Kaposi's Sarcoma in the Era of HAART – An Update on Mechanisms, Diagnostics and Treatment

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Abstract

Kaposi's Sarcoma (KS) signified the AIDS epidemic in the 1980's and led to the discovery of the eighth human herpesvirus, KS-associated herpesvirus (KSHV), as the causative agent for this disease. Today we know a lot about KSHV and can begin to understand, diagnose and treat KS as a viral disease rather than another sarcoma. **(AIDS Reviews 2005;7:56-61)**

Key words

Kaposi's sarcoma. HHV-8. KSHV. Ganciclovir.

Introduction

One quarter to one third of all human cancers are associated with infectious agents[1] that are normally contained by the host immune system. Hence, patients that are immunodeficient, such as AIDS patients or patients receiving immunosuppressive drugs following organ transplantation, are at risk for immunodeficiency-associated cancers. Kaposi's sarcoma (KS) is an AIDS-defining cancer, which is caused by Kaposi's sarcoma-associated herpesvirus (KSHV).

Kaposi's sarcoma is divided into several subtypes with varying clinical manifestations[2,3]. Classic KS was first described in 1872 as a fatal, disseminated sarcoma of the skin. KS is endemic in parts of equatorial Africa where it is responsible for an estimated 1% of all adult tumors. In these regions, transmission of KSHV proceeds from mother to child before puberty and KS is often fatal by an early age. Widespread HIV-1 infection has now turned KS into an epidemic disease on the continent. Prevalence levels for KSHV antibodies reach 30% in black South African HIV patients and childhood KS has become the most common neoplasm in many regions of sub-Saharan Africa. KS has also been documented in organ transplant recipients (iatrogenic KS) in whom it comprises an estimated 3% of all tumors[4]. This is seen particularly in regions of high KSHV prevalence, such as southern Italy, Turkey, and Saudi Arabia. Under these circumstances, KSHV may be already present in the recipient, acquired during immunodeficiency after transplantation, or transmitted through the graft[5]. In 1981 KS was recognized as the signature pathology of AIDS. Highly active antiretroviral therapy (HAART) has led to a decline of AIDS-related KS in the USA, although data indicating a failure rate of HAART of up to 22% (primarily due to noncompliance) suggests that KS represents a permanent health problem for years to come[6,7].

Figure 1 A plots the annual incidence rates for KS in the San Francisco Bay area from the National Cancer Institute SEER database (http://seer.cancer.gov). In the mid-1980s, incidence rates for KS showed a greater than exponential increase, while incidence rates for other human cancers remained largely level. This, of course, marked the onset of the AIDS epidemic in the USA[2]. During the initial phase of the AIDS epidemic, KS was only observed in men who had sexual relations with other men. KS has since been reported in women. African KS affects both genders, but classic (Mediterranean) KS affects predominantly older men. As seen in figure 1 B, incidence rates of KS per age group follow a bimodal distribution that peaks at ages 30 to 36 years and again at ages > 70 years. In contrast, incidence rates for most spontaneously occurring cancers increase exponentially with age as the number of spontaneous mutations accumulates.

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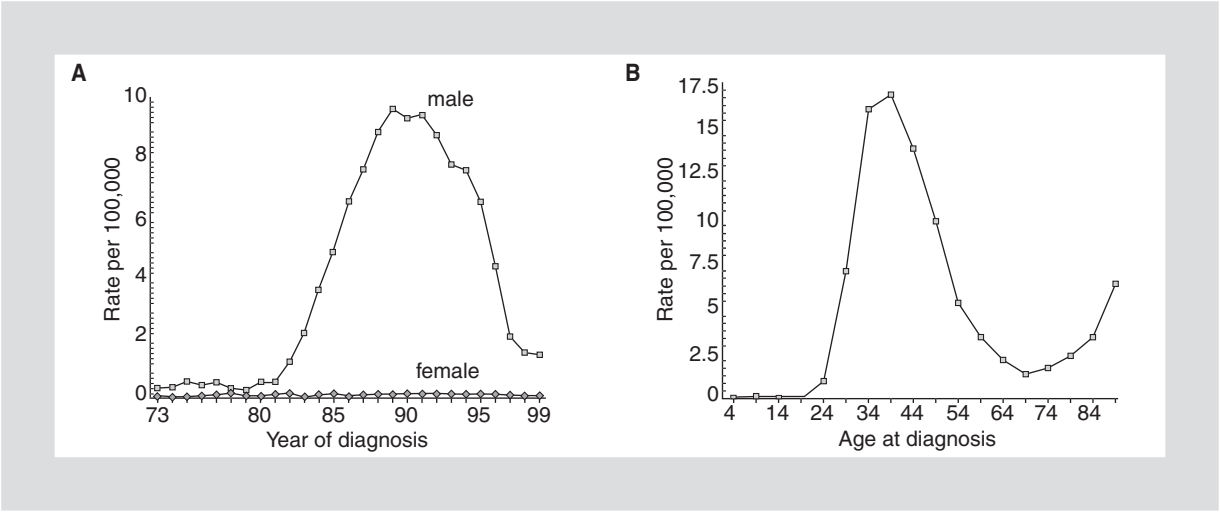

Figure 1. *A. Incidence rates of Kaposi's sarcoma in the San Francisco Bay area. B. Age-specific incidence rates of Kaposi's sarcoma (from SEER).*

Cancers with a strong association to one genetically predisposing mutation, such as loss of Rb alleles in the case of retinoblastoma, also exhibit a bimodal age distribution. However, in cases of genetic predisposition, a familial pattern is also present and the typical age of onset is early childhood. By contrast, to date, only a single report has suggested a genetic factor for KS[8]. In AIDS KS, incidence rates correlated significantly with the lifetime number of male sexual partners[9], which corroborated KSHV as the sexually transmitted agent that caused this cancer.

Kaposi's sarcoma-associated herpesvirus

Attempts to culture a virus directly from KS tumors failed, but in 1994 Chang, et al.[10] used representational difference analysis to demonstrate the presence of this novel human herpesvirus in KS lesions, but not in normal skin of the same patient, or in KS-negative patients. Hence, for the definite diagnosis of KS and the detection of KSHV it is essential to biopsy the lesion. The cloning of KSHV established a new paradigm for the discovery of uncultivatable agents[11,12]. Traditionally, infectious causes of disease had to fulfill Koch's postulates: a) they needed to be found exclusively in the affected patients (cases), but not in unaffected control individuals (controls); b) they needed to be isolated and grown clonally in the laboratory; and c) they needed to be able to induce disease in the original or an experimental host. These rules were softened for the discovery of KSHV because of the long latency period (approximately seven years) between initial infection and clinically recognized malignancy. KSHV viral load typically rises in the one-to-six months that precede lesion formation[13,14] and a rise in viral loads may predict immanent clinical lesions. These can be internal as well as on the skin.

The presence of anti-KSHV antibodies documents prior exposure, but does not allow a prediction of KS development within the next six months (Dittmer and Martin, unpublished), since in HIV-positive individuals the median time from seroconversion to disease is seven years[15]. We do not know whether a temporal change in antibody titers is of prognostic value for KS, or merely reveals sporadic viral reactivation that is sufficiently controlled by the immune system, since most immunodeficient AIDS patients are expected to also be deficient in their CD4-dependent humoral response.

By definition, all KS cases (and all other KSHV-associated cancers such as primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD)[2,16], contain KSHV. However, KS lesions are heterogeneous. The tumor is characterized by a mixture of infiltrating inflammatory leukocytes and spindle-formed endothelial cells, which constitute the malignant cells[17,18]. KSHV genomes have been found exclusively in the CD34-positive endothelial lineage-derived cells by *in situ* hybridization[18,19] and KSHV mRNA and proteins have been found in the CD34 endothelial-derived cells[20,21], although tumor-infiltrating monocytes may also harbor KSHV[22].

KS lesions are classified as plaque, patched, and nodular based upon dermatological examination[23]. In its most aggressive form, KS forms foci in the visceral organs leading to tissue destruction, bleeding, and ultimately death. Several studies found that as the tumor progresses, the numbers of virally infected cells increase and spindle-formed endothelial cells expand[24,25]. However, KSHV has never been cultured from KS lesions, and tumor explants lose the virus after

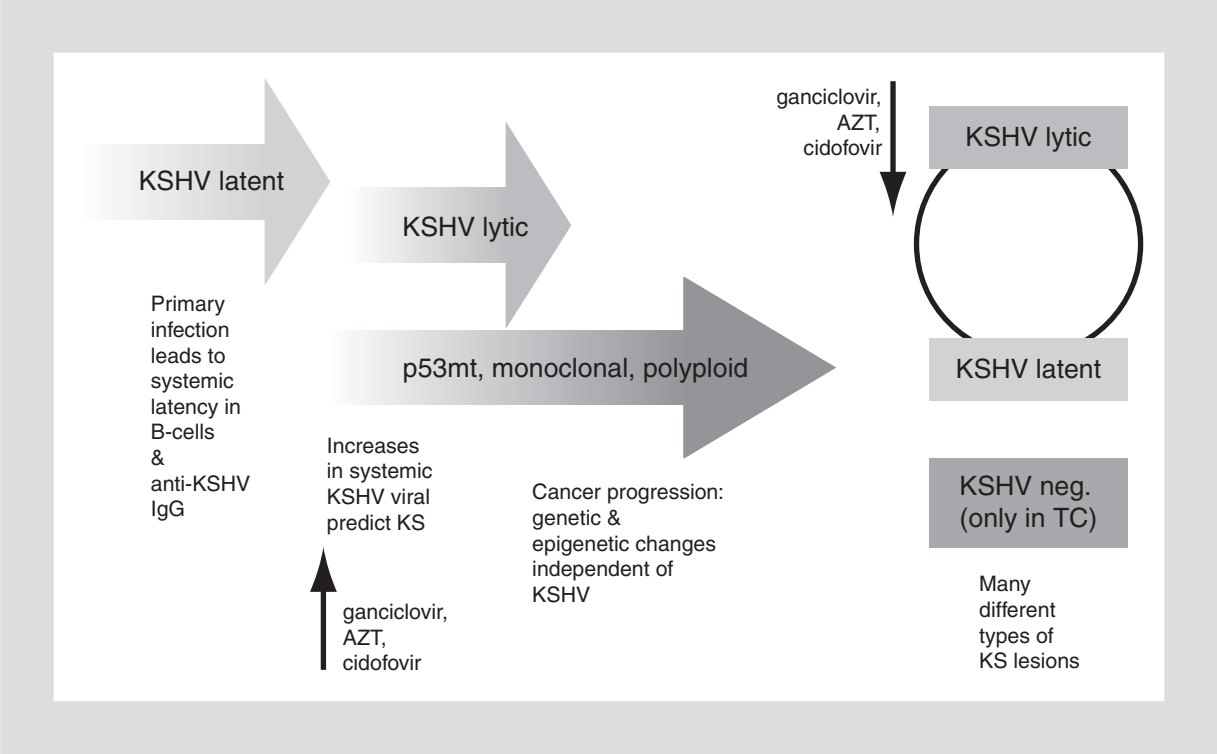

Figure 2. *Model of KS development and possible intervention points.*

passage in culture. Presumably *in vitro* conditions select for cells that no longer depend on viral oncogenes for survival. These could be KSHV-infected cells that have acquired additional mutations that substitute for KSHV oncogenes, or cells within the KS lesions that were never infected[26]. This may be mechanistically similar to lymphomas at advanced stages where mutations in cellular genes can substitute for the viral oncoproteins. This has not been documented for KSHV-associated PEL, but may be one explanation why histologically identical Burkitt's lymphomas (BL) can occur either as Epstein-Barr Virus (EBV)-positive (classic BL) or as EBV-negative (most AIDS-associated BL) varieties. PEL always contain KSHV, but may or may not be coinfected with EBV.

High-throughput genomic profiling offers the chance to accelerate our investigations into AIDS-associated cancers as much as it has benefited research into cancers of hereditary or spontaneous origin[27]. Microarray analyses[28-30] proved that KSHV-positive PEL differ from KSHV/EBV-positive PEL, from EBV-positive BL, and EBV-negative lymphoma. This is consistent with the idea that KSHV reprograms the tumor cell. To date, no transcriptional profile has been reported for KS, presumably since too little biopsy material is available for a thorough analysis. Recently it was shown that KSHV infection reprograms endothelial cells towards a specialized cell fate – that of lymphatic endothelium, which expresses characteristic lineage markers[31,32].

Pathogenesis and treatment approaches

Several studies have ascertained the transcription profile in tissue culture models of KSHV infection[33-36].

Potentially interesting drug targets emerged in each of these studies, but a consensus has yet to be generated. These tissue culture results may or may not relate to cellular transcription in the primary KS lesion; however, KS will almost certainly have a cellular transcription signature that is distinct from other cancers and tied to the pathology of this disease. Finding upregulation of c-Kit and other growth-factor receptors in micro-array studies of KSHV-infected endothelial cells led to a successful pilot study using the kinase inhibitor Gleevec (Imatinib)[37].

The KSHV viral genome also presents a well-defined target for genomic explorations[38,39]. Since the KSHV genome is orders of magnitude smaller than the human genome, it has been feasible to develop whole genome arrays based upon real-time quantitative RT-PCR rather than hybridization[40,41] for all individual viral genes. This novel technique yielded the same result as the more expensive arrays and was sensitive enough to be used on a 2 x 2 mm fine-needle KS biopsy directly, without amplification of either the tumor cells in culture

or the tumor RNA. Every KS tumor transcribed high levels of the canonical KSHV latency genes LANA, vFLIP, vCyclin and kaposin. LANA, vFLIP and vCyclin are under control of the same promoter and are expressed in every KS tumor cell[20,21]. Kaposin is located immediately downstream of these three genes and regulated by a promoter located between LANA and cyclin[42]. Kaposin too is expressed in every tumor cell[18] and has recently been shown to stabilize cellular cytokine mRNAs[43]. Hence, these four genes are required for KS tumorigenesis.

We were able to separate histologically indistinguishable primary tumors into distinct subsets based upon the extent of lytic viral gene expression, including expression of the KSHV interferon regulatory factor (vIRF-1) and G-coupled receptor (vGPCR) homologs[41], suggesting that a subset of KS phenotypes may be attributable to these genes[44-46]. Interestingly, vIRF-3, a duplicated KSHV-IRF homolog, is constitutively (latently) transcribed in KSHV-infected PEL[47], but not KS. Most likely, KSHV has to interfere with the host cell's innate interferon response in every infected cell and has thus placed copies of the vIRFs, which both interfere with normal IRF signaling, under different control elements: one vIRF-3 specific for B-cells, and one vIRF-1 specific for endothelial cells. Therefore, in KS both latent and select lytic genes can be considered tumor-specific therapy targets.

How do we treat KS and what future therapies are on the horizon? Current therapies do not take into account the viral association of KS. Instead KS is treated with local irradiation or conventional chemotherapy including anthracyclines and paclitaxel[48]. Interferon (IFN)-α is also approved for treatment of KS. IFN-α inhibits KSHV replication directly, but may have more important anti-tumor effects independent of KSHV[49-55]. The exact mechanism of action has yet to be elucidated, since KSHV encodes a slew of viral interferon regulatory factors, which inhibit IFN-α signaling *in vitro*[56-59]. Thalidomide also has shown activity against KS[60,61]. In addition to cytotoxic therapies, new KS-specific targets and new KS-specific drugs have entered clinical trials.

Several clinical trials on AIDS KS are currently being conducted through the AIDS-associated malignancies clinical trials consortium (http://www.amc.uab.edu/) and the intramural branch of the US National Cancer Institute (http://www-dcs.nci.nih.gov/branches/aidstrials/index.html). These include: a) the aforementioned Gleevec/Imatinib, which targets cKit/PDGFr (AMC042); b) bevacizumab, which targets VEGFr (NCI-03-C-0110E); c) topical halofuginone, which interferes with matrix metalloproteinases (AMC036); d) valproic acids (AMC038), which induce KSHV lytic replication thereby killing the host cell. Other highly selective angiogenesis inhibitors have failed to meet expectations[62]. Only clinical trials will show which molecular target affects clinical outcome for this disease.

Controlling KS disease in HIV patients by restoring CD4+ levels through HAART is very effective. HAART serves two purposes: a) it controls HIV+ viral load, which in the context of AIDS KS may reactivate KSHV[63] or vice versa[64], or exasperate the KS phenotype through changes in the local cytokine milieu[65,66]; b) it enables a functional immune response against KSHV thereby limiting systemic spread[67]. It is important to give HAART therapy time to restore the immune system as some lesions take up to three months to respond.

Every KS tumor cell expresses the viral LANA. LANA alone is necessary and sufficient to maintain the viral episome during latency[68]. In addition, LANA also has been shown to modulate cellular transcription directly[36]. Abolishing LANA (or vFLIP, or vCYC) expression abolishes the transformed phenotype[69], and LANA therefore stands out as a target for novel anti-KS drugs. Since LANA first and foremost binds to the latent viral origin of replication[70,71], high-throughput screens have a high chance of success. However, it will be some time until even experimental drugs will become available that can cure latent KSHV infection.

It is tempting to employ anti-herpesvirus drugs to fight KSHV-associated cancers, since they would be highly selective against the virally infected tumor cells. A single study showed that systemic ganciclovir reduced the incidence of KS[72], yet ganciclovir had no effect on established PEL tumors in a mouse model[73]. Because ganciclovir requires activation by the KSHV thymidine kinase or phosphotransferase[74], cells, like most PEL, that do not express these viral proteins are resistant to this drug. Yet some KS lesions, which express viral lytic genes in a great proportion of cancer cells, would be susceptible. Phase I trials using valganciclovir (MSKCC-04-055) and phase II studies using other herpesvirus inhibitors have been initiated (NCI-00-C-0193). At this point we do not know whether this class of tumors are the most benign or the most aggressive. Ganciclovir, cidofovir and other anti-herpesvirus drugs most certainly limit KSHV replication and peripheral viremia[75], which would explain some clinical results. Furthermore, anti-herpesvirus drugs limit human cytomegalovirus (HCMV) replication, which frequently reactivates in AIDS patients and in turn was shown to induce KSHV[76]. This may either be the direct result of HCMV proteins in coinfected monocytes, or through the upregulation action of inflammatory cytokines, which in turn reactive KSHV[52,77-79].

Acknowledgements

Work on this project was supported by NIH (CA109232). The authors thank William Harrington,

Blossom Damania and Henry Koon for helpful discussions. We regret having had to omit many noteworthy references due to page limitations.

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