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REVIEW ARTICLE

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Human herpes virus 8: a new virus discloses its face

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Abstract The human herpes virus 8 (HHV8) or Kaposi's sarcoma-associated herpes virus (KSHV) is present in all Kaposi's sarcoma, and the detection of the virus using polymerase chain reaction or in situ hybridization is a highly sensitive and specific diagnostic test for the diagnosis of this neoplasm. HHV8 is furthermore invariably present in primary effusion lymphoma (PEL) and has also been detected in patients with acquired immunodeficiency syndrome (AIDS)-associated multicentric Castleman's disease (MCD) as well as, to a lesser extent, in non-AIDS MCD. In contrast to Kaposi's sarcoma, in which the tumor cells show primarily latent HHV8 infection, a higher rate of lytically infected cells can be observed in MCD. Epidemiological surveys indicate that the seroprevalence for HHV8 parallels the risk of developing Kaposi's sarcoma – 5–10% in the general population of the Western world but ranging up to 20–70% in homosexual human immunodeficiency virus (HIV)-infected patients, and the infection precedes the development of Kaposis's sarcoma. Finally, HHV8 has been reported in a number of other diseases, especially in multiple myeloma. However, the highly controversial role of HHV8 in these lesions has to be clarified. Based on the data available today, HHV8 can be assigned as a new human virus, associated with tumors.

Key words Herpes virus · HHV8 · Kaposi's sarcoma · Castleman's disease · Non-Hodgkin's lymphoma · Review

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Introduction

Based on the epidemiology and the clinical course of disease, Kaposi's sarcoma has long been suspected to be either caused by or closely related to an infectious agent, and a variety of viruses and bacteria have been implicated, including cytomegalovirus, hepatitis B, the human immunodeficiency virus (HIV) and mycoplasma [13, 14, 42, 118, 135, 137]. In 1994, Chang and co-workers reported the presence of herpes-virus-like DNA in tumor tissue of patients with Kaposi's sarcoma [33]. Subsequent work confirmed these findings and the detection of a new human herpes virus referred to as Kaposi's sarcoma-associated herpes virus (KSHV) or human herpes virus 8 (HHV8) [88]. In recent years, an increasing body of information has been generated confirming the link between HHV8 and Kaposi's sarcoma as well as with two lymphoproliferative diseases, the primary effusion lymphoma (PEL) and the multicentric Castleman's disease (MCD) [30, 92, 123]. In the present review, the basic data defining HHV8 as a new human tumor virus is presented, and the impact of the virus detection in the diagnosis of HHV8-associated diseases as well as some controversies concerning other diseases possibly associated with HHV8 infection are discussed.

The virus

In contrast to the other human herpes viruses, HHV8 was primarily characterized using molecular biology techniques, i.e., the representational difference analysis [33]. Further analysis and subsequent cloning and sequencing of the complete viral genome revealed that HHV8 belongs to the subfamily of γ-herpes virus, related to Epstein-Barr virus (EBV), and represents the first member of a human γ_2-herpes virus, genus *Rhadinovirus* [88, 93, 112]. HHV8 is most closely related to herpes virus saimiri, an animal virus causing lymphoproliferative disorders in new-world primates. The genomic structure of HHV8 is shared by all rhadinoviruses and consists of a

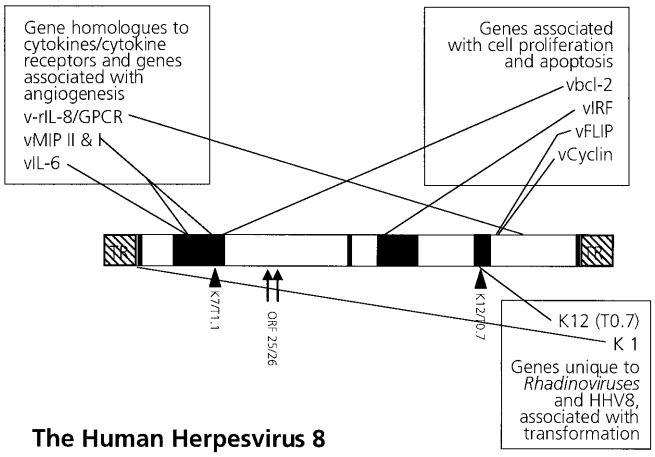

Fig. 1 Schematic representation of the human herpes virus 8 (HHV8) genome showing blocks of coding regions conserved in other herpes viruses (*black areas*) and regions unique to *Rhadinovirus* or HHV8 (*white areas*). The position of various genes is indicated as well as the gene position of ORF 25 and 26 used for polymerase chain reaction (PCR) (*arrows*) and two probes K12/T0.7 and K7/T1.1 suitable for in situ hybridization (*arrowheads*) [88, 93, 112]. *v* viral; *rIL8/GPCR* interleukin-8 receptor/G-coupled protein receptor; *MIP* macrophage inflammatory protein; *IL-6* interleukin 6; *IRF* interferon regulatory factor; *FLIP* Flice inhibitory protein

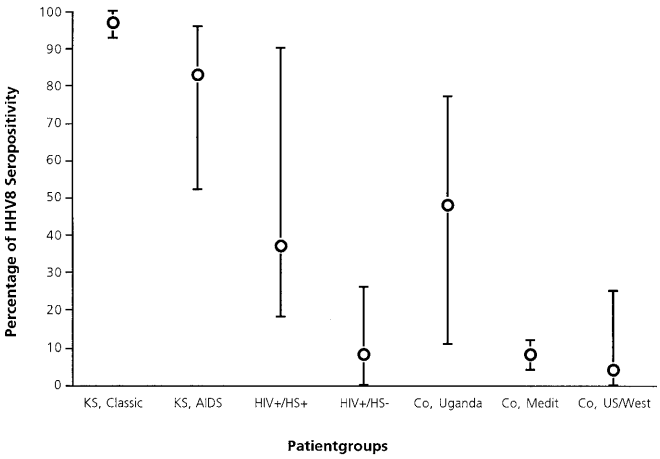

Fig. 2 Human herpes virus 8 (HHV8) seroepidemiology in various patient groups: the *circles* represent the mean of six studies including different techniques using latent and lytic antigens [34, 57, 66, 70, 106, 119]. The *error bars* indicate the maximum and minimum values reported by these studies. *KS, classic* classic or endemic Kaposi's sarcoma; *KS, AIDS* Kaposi's sarcoma associated with human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS); *HIV+/HS+* homosexual patients infected with HIV without Kaposi's sarcoma; *HIV+/HS–* HIV-infected patients without known homosexual or bisexual behavior; *Co, Uganda* control groups originating from Uganda; *Co, Medit* control groups originating from the Mediterranean area; *Co, US/West* control groups originating form the US or Western Europe

central unique gene region flanked by repetitive sequences, containing a high GC DNA content (Fig. 1). The linear viral genome of HHV8 includes the 140.5-kb central coding region and the terminal repeats containing several 801-bp units [93, 112]. In HHV8, two types of genes have been described within the genome: conserved genes similar to other herpes virus and coding for structural and replication-associated proteins, being arranged in blocks (Fig. 1). In the gene regions between these conserved blocks, the viral genome reveals open reading frames (ORFs) unique to rhadinoviruses and HHV8, including a variety of homologues to human genes [93, 112], of which many revealed in vitro functional activity similar to that seen in their natural counterpart. These genes include viral homologues to cytokines and cytokine receptors, such as interleukin 6 (vIL-6) [86, 94], the macrophage inflammatory proteins I and II (vMIP I/II) [19, 86, 94], and the interleukin-8 receptor/G-protein-coupled receptor (vIL-8/GPCR) [7, 9], as well as genes associated with cell proliferation and anti-apoptosis, such as a viral Cyclin D [72], an interferon response factor (vIRF) [55, 86, 140], vbcl-2 [36, 115] and Flice inhibitory protein (vFLIP) [133] (for review see also [117]). Furthermore, HHV8-encoded genes have been shown to induce angiogenesis [5, 9, 19]. Genes unique to HHV8 have been characterized and assigned with the prefix "K" and, using in vitro analysis, at least two of them, K1 and K12 (T0.7/Kaposin), revealed transforming potential in vitro [68, 91]. Transforming activity was further observed in long-term culture of HHV8-infected endothelial cells [49, 90].

Most of the in vitro data have been generated by analyzing permanently HHV8-infected lymphoma cell lines [8, 82, 110]. The expression of viral genes in these cell lines can be induced by butyrate or phorbol esters and, based on this response, three classes of HHV8 transcripts have been distinguished [116]. Whereas class-I genes, such as vFLIP or vCyclin D, are constitutively expressed and unaffected by induction, class-III genes, representing predominantly structural viral genes, can be detected only following appropriate stimulation. Class-II genes, finally, as exemplified by K7 (T1.1/nut-1), are constitutively transcribed at low level, but expression increases following induction. In general, class-I genes are basically thought to be associated with latent, class III with lytic infection, whereas class-II genes have been associated with both latent and lytic infection [139]. Although successful primary cultivation of HHV8 from Kaposi's sarcoma tissue has been reported, the use of cell culture systems other than the lymphoma cell lines is very limited so far [51, 109].

Seroepidemiology of HHV8

As for other infectious agents, the detection of antibodies against HHV8 proteins analyzed in various populations provides important data concerning the general epidemiology of the infection as well as important hints to the mode of transmission. In addition, as is the case for other herpes viruses, it is very likely that, following primary infection, HHV8 establishes a latent or persistent infection with the potential to be reactivated under certain

conditions, such as immunosuppression. A number of serosurveys have been performed analyzing various patient groups and cohorts all over the world [34, 66, 70, 106, 119, 120]. To perform the serological assays, basically two sources of antigen have been used: recombinant proteins and HHV8-infected lymphoblastoid cell lines, derived from PEL [102]. With respect to the antigen itself, viral proteins associated with lytic or latent infection have been used, manufactured either as recombinant proteins [119] or derived from uninduced (latent infection) and induced (lytic infection) HHV8-infected lymphoblastoid cell lines [57, 66]. So far, the antibody tests have not been standardized and considerable differences in the seroprevalence of HHV8 within a given population as well as in the interassay correlation have been reported [102]. Nevertheless, most studies gave concordant results, demonstrating that HHV8 is, in contrast to most other herpes viruses, not a ubiquitous human infection, and HHV8 seroprevalence parallels the risk of developing Kaposi's sarcoma (Fig. 2).

Antibodies against HHV8 have been detected in 70–100% of individuals with Kaposi's sarcoma, whereby the detection rate is slightly lower in patients with acquired immunodeficiency syndrome (AIDS)-associated disease than in patients with classic Kaposi's sarcoma [119]. In the general population of the Western World, a range of seroprevalences of 0–20% has been reported [34, 66, 70, 106, 119]. The majority of studies, however, report a seroprevalence of 5–10% in this population and the discrepancies reported may indeed be associated with a lower specificity of some of these tests. Although some studies suggest that assays detecting antibodies against lytic antigen may find more seropositive samples, this could not generally been confirmed [102]. One should keep in mind, however, that as none of the tests detect HHV8 antibodies in all patients with Kaposi's sarcoma, but HHV8 DNA can be detected in all these lesions (see below), the true rate of HHV8 infection in low-risk populations remains to be elucidated. A similar problem can be observed in the typical low-risk population of children in the Western World. Most studies, however, reported the absence or at least a low prevalence of HHV8 antibodies in young children [18, 70, 119].

In high endemic areas, such as Central Africa, a higher seroprevalence in the general population has been reported ranging from 32% to 100% [57, 59, 70, 119, 120]. This is not surprising, as in some areas in Central Africa Kaposi's sarcoma is one of the most common tumors [136]. A higher rate of seroprevalence has further been reported in patients attending clinics for sexually transmitted diseases or in patients with a well-known risk of Kaposi's sarcoma, especially in HIV-positive homosexual men, ranging from 20% to 70% and supporting the concept that HHV8 is a sexually transmitted disease [16, 57, 66, 70, 106, 119]. However, in HIV-infected and uninfected hemophiliacs, the HHV8 seroprevalence does not significantly exceed the prevalence reported for the general population [57, 66, 70, 119].

Generally, seroconversion and seropositivity for HHV8 predicts the risk to develop Kaposi's sarcoma [56, 78, 108, 121]. In a large cohort of 593 men with a seropositivity of 37.6%, the 10-year probability of developing Kaposi's sarcoma was 49.6% [78]. HHV8 can also be transmitted by solid organ and bone-marrow transplantation. Following transplantation, primary infection and reactivation may lead to Kaposi's sarcoma, and these patients have an increased risk of developing the tumor [48, 58, 61, 100, 108]. In a study of renal transplant recipients, within 4 years, Kaposi's sarcoma developed in 2 of 39 HHV8-seropositive patients, all showing seroconversion following transplantation, but in none of the 181 seronegative kidney recipients [108].

Taken together, serological data confirm the strong correlation between Kaposi's sarcoma and HHV8 infection and suggest that the virus is primarily sexually transmitted in countries with low Kaposi's sarcoma prevalence. Other ways of transmission, however, must be present, as suggested by the fact that HHV8 can be detected in young children from endemic areas [3].

HHV8 in Kaposi's sarcoma tissue

Since the original description of Kaposi's sarcoma in 1872, a variety of different clinical forms of this lesion have been described [64]. The classic or endemic form of Kaposi's sarcoma, originally found in older men of Eastern Europe or of Mediterranean origin, usually shows an indolent clinical course. In contrast, the more recently described forms associated with prolonged immunosuppression, as seen in solid organ transplant recipients and especially in patients infected with HIV, show a more aggressive clinical behavior and often disseminate and involve inner organs such as the lung or the gastrointestinal tract. Furthermore, additional variants of Kaposi's sarcoma in Africa have been described. Histologically, all clinical forms of Kaposi's sarcoma show similar features. In the early or patchy stage, jagged and dilated vascular spaces with interstitial inflammatory cells and extravasated red blood cells can be seen. In the more characteristic plaque and finally nodular stage, the tumor is made up of plump spindle cells with irregular slit-like vascular spaces aligned by a recognizable endothelium and filled with erythrocytes [46].

In the original description of the herpes virus-like DNA sequences of HHV8 by Chang and coworkers, a polymerase chain reaction (PCR)-based method, the representational difference analysis, has been used to detect the viral sequences [33]. Although HHV8 can be detected in Kaposi's sarcoma tissue using Southern blots, in the vast majority of subsequent analyses, PCR has been performed to detect fragments of the viral DNA either in fresh tissue samples or formalin-fixed biopsy material [2, 24, 33, 44, 60, 87]. In addition, to increase the sensitivity of the test, a nested PCR, i.e., two consecutive PCR reactions with an inner and outer primer pair, has been used. Although nested PCR can be performed for diagnostic purposes, rigorous precautions and appropri-

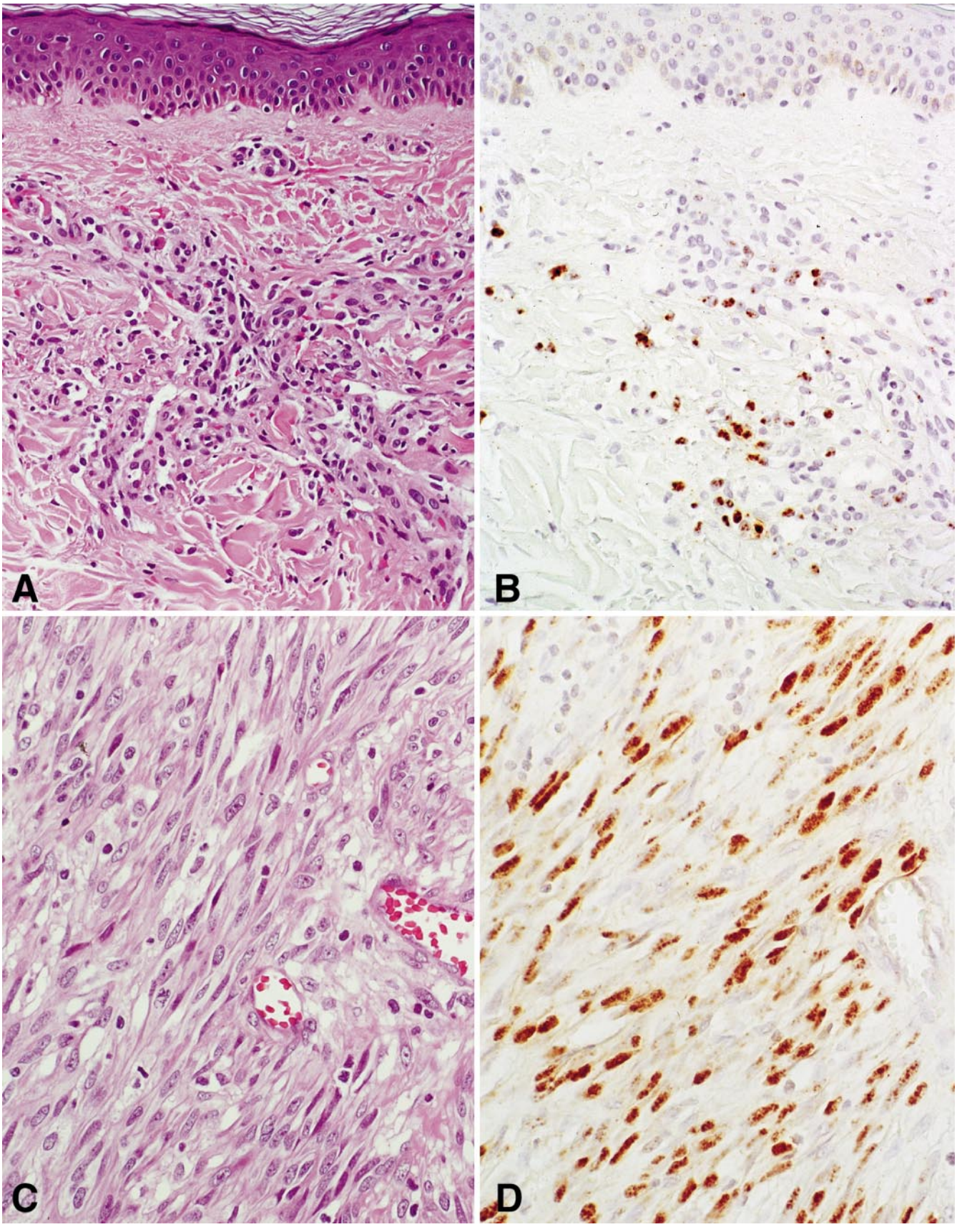

Fig. 3 Human herpes virus 8 (HHV8) and Kaposi's sarcoma: patch or macula stage (**A**) and nodular stage (**C**) of Kaposi's sarcoma (hematoxylin and eosin) and in situ hybridization using biotinylated tyramide signal amplification (**B**, **D**). A few positive cells can be seen in the macula stage (**B**) and a strong staining of most of the spindle cells in the nodular form of Kaposi's sarcoma (**D**)

ate controls are mandatory to avoid false-positive results. However, so far, no other test achieves equal sensitivity. Using nested PCR, HHV8 DNA can be detected in virtually all Kaposi's sarcoma tissues, including formalin-fixed, paraffin embedded tissue, and in all clinical forms, including the classic, endemic, transplantation-associated and African tumors [2, 22, 23, 24, 33, 44, 60, 63, 87, 95, 127]. Many studies used primer from the minor capsid protein region (ORF26), the originally described region of the HHV8 genome [33]. Primer pairs of other gene regions, especially ORF 25, have been used equally successfully and, although variation within the HHV8 genome has been described, most of these gene regions revealed no major sequence variability and subsequent decrease of the sensitivity of the assay [11, 21, 28]. Most studies were unable to detect HHV8 DNA in mesenchymal and vascular tumors other than Kaposi's sarcoma, including hemangiomas, hemangioendotheliomas, infantile capillary hemangioma and hemangiosarcomas [24, 63, 67, 73, 77, 122], although some controversies exist with respect to hemangiosarcomas [79].

To overcome the problems of false-positive results and to increase the specificity, an in situ hybridization assay can be performed. Using in situ hybridization and PCR in situ hybridization, HHV8 DNA has been shown to be present in the spindle and vascular cells of Kaposi's sarcoma [20, 50, 71]. In addition, using the same technique, HHV8 DNA has been reported to be present in the epidermal cells overlaying Kaposi's sarcoma tissue as well as in pneumocytes of patients with the tumor [50]. Using isotopic in situ hybridization with probes directed against HHV8 RNA, it has been further shown that viral RNA associated with latent infection is expressed in the majority of tumor spindle cells as well as in endothelial cells lining atypical blood vessels [125, 126]. In contrast, only few cells express transcripts associated with lytic infection and at least a subgroup of these cells are of monocytic origin [17, 125]. Nevertheless, for routine use, neither PCR in situ hybridization nor isotopic in situ hybridization are well suited as a diagnostic test. Therefore, non-radioisotopic, colorimetric assays have been applied to detect HHV8 in routinely processed tissue samples [105] (Cathomas et al, unpublished observations). ORF K12 (T0.7/Kaposin) RNA is a suitable target as it is expressed in latently HHV8-infected spindle and endothelial cells of Kaposi's sarcoma. Another target associated with lytic HHV8 infection is ORF K7 (T1.1/nut-1); this transcript is abundantly expressed, albeit in a small number of cells [97, 124, 139] (Cathomas et al, unpublished observations). Using digoxigenin-labeled oligonucleotides, the test revealed a rather low sensitivity, detecting K7 (T1.1/nut-1) RNA in only 63.2% and K12 (T0.7/Kaposin) RNA in 19.2% of Kaposi's sarcoma tissues, and early lesions were rarely positive [75]. To increase the sensitivity, signal amplification using biotinylated thyramide has been successfully applied and, using this more-sensitive technique, latency-associated HHV8 RNAs such as K12 (T0.7/Kaposin) or v-cyclin D can be detected in all forms and stages of Kaposi's sarcoma samples (Fig. 3) [105] (Cathomas et al, unpublished observations). Using immunohistochemistry, latency-associated HHV8 proteins have also been detected [43, 104], reporting a detection rate of 11 (78.6%) of 14 samples analyzed [43]. Finally, herpes virus particles have been visualized by electron microscopy in Kaposi's sarcoma tissue [97].

In Kaposi's sarcoma tissues, as outlined above, HHV8 can been detected using PCR in 95–100% of cases. It can therefore be concluded that HHV8 DNA is present in all variants of the tumor, and the detection of HHV8 DNA can be used as a diagnostic test for the disease. Failure to detect HHV8 in a given lesion (and a positive internal control to confirm the presence of appropriate DNA) should shed doubts on the primary diagnosis. The detection of HHV8 can further be used as a diagnostic tool in clinical specimens for which the diagnosis of Kaposi's sarcoma is difficult to achieve due to the small size of the biopsy and crash artifacts, as in biopsies of the lung and the gastrointestinal tract (Fig. 4). In addition, the assay can also be applied to fine-needle aspirates [1] and bronchoalveolar lavage fluids (BAL). The detection of HHV8 DNA in BAL of immunocompromised patients has been shown to have a high sensitivity and specificity for the diagnosis of pulmonary Kaposi's sarcoma and the virus may be detected prior to manifest lesions and disappear following successful therapy [12, 25, 128].

Detection of HHV8 in non-tumorous specimens of patients with and without Kaposi's sarcoma

HHV8 DNA can be detected in the blood, especially in the lymphocyte fraction of infected patients and the presence of viral DNA is predictive of a significantly increased risk of developing Kaposi's sarcoma [69, 89, 138]. In non-tumorous tissue of patients with Kaposi's sarcoma elsewhere, HHV8 DNA can be detected by means of PCR in up to 50%; the viral load in these non-tumorous tissues is, however, lower than in tumor tissue and may represent circulating HHV8-infected cells [22, 28, 38, 44]. Nevertheless, the detection of HHV8 DNA in non-tumorous tissue may also lower the specificity of the HHV8 detection by means of PCR as a diagnostic test in patients with Kaposi's sarcoma in another location [28].

Intriguingly, the presence of HHV8 DNA detected using PCR has been reported not only in semen of HIV-infected homosexual men but also in 23–91% of semen and 63% of non-neoplastic prostatic tissue of normal healthy men or patients without evidence of immunosuppression [74, 84]. These findings are important as they would support the concept that HHV8 is transmitted by sexual contact and implicate a widespread infection within the general population. However, subsequent studies were unable to confirm these data in healthy subjects and seroepidemiology makes a widespread infection in healthy, non-homosexual men unlikely [28, 41, 74, 131].

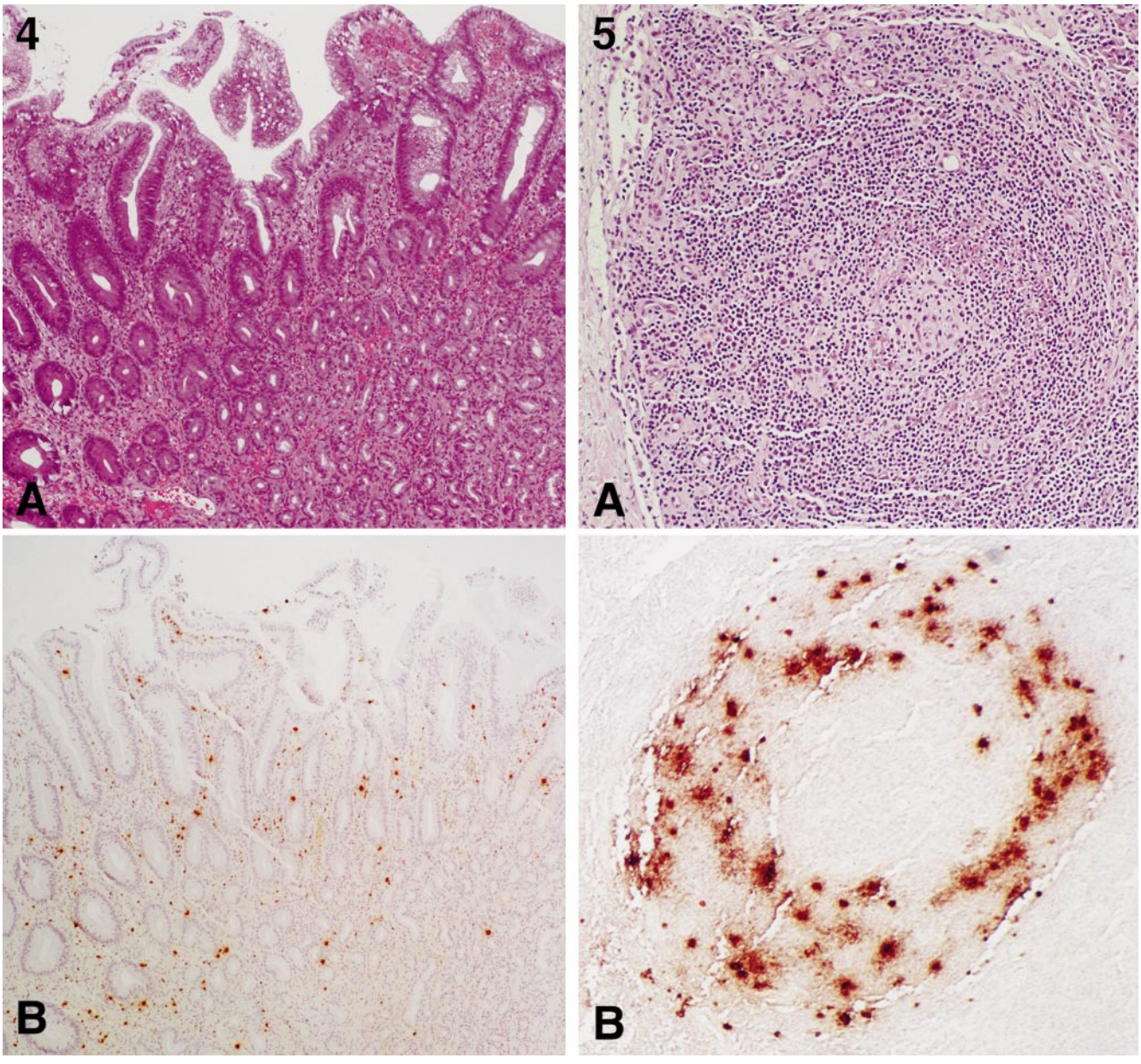

Fig. 4 Gastric involvement of a Kaposi's sarcoma in a patient with acquired immunodeficiency syndrome (AIDS): hematoxylin and eosin and in situ hybridization to detect latency-associated human herpes virus 8 (HHV8) RNA

Fig. 5 Acquired immunodeficiency syndrome (AIDS)-associated multicentric Castleman's disease (**A**, hematoxylin and eosin, frozen section) and in situ hybridization using a probe directed against a RNA transcript associated with lytic infection (**B**). The probe reveals a large number of positive cells in the mantle zone of a lymphoid follicle

PEL and MCD

Two additional diseases have been associated with HHV8, the body-cavity-based-lymphoma (BCBL), later referred to as PEL and the MCD. PEL is a rare malignant non-Hodgkin's lymphoma, emerging as serous pleural and pericardial effusion or ascites, usually without a detectable mass or lymph-node involvement [4, 30, 65, 92]. However, solid tumor masses of PEL have been described in lymph nodes, lung and the gastrointestinal tract [39, 65, 92]. Meta- or synchrone Kaposi's sarcoma is common [4, 30, 65]. The tumor is associated with HIV-infection but PEL in HIV-negative patients has been described [32, 92]. PEL often shows co-infection with EBV, but EBV-negative forms also exist, especially in HIV-negative patients [8]. Morphologically, the PEL bridges large-cell immunoblastic lymphoma and anaplastic large-cell lymphoma [4, 30, 65, 92]. The tumor cells are polymorphous, with abundant cytoplasm often with a clear perinuclear Golgi zone. Most of the nuclei are round to oval with prominent nucleoli, a subset of cells show highly irregular, polylobular or multiple nuclei. The tumor cells express CD45, but there is frequently an absence of other B-cell-associated antigens. As the ma-

jority of tumors are also positive for CD30, CD38 and epithelial membrane antigene (EMA), it was suggested to put PEL in the group of mature plasma cell neoplasms [62]. Clonal immunoglobulin gene rearrangement is present, but a c-myc gene rearrangement is missing [4, 30, 65, 92]. In addition, a PEL of the T-cell phenotype has been reported [114]. HHV8 is thought to be invariably associated with this lymphoma, and the detection of HHV8 can therefore be used for the diagnosis of this non-Hodgkin's lymphoma. The presence of HHV8 may further be helpful to distinguish PEL from pyothorax-associated lymphoma (PAL), another malignant non-NHL associated with malignant effusions [6]. A number of cell lines deriving from PEL have been established, which can maintain the virus in vitro [8, 31, 110]. These cell lines have been important in the study of viral replication and as a base for serological assays (see above). In contrast, HHV8 DNA has rarely been detected in other forms of malignant non-Hodgkin's lymphoma in patients with or without immunosuppression [26, 30, 52].

HHV8 DNA has been further detected in most patients with MCD associated with HIV infection [123]. In HIV-negative patients, HHV8 was detected in about 40% of the multicentric variant of this disease, but in the localized form of Castleman's disease, HHV8 can only rarely been detected [10, 123]. MCD, a non-neoplastic angiofollicular lymphoid hyperplasia, usually of the plasma cell or mixed plasma cell/hyaline vascular type [96, 123], affects multiple lymphoid organs and may be associated with a number of diseases showing immunosuppression or immuno-dysregulation (reviewed in [101]). Recently, the presence of HHV8 has further been reported in 6 of 7 patients with MCD in POEMS' syndrome [11]. Although the precise pathogenesis of the disease remains to be elucidated, extensive production of cytokines has been reported in MCD, especially of IL-6, and HHV8-derived vIL-6 was detected in the lymph node of HHV8 by means of immunohistochemistry [98] and PCR [132]. In contrast to Kaposi's saracoma, lymph nodes of HHV8-associated MCD reveal in the mantle zone of lymphoid follicles a significant expression of HHV8 RNA associated with lytic infection, and the pattern of expression is characteristic of HHV8-associated MCD (Fig. 5) [124] (Cathomas et al, unpublished observations).

HHV 8 in other diseases

There are a number of data reporting the presence of HHV8 DNA in various other diseases and lesions. The most intriguing is the presence of HHV8 in cultured bone-marrow stromal dendritic cells [111] and bone-marrow biopsies [113] of patients with multiple myeloma and in a proportion of patients with monoclonal gammopathy of unknown significance (MGUS) [111]. These data, have been confirmed by some groups [35, 54], whereas others have been unable to detect evidence of HHV8 infection in these patients, either using PCR or serological tests [27, 99, 130, 134], and a fierce controversy has emerged concerning the role of HHV8 in multiple myeloma [15, 129]. To explain these conflicting results, differences in the techniques applied by the various groups or other so-far unknown viruses have been incriminating, but further data are urgently needed to define the role of HHV8 in this disease.

Additional reports describe the detection of HHV8 DNA in epithelial skin tumors following solid organ transplantation [103], pemphigus vulgaris/foliaceus [80], sarcoidosis [40] and angiolymphoid hyperplasia [76]. Most of these findings have, however, not been confirmed by other groups [21, 26, 29, 37, 85, 107]. It remains to be seen whether these controversial reports, often based on the detection of HHV8 DNA by means of PCR, originate in false-positive results generated by nested PCR or are due to marked regional differences in the prevalence of HHV8 [85]. Nevertheless, based on the discrepancies reported and the fact that, in contrast to Kaposi's sarcoma, many groups failed to regularly detect HHV8 in these lesions, it is unlikely that HHV8 is involved in the basic pathogenesis of these diseases.

HHV8, a human tumor virus?

Due to the long incubation period and the fact that only a subgroup of infected patients finally develop a tumor, it is difficult to prove the link between a given virus and an associated neoplasm. Based on the famous postulate of Robert Koch, Evans and Müller proposed a number of criteria to prove the causal relationship of virus and tumor [47]: the geographic distribution of the infection should cover similar areas as the tumor; markers of viral infection should precede the development of the tumor; and the rate of infection should be higher in patients with than without the tumor [47]. In addition, the quantity of viral markers indicated by antibody titers or viremia should be increased in the tumor population relative to the matched controls, and prevention of the infection, such as by vaccination, should decrease the tumor incidence. Besides these epidemiological points, virological aspects should also be fulfilled, including the presence of viral nucleic acids in most tumor tissue samples (but not in normal cells) as well as experimental evidence, such as the transformation capacity of the virus (or viral genes) and the possibility of experimental induction of tumors in a susceptible animal system [47].

As outlined above, for HHV8, a number of these criteria have been already fulfilled. The seroepidemiology parallels the risk of Kaposi's sarcoma in terms of geographical distribution as well as within different patient groups with a well-known risk of developing the tumor. In addition, it has been shown that the HHV8 antibody as well as the detection of HHV8 DNA by means of PCR precedes the development of Kaposi's sarcoma, and patients with HHV8 infection have a significantly increased risk of developing the tumor, as has been shown in HIV-infected patients and in transplant recipients [78, 108]. HHV8 DNA can be detected in all tumor tissue,

Table 1 Viruses associated with human tumors. *HPV* human papillomavirus; *HBV* hepatitis B virus; *HCV* hepatitis C virus; *HTLV I* human T-cell leukemia virus I; *EBV, HHV5* Epstein-Barr-virus; *PTLD* post-transplantation lymphoproliferative disease;

Virus	Family	Non-malignant diseases	Associated malignancies	Co-factors
HPV	Papovaviridiae	Warts, laryngeal papillomatosis	Epidermoid cancer of cervix, anus, vulva and penis	
HBV	Hepadnavirus	Chronic hepatitis Cirrhosis	Hepatocellular carcinoma	Aflatoxin, Alcoholism Co-infection with HCV
HCV	Flaviviridiae	Chronic hepatitis Cirrhosis	Hepatocellular carcinoma	Alcoholism Co-infection with HBV
HTLV I	C-Retrovirus	Tropical spastic paresis	Adult T-cell leukemia	
EBV, HHV5	γ-Herpes virus	Infectious mononucleosis Oral hairy leukoplakia	Burkitt's lymphoma (endemic form) PTLD X-linked lymphoproliferative disease Nasopharyngeal carcinoma Hodgkin's disease AIDS-associated CNS-lymphoma Leiomyosarcoma in children with AIDS	Malaria Immunosuppression Gene defects
Human herpes virus 8 (HHV8)	γ_2-Herpes virus	Multicentric Castleman's disease	Kaposi's sarcoma	Immunosuppression
Kaposi's sarcoma-associated herpes virus (KSHV)			Primary effusion lymphoma	

and there is at least some evidence that HHV8 has a transforming capacity in vitro [49, 68, 91].

Kaposi's sarcoma itself, however, is a rather peculiar lesion and even the question whether Kaposi's sarcoma is really a neoplastic or basically a reactive proliferation remains a controversial issue [53]. Several studies have shown high levels of cytokines and chemokines within Kaposi's sarcoma lesions and especially the dependence on these factors to maintain cell proliferation (reviewed in [45]). It is therefore very likely that, as for other tumors, cofactors are an important prerequisite for the development of the lesion, of which immunosuppression is the most obvious. A possible explanation for the difference between HHV8 infection and the traditional viral transforming pathway of other tumor viruses is the recent finding that inflammatory cytokines found in Kaposi's sarcoma lesions reactivate latent HHV8 infection [83]. It has therefore been proposed that latently infected cells exposed to sites of inflammation with an increased cytokine environment leads to the reactivation of HHV8 and subsequent expression of potential pathogenic genes as vIL6. This lytic infection subsequently re-infects other cells, maintaining, especially in the context of immunosuppression and immuno-dysregulation, a cycle of lytic and latent infection, sustaining the proliferative stage of Kaposi's sarcoma [81].

Much less is known about the pathogenesis of PEL, a rare disease. However, the fact that cell lines of this type of lymphoma maintain replicative virus also in the EBV-negative forms makes it likely that this tumor is also basically caused by HHV8. Taken together, the data available today points in the direction that HHV8 is indeed the cause of Kaposi's sarcoma and PEL, and can be seen as a new virus associated with human tumors (Table 1).

In conclusion, the most recently detected human herpes virus, HHV8, is closely linked to all forms of Kaposi's sarcoma. The total of epidemiological evidence and the molecular and cellular biology data strongly suggest that HHV8 is a new human oncogenic virus directly involved in the pathogenesis of Kaposi's sarcoma. This close correlation makes the detection of HHV8 a useful diagnostic marker, especially using in situ hybridization and eventually immunohistochemistry. In addition, there is strong evidence that HHV8 is the cause of a rare malignant non-Hodgkin's lymphoma, PEL, and subgroup of MCDs. Again, the detection of the virus in this lesion is a helpful tool in the diagnosis of these diseases. A number of questions, however, remain to be answered, especially relating to the precise epidemiology, the mode of transmission in the general population, and the cofactors leading to the development of HHV8-associated diseases in persons infected with the virus. In addition, the role of HHV8 in other diseases, especially in the multiple myeloma, has to be clarified. Finally, the better understanding of the pathogenesis of HHV8 infection will ultimately lead to more specific therapeutic approaches and, hopefully, to a vaccine. In any case, the detection of HHV8 has opened a new exciting field in viral oncogenesis.

References

1. Alan S, Eltoum IA, Tabbara S, Day E, Karcher DS (1999) Usefulness of molecular detection of human herpesvirus-8 in the diagnosis of Kaposi sarcoma by fine-needle aspiration. Am J Clin Pathol 111:91–96
2. Ambroziak JA, Blackbourn DJ, Herndier BG, Glogau RG, Gullet JH, McDonald AR, Lenette ET, Levy JA (1995) Herpes-like sequences in HIV-infected and uninfected Kaposi's sarcoma patients. Science 268:582–583

3. Andreoni M, El-Sawaf G, Rezza G, Ensoli B, E. N, Ventura L, Ercoli L, Sarmati L, Rocchi G (1999) High seroprevalence of antibodies to human herpesvirus-8 in Egyptian children: evidence of nonsexual transmission. J Natl Cancer Inst 91:465–469
4. Ansari MQ, Dawson DB, Nador R, Rutherford C, Schneider NR, Latimer JM, Picker L, Knowles DM, McKenna RW (1996) Primary body cavity-based AIDS-related lymphomas. Am J Clin Pathol 105:221–229
5. Aoki Y, Jaffe ES, Chang Y, Jones K, Teruya-Feldstein J, Moore PS, Tosato G (1999) Angiogenesis and hematopoiesis induced by Kaposi's sarcoma-associated herpesvirus-encoded interleukin-6. Blood 93:4034–4043
6. Aozasa K, Ohsawa M, Kanno H (1997) Pyothorax-associated lymphoma: a distinctive type of lymphoma strongly associated with Epstein-Barr Virus. Adv Anat Pathol 4:58–63
7. Arvanitakis L, Geras RE, Varma A, Gershengorn MC, Cesarman E (1997) Human herpesvirus KSHV encodes a constitutively active G-protein-coupled receptor linked to cell proliferation. Nature 385:347–350
8. Arvanitakis L, Mesri EA, Nador RG, Said JW, Asch SA, Knowles DM, Cesarman E (1996) Establishment and characterization of a primary effusion (body cavity-based) lymphoma cell line (BC-3) harboring Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) in the absence of Epstein-Barr virus. Blood 88:2648–2654
9. Bais C, Santomasso B, Coso O, Arvanitakis L, Raaka EG, Gutkind JS, Asch AS, Cesarman E, Gerhengorn MC, Mesri EA (1998) G-protein-coupled receptor of Kaposi's sarcoma-associated herpesvirus is a viral oncogene and angiogenesis activator. Nature 391:86–89
10. Barozzi P, Luppi M, Marasca R, Savarino M, Morselli M, Ferrari MG, Bevini M, Bonacorsi G, Torelli G (1996) Lymphotropic herpes virus (EBV, HHV-6, HHV-8) DNA sequences in HIV negative Castleman's disease. J Clin Pathol Mol Pathol 49:M232–M235
11. Bélec L, Mohamed AS, Authier F-J, Hallouin M-C, Soe AM, Cotigny S, Gaulard P, Gherardi RK (1999) Human herpesvirus 8 infection in patients with POEMS syndrome-associated multicentric Castleman's disease. Blood 93:3643–3653
12. Benfield TL, Dodt KK, Lundgren JD (1997) Human herpes virus-8 DNA in bronchoalveolar lavage samples from patients with AIDS-associated pulmonary Kaposi's sarcoma. Scand J Infect Dis 29:13–16
13. Beral V, Bull D, Darby S, Weller I, Carne C, Beecham M, Jaffe HW (1992) Risk of Kaposi's sarcoma and sexual practices associated with faecal contact in homosexual or bisexual men with AIDS. Lancet 339:632–635
14. Beral V, Peterman TA, Berkelman RL, Jaffe HW (1990) Kaposi's sarcoma among persons with AIDS: a sexually transmitted infection? Lancet 335:123–128
15. Berenson JR, Vescio RA (1999) HHV-8 is present in multiple myeloma patients. Blood 93:3157–3166
16. Blackbourn DJ, Osmond D, Levy JA, Lennette ET (1999) Increased human herpesvirus 8 seroprevalence in young homosexual men who have multiple sex contacts with different partners. J Infect Dis 179:237–239
17. Blasig C, Zietz C, Haar B, Neipel F, Esser S, Brockmeyer NH, Tschachler E, Colombini S, Ensoli B, Stürzl M (1997) Monocytes in Kaposi's sarcoma lesions are productively infected by human herpesvirus 8. J Virol 71:7963–7968
18. Blauvelt A, Sei S, Cook PM, Schulz TF, Jeang K-T (1997) Human herpesvirus 8 infection occurs following adolescence in the United States. J Infect Dis 176:771–774
19. Boshoff C, Endo Y, Collins PD, Takeuchi Y, Reeves JD, Schweickart VL, Siani MA, Sasaki T, Williams TJ, Gray PW, Moore PS, Chang Y, Weiss RA (1997) Angiogenic and HIV-inhibitory functions of KSHV-encoded chemokines. Science 278: 290–294
20. Boshoff C, Schulz TF, Kennedy MM, Graham AK, Fisher C, Thomas A, McGee JOD, Weiss RA, O'Leary JJ (1995) Kaposi's sarcoma-associated herpesvirus infects endothelial and spindle cells. Nat Med 1:1274–1278
21. Boshoff C, Talbot S, Kennedy M, O'Leary J, Schulz T, Chang Y (1996) HHV8 and skin cancers in immunosuppressed patients. Lancet 347:338–339 (published erratum Lancet 348:138)
22. Boshoff C, Whitby D, Hatziioannou T, Fisher C, van der Walt J, Hatzakis A, Weiss R, Schulz T (1995) Kaposi's sarcoma associated herpesvirus in HIV-negative Kaposi's sarcoma. Lancet 345:1043–1044
23. Buonaguro FM, Tornesello ML, Beth-Giraldo E, Hatzakis A, Mueller N, Downing R, Biryamwaho B, Sempala DK, Giraldo G (1996) Herpesvirus-like DNA sequences detected in endemic classic, iatrogenic and epidemic Kaposi's sarcoma (KS) biopsies. Int J Cancer 65:25–28
24. Cathomas G, McGandy CE, Terracciano LM, Itin PH, DeRosa G, Gudat F (1996) Detection of herpesvirus-like DNA by nested polymerase chain reaction in archival skin biopsies of various forms of Kaposi's sarcoma. J Clin Pathol 49:631–633
25. Cathomas G, Tamm M, McGandy CE, Perruchoud AP, Mihatsch MJ, Dalquen P (1996) Detection of herpesvirus like DNA in the bronchoalveolar lavage fluid of patients with pulmonary Kaposi's sarcoma. Eur Respir J 9:1743–1746
26. Cathomas G, Tamm M, McGandy CE, Hin PI, Gudat F, Thiel G, Mihatsch MJ (1997) Transplantation-associated malignancies: restriction of human herpes virus 8 (HHV8) to Kaposi's sarcoma. Transplantation 64:175–178
27. Cathomas G, Stalder A, Kurrer MO, Regamey N, Erb P, Joller-Jemelkt HI (1998) Multiple myeloma and HHV8 infection. Blood 91:4391–4393
28. Cathomas G, Stalder A, McGandy CE, Mihatsch MJ (1998) Distribution of human herpes virus 8 (HHV-8) DNA in tumorous and non-tumorous tissue of AIDS patients with and without Kaposi's sarcoma. Mod Pathol 11:415–420
29. Cathomas G, Regamey N, Stalder A, Erb P, Itin HP (1998) No evidence of HHV8 infection in patients with pemphigus vulgaris/foliaceus. Arch Dermatol 134:1162
30. Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM (1995) Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. N Engl J Med 332:1186–1191
31. Cesarman E, Moore PS, Rao PH, Inghirami G, Knowles DM, Chang Y (1995) In vitro establishment and charcterization of two acquired immunodeficiency syndrome-related lymphoma cell lines (BC-1 and BC-2) containing Kaposi's sarcoma-associated herpesvirus-like (KSHV) DNA sequences. Blood 86: 2708–2714
32. Cesarman E, Nador RG, Aozasa K, Delsol G, Said JW, Knowles DM (1996) Kaposi's sarcoma-associated herpesvirus in non-AIDS-related lymphomas occurring in body cavities. Am J Pathol 149:53–57
33. Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS (1994) Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 266:1865–1869
34. Chatlynne LG, Lapps W, Handy M, Huang YQ, Masood R, Hamilton AS, Said JW, Koeffler HP, Kaplan MH, Friedman-Kien A, Gill PS, Whitman JE, Ablashi DV (1998) Detection and titration of human herpesvirus-8-specific antibodies in sera from blood donors, acquired immunodeficiency syndrome patients, and Kaposi's sarcoma patients using a whole virus enzyme-linked immunosorbent assay. Blood 92:53–58
35. Chauhan D, Bharti A, Raje N, Gustafson E, Pinkus GS, Pinkus JL, Teoh G, Hideshima T, Treon SP, Fingerroth JD, Anderson KC (1999) Detection of Kaposi's sarcoma herpesvirus DNA sequences in multiple myeloma bone marrow stromal cells. Blood 93:1482–1486
36. Cheng EH, Nicholas J, Bellows DS, Hayward GS, Guo HG, Reitz MS, Hardwick JM (1997) A Bcl-2 homolog encoded by Kaposi sarcoma-associated virus, human herpesvirus 8, inhibits apoptosis but does not heterodimerize with Bax or Bak. Proc Natl Acad Sci U S A 94:690–694
37. Cohen SS, Weinstein MD, Herndier BG, Anhalt GJ, Blauvelt A (1998) No evidence of human herpesvirus 8 infection in patients with paraneoplastic pemphigus, pemphigus vulgaris, or pemphigus foliaceus. J Invest Dermatol 111:781–783

38. Corbellino M, Poirel L, Bestetti G, Pizzuto M, Aubin JT, Capra M, Bifulco C, Berti E, Agut H, Rizzardini G, Galli M, Parravicini C (1996) Restricted tissue distribution of extralesional Kaposi's sarcoma associated herpesvirus-like DNA sequences in AIDS patients with Kaposi's sarcoma. AIDS Res Hum Retroviruses 12:651–657
39. DePond W, Said JW, Tasaka T, Devos S, Kahn D, Cesarman E, Knowles DM, Koeffler HP (1997) Kaposis sarcoma associated herpesvirus and human herpesvirus 8 (KSHV/HHV8) associated lymphoma of the bowel: report of two cases in HIV positive men with secondary effusion lymphomas. Am J Surg Pathol 21:719–724
40. DiAlberti L, Piattelli A, Artese L (1998) Human herpes virus 8 variants in sarcoid tissue. Lancet 350:1655–1660
41. Diamond C, Huang ML, Kedes DH, Speck C, Rankin GW, Ganem D, Rose TM, Krieger JN, Corey L (1997) Absence of detectable human herpes virus 8 in the semen of human immunodeficiency virus-infected men without Kaposi's sarcoma. J Infect Dis 176:775–777
42. Drew WL, Conant MA, Miner RC, Huang E-S, Ziegler JL, Groundwater JR, Gullett JH, Volberding P, Abrams DI, Mintz L (1982) Cytomegalovirus and Kaposi's sarcoma in young homosexual men. Lancet ii:125–126
43. Dupin N, Fisher C, Kellam P, Ariad S, Tulliez M, Franck N,Marck E van, Salmon D, Gorin I, Escade J-P, Weiss RA, Alitalo K, Boshoff C (1999) Distribution of human herpesvirus-8 latently infected cells in Kaposi's sarcoma, multicentric Castleman's disease, and primary effusion lymphoma. Proc Natl Acad Sci U S A 96:4546–4551
44. Dupin N, Grandadam M, Calvez V, Gorin I, Aubin JT, Havard S, Lamy F, Leibowitch M, Huraux JM, Escande JP, Agut H (1995) Herpesvirus-like DNA sequences in patients with mediterranean Kaposi's sarcoma. Lancet 345:761–762
45. Ensoli B, Stürzl M (1998) Kaposi's sarcoma: a result of the interplay among inflammatory cytokines, angiogenetic factors and viral antigens. Cytokine Growth Factors Rev 9:63–83
46. Enzinger FM, Weiss SW (1995) Malignant vascular tumors, In: Soft tissue tumors. 3rd edn. Mosby, St.Louis, pp 641–677
47. Evans AS, Müller NE (1990) Viruses and cancer: causal associations. Ann Epidemiol 1:71–92
48. Farge D, Lebbé C, Marjanovic Z, Tuppin P, Mouquet C, Peraldi M-N, Lang P, Hiesse C, Antoine C, Legendre C, Bedrossian J, Gagnadoux MF, Loirat C, Pellet C, Sheldon J, Golmard J-L, Agbalika F, Schulz TF (1999) Human herpes virus-8 and other risk factors for Kaposi's sarcoma in kidney transplant recipients. Transplantation 67:1236–1242
49. Flore O, Rafii S, Ely S, O'Leary JJ, Hyjek EM, Cesarman E (1998) Transformation of primary human endothelial cells by Kaposi's sarcoma-associated herpesvirus. Nature 394:588–592
50. Foreman KE, Bacon PE, Hsi ED, Nickoloff BJ (1997) In situ polymerase chain reaction-based localization studies support role of human herpesvirus-8 as the cause of two AIDS-related neoplasms: Kaposi's sarcoma and body cavity lymphoma. J Clin Invest 99:2971–2978
51. Foreman KE, Friborg J, Kong W-P, Woffendin C, Polverini PJ, Nickoloff BJ, Nabel GJ (1997) Propagation of human herpesvirus from AIDS-associated Kaposi's sarcoma. N Engl J Med 336:163–171
52. Gaidano G, Pastore C, Gloghini A, Cusini M, Nomdedeu J, Volpe G, Capello D, Vaccher E, Bordes R, Tirelli U, Saglio G, Carbone A (1996) Distribution of human herpesvirus-8 sequences throughout the spectrum of AIDS-related neoplasia. AIDS 10:941–949
53. Gallo RC (1998) The enigmas of Kaposi's sarcoma. Science 282:1837–1839
54. Gao S-J, Alsina M, Deng J-J, Harrison CR, Montalvo EA, Leach CT, Roodman GD, Jenson HB (1998) Antibodies to Kaposi's sarcoma -associated herpesvirus (human herpesvirus 8) in patients with multiple myeloma. J Infect Dis 178:846–849
55. Gao S-J, Boshoff C, Jayachandra S, Weiss RA, Chang Y, Moore PS (1997) KSHV ORF K9 (vIRF) is an oncogene which inhibits the interferon signaling pathway. Oncogene 15: 1979–1985
56. Gao S-J, Kingsley L, Hoover D, Spira TJ, Rinaldo C, Saah A, Phair J, Detels R, Parry P, Chang Y, Moore PS (1996) Seroconversion to antibodies against Kaposi's sarcoma-associated herpesvirus-related latent nuclear antigens before the development of Kaposi's sarcoma. N Engl J Med 335:233–241
57. Gao S-J, Kingsley L, Li M, Zheng W, Parravicini C, Ziegler J, Newton R, Rinaldo C, Saah A, Phair J, Detels R, Chang Y, Moore PS (1996) KSHV antibodies among Americans, Italians and Ugandans with and without Kaposi's sarcoma. Nat Med 2:925–928
58. Gluckman E, Parquet N, Scieux C, Deplanche M, Traineau R, Betheau P, Morinet F (1995) KS-associated herpesvirus-like DNA sequences after allogeneic bone-marrow transplantation. Lancet 346:1557–1558
59. He J, Bhat G, Kankasa C, Chintu C, Mitchell C, Duan W, Wood C (1998) Seroprevalence of human herpesvirus 8 among Zambian women of childbearing age without Kaposi's sarcoma (KS) and mother-child pairs with KS. J Infect Dis 178:1787–1790
60. Huang YQ, Li JJ, Kaplan MH, Poiesz B, Katabira E, Zhang WC, Feiner D, Friedman-Kien AE (1995) Human herpesvirus-like nucleic acid in various forms of Kaposi's sarcoma. Lancet 345:759–761
61. Hudnall SD, Rady PL, Tyring SK, Fish JC (1998) Serologic and molecular evidence of human herpesvirus 8 activation in renal transplant recipients. J Infect Dis 178:1791–1794
62. Jaffe ES (1996) Primary body cavity-based AIDS-related lymphomas: evolution of a new disease entity. Am J Clin Pathol 105:141–143
63. Jin Y-T, Tsai S-T, Yan J-J, Hsia J-H, Lee Y-Y, Su I-J (1996) Detection of Kaposi's sarcoma-associated herpesvirus-like DNA sequence in vascular lesions. Am J Clin Pathol 105:360–363
64. Kaposi M (1872) Idiopathisches multiples Pigmentsarkom der Haut. Arch Dermatol Syphilis 4:742–749
65. Karcher DS, Alkan S (1997) Human herpesvirus-8-associated body cavity-based lymphoma in human immunodeficiency virus-infected patients: a unique B-cell neoplasm. Hum Pathol 28: 801–808
66. Kedes DH, Operskalski E, Busch M, Kohn R, Flood J, Ganem D (1996) 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 2:918–924
67. Lasota J, Miettinen M (1999) Absence of Kaposi's sarcoma-associated virus (human herpesvirus-8) sequences in angiosarcoma. Virchows Arch 434:51–56
68. Lee H, Veazey R, Williams K, Li M, Guo J, Neipel F, Fleckenstein B, Lackner A, Desroisiers RC, Jung JU (1998) Deregulation of cell growth by the K1 gene of Kaposi's sarcoma-associated herpesvirus. Nat Med 4:435–440
69. Lefrere JJ, Meyohas MC, Mariotti M, Meynard JL, Thauvin M, Frottier J (1996) Detection of human herpesvirus 8 DNA sequences before the appearance of Kaposi's sarcoma in human immunodeficiency virus (HIV)-positive subjects with a known date of HIV seroconversion. J Infect Dis 174:283–287
70. Lennette ET, Blackbourn DJ, Levy JA (1996) Antibodies to human herpesvirus type 8 in the general population and in Kaposi's sarcoma patients. Lancet 348:858–861
71. Li JJ, Huang YQ, Cockerell CJ, Friedman KA (1996) Localization of human herpes-like virus type 8 in vascular endothelial cells and perivascular spindle-shaped cells of Kaposi's sarcoma lesions by in situ hybridization. Am J Pathol 148: 1741–1748
72. Li M, Lee H, Yoon D-W, Albrecht J-C, Fleckenstein B, Neipel F, Jung JU (1997) Kaposi's sarcoma-associated herpesvirus encodes a functional cyclin. J Virol 71:1984–1991
73. Lin BT-Y, Chen Y-Y, Battifora H, Weiss LM (1996) Absence of Kaposi's sarcoma-associated herpesvirus-like DNA sequences in malignant vascular tumors of the serous membranes. Mod Pathol 9:1143–1146
74. Lin J-C, Lin S-C, Mar EC, Pellett PE, Stamey FR, Stewart JA, Spira TJ (1995) Is Kaposi's-sarcoma-associated herpesvi-

rus detectable in semen of HIV-infected homosexual men? Lancet 346:1601–1602 (Retraction Lancet 1998; 351:1365)
75. Linderoth J, Rambech E, Dictor M (1999) Dominant human herpesvirus type 8 RNA transcripts in classical and AIDS-related Kaposi's sarcoma. J Pathol 187:582–587
76. Luppi M, Barozzi P, Maiorana A, Artusi T, Trovato R, Marasca R, Savarino M, Ceccherini NL, Torelli G (1996) Human herpesvirus-8 DNA sequences in human immunodeficiency virus- negative angioimmunoblastic lymphadenopathy and benign lymphadenopathy with giant germinal center hyperplasia and increased vascularity. Blood 87:3903–3909
77. Maiorana D, Luppi M, Borozzi P, Collina G, Fano RA, Torelli G (1997) Detection of human herpes virus type 8 DNA sequences as a valuable aid in the differential diagnosis of Kaposi's sarcoma. Mod Pathol 10:182–187
78. Martin JN, Ganem DE, Osmond DH, Page-Shafer KA, Macrae D, Kedes DH (1998) Sexual transmission and the natural history of human herpesvirus 8 infection. N Engl J Med 338:948–954
79. McDonagh DP, Liu J, Gaffey MJ, Layfield LJ, Azumi N, Traweek ST (1996) Detection of Kaposi's sarcoma-associated herpesvirus-like DNA sequences in angiosarcoma. Am J Pathol 149:1363–1368
80. Memar OM, Rady PL, Goldblum RM, Yen A, Tyring SK (1997) Human herpesvirus 8 DNA sequences in blistering skin from patients with pemphigus. Arch Dermatol 133:1247–1251
81. Mesri EA (1999) Inflammatory reactivation and angiogenicity of Kaposi's sarcoma-associated herpesvirus/HHV8: a missing link in the pathogenesis of acquired immunodeficiency syndrome-associated Kaposi's sarcoma. Blood 93:4031–4033
82. Miller G, Rigby MO, Heston L, Grogan E, Sun R, Metroka C, Levy JA, Gao S-J, Chang Y, Moore P (1996) Antibodies to butyrate-inducible antigens of Kaposi's sarcoma-associated herpesvirus in patients with HIV-1 infection. N Engl J Med 334: 1292–1297
83. Monini P, Colombini S, Stürzl M, Goletti D, Cafaro A, Sgadari C, Buttò S, Franco M, Leone P, Fais S, Leone P, Melucci-Vigo G, Chiozzini C, Carlini F, Ascherl G, Cornali E, Zietz C, Ramazzotti E, Ensoli F, Andreoni M, Pezzotti P, Rezza G, Yarchoan R, Gallo RC, Ensoli B (1999) Reactivation and persistence of human herpesvirus-8 infection in B cells and monocytes by Th-1 cytokines increased in Kaposi's sarcoma. Blood 93:4044–4058
84. Monini P, DeLellis L, Fabris D, Rigolin F, Cassai E (1996) Kaposi's sarcoma-associated herpesvirus DNA sequences in prostate tissue and human semen. N Engl J Med 334:1168–1172
85. Moore PS (1998) Human herpesvirus 8 variants. Lancet 351: 679–680
86. Moore PS, Boshoff C, Weiss RA, Chang Y (1996) Molecular mimicry of human cytokines and cytokine response pathway genes by KSHV. Science 274:1739–1744
87. Moore PS, Chang Y (1995) Detection of herpesvirus-like DNA sequences in Kaposi's sarcoma in patients with and those without HIV infection. N Engl J Med 332:1181–1185
88. Moore PS, Gao S-J, Dominguez G, Cesarman E, Lungu O, Knowles DM, Garber R, McGeoch DJ, Pellett P, Chang Y (1996) Primary charcterization of a herpesvirus-like agent assiciated with Kaposi's sarocma. J Virol 70:549–558
89. Moore PS, Kingsley LA, Holmberg SD, Spira T, Gupta P, Hoover DR, Parry JP, Conley LJ, Jaffe HW, Chang Y (1996) Kaposi's sarcoma-associated herpesvirus infection prior to onset of Kaposi's sarcoma. AIDS 10:175–180
90. Moses AV, Fish KN, Ruhl R, Smith PP, Strussenberg JG, Zhu L, Chandran B, Nelson JA (1999) Long-term infection and transformation of dermal microvascular endothelial cells by human herpesvirus 8. J Virol 73:6892–6902
91. Muralidhar S, Pumfery AM, Hassani M, Seadie R, Azumi N, Kishishita M, Brady JN, Doniger J, Medveczky P, Rosenthal LJ (1998) Identification of Kaposin (open reading frame K12) as a human herpesvirus 8 (Kaposi's sarcoma-associated herpesivrus) transforming gene. J Virol 72:4980–4988
92. Nador RG, Cesarman E, Chadburn A, Dawson DB, Ansari MQ, Said JW, Knowles DM (1996) Primary effusion lymphoma: a distinct clinicopathologic entity associated with the Kaposi's sarcoma-associated herpes virus. Blood 88:645–656
93. Neipel F, Albrecht J-C, Fleckenstein B (1997) Cell-homologous genes in Kaposi's sarcoma-associated rhadinovirus human hepresvirus 8: determinants of its pathogenicity? J Virol 71:4187–4192
94. Nicholas J, Ruvolo VR, Burns WH, Sandford G, Wan X, Ciufo D, Hendrickson SB, Guo HG, Hayward GS, Reitz MS (1997) Kaposi's sarcoma-associated human herpesvirus-8 encodes homologues of macrophage inflammatory protein-1 and interleukin-6. Nat Med 3:287–292
95. Noel JC, Hermans P, Andre J, Fayt I, Simonart Th, Verhest A, Haot J, Burny A (1996) Herpesvirus-like DNA sequences and Kaposi's sarcoma: relationship with epidemiology, clinical spectrum, and histologic features. Cancer 77:2132–2136
96. Oksenhendler E, Duarte M, Soulier J, Cacoub P, Weler Y, Cadranel J, Cazals-Hatem D, Autran B, Clauvel J-P, Raphel M (1996) Multicentric Castleman's disease in HIV infection: a clinical and pathological study of 20 patients. AIDS 10: 61–67
97. Orenstein JM, Alkan S, Blauvelt A, Jeang KT, Weinstein MD, Ganem D, Herndier B (1997) Visualization of human herpesvirus type 8 in Kaposi's sarcoma by light and transmission electron microscopy. AIDS 11:F35–45
98. Parravicini C, Corbellino M, Paulli M, Magrini U, Lazzarino M, Moore PS, Chang Y (1997) Expression of a virus-derived cytokine, KSHV vIL-6 in HIV-seronegative Castleman's disease. Am J Pathol 151:1517–1522
99. Parravicini C, Lauri E, Baldini L, Negri N, Poli F, Sirchia G, Moroni M, Galli M, Corbellino M (1997) Kaposi's sarcoma-associated herpesvirus infection and multiple myeloma. Science 278:1969–1970
100. Parravicini C, Olsen SJ, Capra M, Poli F, Sirchia G, Gao S-J, Bert E, Nocera A, Rossi E, Bestetti G, Pizzuto M, Galli M, Moroni M, Moore PS, Corbellino M (1997) Risk of Kaposi's sarcoma-associated herpes virus transmission from donor allografts among Italian posttransplant Kaposi's sarcoma patients. Blood 90:2826–2829
101. Peterson BA, Frizzera G (1993) Multicentric Castleman's disease. Semin Oncol 20:636–647
102. Rabkin CS, Schulz TF, Whitby D, Lenette ET, Magpantay LI, Chatlynne L, Biggar RJ (1998) Interassay correlation of human herpesvirus 8 serologic tests. J Infect Dis 178: 304–309
103. Rady PL, Yen A, Rollefson JL, Orengo I, Bruce S, Hughes TK, Tyring SK (1995) Herpesvirus-like DNA sequences in non-Kaposi's sarcoma skin lesions of transplant patients. Lancet 345:1339–1340
104. Rainbow L, Platt GM, Simpson GR, Sarid R, Gao S-J, Stoiber H, Herrington CS, Moore PS, Schulz TF (1997) The 222- to 234-kilodalton latent nuclear protein (LNA) of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) is encoded by orf73 and is a component of the latency-associated nuclear antigen. J Virol 71:5915–5921
105. Reed JA, Nador RG, Spauling D, Tani Y, Cesarman E, Knowles DM (1998) Demonstration of Kaposi's sarcoma associated herpes virus cycline D homolog in cutaneous Kaposi's sarcoma by colorimetric in situ hybridization using a catalyzed signal amplification system. Blood 91:3825–3832
106. Regamey N, Cathomas G, Schwager M, Wernli M, Harr T, Erb P (1998) High human herpes virus 8 seroprevalence in the homosexual population in Switzerland. J Clin Microbiol 36: 1784–1786
107. Regamey N, Erb P, Tamm M, Cathomas G (1998) Human herpesvirus 8 variants. Lancet 351:680
108. Regamey N, Tamm M, Schwager M, Wernli M, Cathomas G, Erb P (1998) Transmission of human herpes virus 8 (HHV8) infection from renal transplant donors to recipients. N Engl J Med 339:1358–1363

109. Renne R, Blackburn D, Whitby D, Levy J, Ganem D (1998) Limited transmission of Kaposi's sarcoma-associated herpesvirus in cultured cells. J Virol 72:5182–5188
110. Renne R, Zhong W, Herndier B, McGrath M, Abbey N, Kedes D, Ganem D (1996) Lytic growth of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in culture. Nat Med 2:342–346
111. Retting MB, Ma HJ, Vesico RA, Pold M, Schiller G, Belson D, Savage A, Nishikubo C, Wu C, Fraser J, Said JW, Berenson JR (1997) Kaposi's sarcoma-associated herpesvirus infection in bone marrow dendritic cells from multiple myeloma patients. Science 276:1851–1854
112. Russo JJ, Bohenzky RA, Chien M-C, Chien J, Yan M, Maddalena D, Parry JP, Peruzzi D, Edelman IS, Chang Y, Moore PS (1996) Nucleotide sequence of the Kaposi sarcoma-associated herpesvirus 8. Proc Natl Acad Sci U S A 93: 14862–14867
113. Said JW, Rettig MR, Heppner K, Vescio RA, Schiller G, Ma HJ, Belson D, Savage A, Shintaku IP, Koeffler HP, Asou H, Pinkus G, Pinkus J, Schrag M, Green E, Berenson JR (1997) Localization of Kaposi's sarcoma-associated herpesvirus in bone marrow biopsy samples from patients with multiple myeloma. Blood 90:4278–4282
114. Said JW, Shintaku IP, Asou H, deVos S, Baker J, Hanson G, Cesarman E, Nador R, Koeffler P (1999) Herpesvirus 8 inclusions in primary effusion lymphoma: report of a unique case with T-cell phenotype. Arch Pathol Lab Med 123: 257–260
115. Said R, Sato T, Bohenzky RA, Russo JJ, Chang Y (1997) Kaposi's sarcoma-associated herpesvirus encodes a functional Bcl-2 homologue. Nat Med 3:293–298
116. Sarid R, Flore O, Bohenzky RA, Chang Y, Moore PS (1998) Transcription mapping of the Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) genome in a body cavity-based lymphoma cell line (BC-1). J Virol 72:1005–1012
117. Schulz TF (1998) Kaposi's sarcoma associated herpesvirus (human herpesvirus-8). J Gen Virol 79:1573–1591
118. Siddiqui A (1983) Hepatitis B virus in Kaposi's sarcoma. Proc Natl Acad Sci U S A 80:4861–4864
119. Simpson GR, Schulz TF, Whitby D, Cook PM, Boshoff C, Rainbow L, Howard MR, Gao S-J, Bohenzky RA, Simmonds P, Lee C, deRuiter A, Hatzakis A, Tedder RS, Weller IVD, Weiss RA, Moore PS (1996) Prevalence of Kaposi's sarcoma associated herpesvirus infection measured by antibodies to recombinant capsid protein and latent immunofluorescence antigen. Lancet 348:1133–1138
120. Sitas F, Carrara H, Beral V, Newton R, Reeves G, Bull D, Jentsch U, Pacella-Norman R, Bourboulia D, Whitby D, Boshoff C, Weiss R (1999) Antibodies against human herpesvirus 8 in black South African patients with cancer. N Engl J Med 340:1863–1871
121. Smith MS, Bloomer C, Horvat R, Goldstein E, Casparian JM, Chandran BRA (1997) Detection of human herpesvirus 8 DNA in Kaposi's sarcoma lesions and peripheral blood of human immunodeficiency virus positive patients and correlation with serologic measurements. J Infect Dis 176:84–93
122. Smoller BR, Chang PP, Kamel OW (1997) No role for human herpes virus 8 in the etiology of infantile capillary hemangioma. Mod Pathol 10:675–678
123. Soulier J, Grollet L, Oksenhendler E, Cacoub P, Cazals-Hatem D, Babinet P, d'Agay M-F, Clauvel J-P, Raphael M, Degos L, Sigaux F (1995) Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castelman's disease. Blood 86:1276–1280
124. Staskus KA, Sun R, Miller G, Racz P, Jaslowski A, Metroka C, Brett-Smith H, Haase AT (1999) Cellular tropism and viral interleukin-6 expression distinguish human herpesvirus 8 involvement in Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease. J Virol 73:4181–4187
125. Staskus KA, Zhong W, Gebhard K, Herndier B, Wang H, Renne R, Beneke J, Pudney J, Anderson DJ, Ganem D, Haase AT (1997) Kaposi's sarcoma-associated herpesvirus gene expression in endothelial (spindle) tumor cells. J Virol 71: 715–719
126. Sturzl M, Blasig C, Schreier A, Neipel F, Hohenadl C, Cornali E, Ascherl G, Esser S, Brockmeyer NH, Ekman M, Kaaya EE, Tschachler E, Biberfeld P (1997) Expression of HHV-8 latency-associated T0.7 RNA in spindle cells and endothelial cells of AIDS-associated, classical and African Kaposi's sarcoma. Int J Cancer 72:68–71
127. Su I-J, Hsu YS, Chang Y-C, Wang I-W (1995) Herpesvirus-like DNA sequence in Kaposi's sarcoma from AIDS and non-AIDS patients in Taiwan. Lancet 345:722–723
128. Tamm M, Reichenberger F, McGandy C, Stalder A, Tietz A, Dalquen P, Perruchoud AP, Cathomas G (1998) Diagnosis of pulmonary Kaposi's sarcoma by detection of human herpes virus 8 in bronchoalveolar lavage. Am J Respir Crit Care Med 157:458–463
129. Tarte K, Chang Y, Klein B (1999) Kaposi's sarcoma-associated herpesvirus and multiple myeloma: lack of criteria for causality. Blood 93:3157–3166
130. Tarte K, Olsen SJ, Yang Z, Legouffe E, Rossi J-F, Cahng Y, Klein B (1998) Clinical-grade functional dendritic cells from patients with multiple myeloma are not infected with Kaposi's sarcoma-associated herpesvirus. Blood 91:1852–1857
131. Tasaka T, Said JW, Morosetti R, Park D, Verbeek W, Nagai M, Takahara J, Koeffler P (1997) Is Kaposi's sarcoma-associated herpesvirus ubiquitous in urogenital and prostate tissues? Blood 89:1686–1689
132. Teruya-Feldstein J, Zauber P, Setsuda JE, Bernman EL, Sorbara L, Raffeld M, Tosato G, Jaffe ES (1998) Expression of human herpesvirus-8 oncogene and cytokine homologues in an HIV-seronegative patient with multicentric Castleman's disease and primary effusion lymphoma. Lab Invest 78: 1637–1642
133. Thome M, Schneider P, Hofmann K, Fickenscher H, Meinl E, Neipel F, Mattmann C, Burns K, Bodmer J-L, Schröter M, Scaffidi C, Krammer PH, Peter ME, Tschopp J (1997) Viral FLICE-inhibitory proteins (FLIPs) prevent apoptosis induced by death receptor. Nature 386:517–521
134. Tisdale JF, Stewart K, Dickstein B, Little RF, Dubé I, Cappe D, Dunbar CE, Brown KE (1998) Molecular and serological examination of the relationship of human herpesvirus 8 to multiple myeloma: orf 26 sequences in bone marrow stroma are not restricted to myeloma patients and other regions of the genome are not detected. Blood 92:2681–2687
135. Vogel J, Hinrichs SH, Reynolds RK, Luciw PA, Jay G (1988) The HIV tat gene induces dermal lesions resembling Kaposi's sarcoma in transgenic mice. Science 335:606–611
136. Wabinga HR, Parkin DM, Wabwire-Mangen F, Mugerwa JW (1993) Cancer in Kampala, Uganda, in 1989–1991: changes in the incidence in the era of AIDS. Int J Cancer 54:26–36
137. Wang RY-H, Shih JW-K, Weiss SH, Grandinetti T, Pierce PF, Lange M, Alter HJ, Wear DJ, Davies CL, Mayur RK, Lo S-C (1993) Mycoplasma penetrans infection in male homosexuals with AIDS: high seroprevalence and association with Kaposi's sarcoma. Clin Infect Dis 17:724–729
138. Whitby D, Howard MR, Tenant-Flowers M, Brink NS, Copas A, Boshoff C, Hatzioannou T, Suggett FEA, Aldam DM, Denton AS, Miller RF, Weller IVD, Weiss RA, Tedder RS, Schulz TF (1995) Detection of Kaposi's sarcoma associated herpesvirus in peripheral blood of HIV-infected individuals and progression to Kaposi's sarcoma. Lancet 346: 799–802
139. Zhong W, Wang H, Herndier B, Ganem D (1996) Restricted expression of Kaposi sarcoma-assocaited herpesvirus (human herpesvirus 8) genes in Kaposi sarcoma. Proc Natl Acad Sci U S A 93:6641–6646
140. Zimring JC, Goodbourn S, Offermann MK (1998) Human herpesvirus 8 encodes an interferon regulatory factor (IRF) homolog that represses IRF-1-mediated transcription. J Virol 72:701–707