of human herpesvirus-8 latently infected cells in Kaposi's sarcoma, multicentric Castleman's disease, and primary effusion lymphoma.** *Proc Natl Acad Sci USA* 96:4546-51, 1999.

Friedman-Kien AE, Laubenstein L, **Marmor M, et al. Kaposi's sarcoma and Pneumocystis pneumonia among homosexual men — New York City and California.** *MMWR* 30:305-308, 1981.

Fuji T, Taguchi J, Katano H, et al. **Seroprevalence of human herpesvirus 8 in human immunodeficiency virus 1-positive and human immunodeficiency virus 1-negative populations in Japan.** *J Med Virol* 57:159-62, 1999.

Gao SJ, Kingsley L, Li M, et al. **KSHV antibodies among Americans, Italians and Ugandans with and without Kaposi's sarcoma.** *Nat Med* 2:925-8, 1996.

Gallo RC. **Some aspects of the pathogenesis of HIV-1 associated Kaposi's sarcoma.** *J Natl Cancer Inst Monogr* 23:55-7, 1998.

Harrington W Jr, Sieczkowski L, Sosa C, et al. **Activation of HHV-8 by HIV-1 tat.** *Lancet* 349(9054):774-5, 1997.

Judde JG, Lacoste V, Briere J, et al. **Monoclonality or oligoclonality of human herpesvirus 8 terminal repeat sequences in Kaposi's sarcoma and other diseases.** *J Natl Cancer Inst* 92(9):729-36, 2000.

Kaposi M. **Idiopathisches muliples pigmensarkom der haut.** *Arch Dermatol Syph* 1872; 4:265-273.

Kedes DH, Ganem D, Ameli N, et al. **The prevalence of serum antibody to human herpesvirus 8 (Kaposi sarcoma-associated herpesvirus) among HIV-seropositive and high-risk HIV-seronegative women.** *JAMA* 12:277(6): 478-81, 1997.

Kedes DH, Operskalski E, Busch M, et al. **The seroepidemiology of human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus): distribution of infection in KS risk groups and evidence for sexual transmission.** *Nat Med* 2(8):918-24, 1996.

Koelle DM, Huang ML, Chandran B, et al. **Frequent detection of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) DNA in saliva of human immunodeficiency virus-infected men: clinical and immunologic correlates.** *J Infect Dis* 176:94-102, 1997.

Martin JN, Ganem DE, Osmond DH, et al. **Sexual transmission and the natural history of human herpesvirus 8 infection.** *N Engl J Med* 338(14):948-54, 1998.

Mayama S, Cuevas LE, Sheldon J, et al. **Prevalence and transmission of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in Ugandan children and adolescents.** *Int J Cancer* 77:817-820, 1998.

Moore P, Chang Y. **Detection of herpesvirus-like DNA sequences in Kaposi's sarcoma patients with and those without HIV infection.** *N Engl J Med* 332:1181-5, 1995.

Olsen SJ, Chang Y, Moore PS, et al. **Increasing Kaposi's sarcoma-associated herpesvirus seroprevalence with age in a highly Kaposi's sarcoma endemic region, Zambia in 1985.** *AIDS* 12:1921-1925, 1998.

Parravicini C, Chandran B, Corbellino M, et al. **Differential viral protein expression in Kaposi's sarcoma-associated herpesvirus-infected diseases: Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease.** *Am J Pathol* 2000 Mar;156(3):743-9, 2000.

Pauk J, Huan M-L, Brodie SJ, et al. **Mucosal shedding of human herpesvirus 8 in men.** *N Engl J Med* 343 (19):1369-77, 2000.

Schalling M, Ekman M, Kaaya EE, et al. **A role for a new herpes virus (KSHV) in different forms of Kaposi's sarcoma.** *Nat Med* 1(7):707-8, 1995.

Sitas F, Carrara H, Beral V, et al. **Antibodies against human herpesvirus 8 in black South African patients with cancer.** *N Engl J Med* 340:1863-71, 1999.

Staskus KA, Zhong W, Gebhard K, et al. **Kaposi's sarcoma-associated herpesvirus gene expression in endothelial (spindle) tumor cells.** *J Virol* 71(1):715-9, 1997

Vieira J, Huang ML, Koelle DM, et al. **Transmissible Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in saliva of men with a history of Kaposi's sarcoma.** *J Virol* 71:7083-7, 1997.