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Kaposi sarcoma herpesvirus was considered by a previous IARC Working Group in 1997 (IARC, 1997). Since that time, new data have become available, these have been incorporated into the Monograph, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Taxonomy, structure, and biology

1.1.1 Taxonomy

First detected by Chang et al. (1994) in Kaposi sarcomas associated with the acquired immune deficiency syndrome (AIDS) (see IARC, 1996) by representational difference analysis, this virus was termed Kaposi-sarcoma-associated herpesvirus, KSHV. KSHV is also associated with primary effusion lymphoma and some cases of multicentric Castleman disease (see Section 2). In keeping with the systematic nomenclature adopted for all human herpesviruses, the formal designation human herpesvirus 8 (HHV-8) was proposed by the herpesvirus subcommittee of the International Committee on the Taxonomy of Viruses. In this Monograph, the term KSHV is used throughout.

On the basis of phylogenetic analyses (Moore et al., 1996a; Russo et al., 1996), KSHV is a gamma-2 herpesvirus (rhadinovirus), and represents the first ‘human’ member of this group. There are many more gamma-2 herpesvirus species in old and new world non-human primates.

1.1.2 Structure of the virion

KSHV has the typical morphological characteristics of a herpesvirus (Fig. 1.1; Arvanitakis et al., 1996; Renne et al., 1996a; Orenstein et al., 1997) with 100–150 nm particles surrounded by a lipid envelope, and an electron-dense central core (Renne et al., 1996b). Cryo-electron microscopy (Cryo-EM) and Cryo-EM tomography studies suggest that KSHV capsomers are hexamers and pentamers of the major capsid protein (encoded by the open reading frame [ORF] 25), with the small capsid protein (encoded by ORF 65), binding around the tips of both hexons and pentons (Trus et al., 2001; Deng et al., 2008).

1.1.3 Structure of the viral genome

KSHV has a double-stranded DNA genome. The genomic structure of the virus (Russo et al., 1996; Neipel et al., 1997a) is similar to that of other primate rhadinoviruses, e.g. Herpesvirus saimiri (Albrecht et al., 1992) or Rhesus Rhadinovirus (Searles et al., 1999; Alexander et al., 2000), with a single, contiguous 140.5-kb-long unique region containing all the identified viral genes (Russo et al., 1996; Neipel et al., 1997a; Fig. 1.2). This region is flanked on either side by a terminal-repeat (TR) region composed of a variable number of repeats of 801-bp length with a high
Fig. 1.1 Electron microscopic view of KSHV capsids in a cross-section of a spleen Kaposi sarcoma

Cytoplasmic viral capsids obtain their lipid outer membrane by budding into cisternae; the electron dense central core represents viral DNA. Original magnification: x53 000.

The orientation of identified open reading frames in the long unique region is denoted by the direction of arrows, with *Herpesvirus saimiri* homologous with open reading frames as shaded areas and those not homologous as lighter areas. Seven blocks (numbered) of conserved herpesviral genes with non-conserved interblock regions (lettered) are shown under the kilobase marker. Features and putative coding regions not specifically designated are shown above the open reading frame map. Repeat regions (frnk, vnct, waka/jwka, zppa, moi, mdsk) are shown as light lines, and putative coding regions and other features are not designated as open reading frames are shown as solid lines.

G:C (84.5%) content. Due to the variable number of repeat subunits (some of the repeat subunits may be truncated), the overall length of the TR region varies, and with it, the overall size of the KSHV genome. The latter has been calculated to be approximately 165 kb on the basis of studies of the viral genome from productive primary effusion lymphoma cells (Arvanitakis et al., 1996; Renne et al., 1996b), and mapping of the whole genome (Russo et al., 1996).

(a) Terminal-repeat region

The TR region is a conserved feature of herpesviruses, and is involved in the packaging of the viral DNA into new virions during the lytic cycle of replication. Depending on the viral strain, KSHV has approximately 20–30 TR units. A particular feature of the KSHV TR unit is that it contains two binding sites for the KSHV latent nuclear antigen (LANA) and the latent (episomal) origin of replication (Garber et al., 2001; Hu et al., 2002). By binding to multiple TR subunits, LANA tethers circular viral episomes to mitotic chromosomes during mitosis (Ballestas et al., 1999; Barbera et al., 2006). LANA is also required for the replication of viral episomes by recruiting a range of cellular factors involved in DNA replication (see Section 4.1). Currently, the TR region is not known to contain any protein-coding ORFs in contrast to, for example, EBV (Longnecker & Neipel, 2007).

(b) Long unique region

The KSHV 140.5-kb long unique region encodes approximately 90 predicted ORFs (Russo et al., 1996; Neipel et al., 1997a; Fig. 1.2). The ORFs were named according to the corresponding herpesvirus saimiri genes with which they share a significant level of sequence similarity. Unique genes that are not homologous with herpesvirus saimiri have a K prefix. The long unique region has blocks of genes conserved among all subfamilies of herpesviruses (Chee et al., 1990), which include genes that encode herpesvirus structural proteins and replication enzymes. Between the conserved herpesvirus gene blocks lie blocks of genes that are either found in rhabdoviruses or are unique to KSHV (Russo et al., 1996). Several of these share significant sequence similarity with cellular genes, and were presumably carried away at some point during the evolution of these viruses.

The long unique region also contains genes for untranslated RNAs. Among these is the PAN/nut-1 transcript, a nuclear untranslated RNA, whose function is not yet clear (Sun et al., 1996; Zhong & Ganem, 1997). In addition, KSHV encodes at least 12 microRNAs, which are generated from one transcript, and located downstream of the ORFK13/vFLIP (Cai et al., 2005; Samols et al., 2005).

The probable function of these genes in the virus life cycle and tumour formation is discussed in Section 4.1.

1.1.4 Host range and tropism

Humans are the natural hosts for KSHV. Epidemiological studies indicate that KSHV is more prevalent in subSaharan Africa, several countries of southern Europe, the North African Mediterranean coast, and several countries of South America compared to northern Europe, North America, and Asia (see Section 1.2).

In vivo, KSHV has been detected in endothelial and spindle cells of Kaposi sarcoma lesions, in circulating endothelial cells, primary effusion lymphoma cells, B cells, macrophages, dendritic cells, oropharynx and prostatic glandular epithelium and keratinocytes (Ambroziak et al., 1995; Boshoff et al., 1995; Cesarman et al., 1995a; Moore & Chang, 1995; Corbellino et al., 1996; Li et al., 1996; Sirianni et al., 1997; Staskus et al., 1997; Stürzl et al., 1997; Reed et al., 1998; Pauk et al., 2000).

Of the cell types targeted by KSHV in vivo, primary endothelial cells of different differentiation (vascular, lymphatic, endothelial precursor...
cells), monocytes, dendritic cells, fibroblasts, epithelial cells and keratinocytes can be infected in vitro (Renne et al., 1998; Blackbourn et al., 2000; Cerimele et al., 2000; Wang et al., 2004a; Rappocciolo et al., 2006). It was shown that B cells can only be infected in vitro at a specific differentiation stage (Rappocciolo et al., 2006, 2008).

1.1.5 Viral life cycle

KSHV, like all herpesviruses, can establish lifelong latent infections in their human host. Latently infected cells provide a perpetual reservoir from which progeny viruses can be amplified for dissemination within the host and transmission between hosts. The peripheral blood CD19-positive B cells have been identified as a long-term latency reservoir for KSHV; other cells such as endothelial cells may also be a site for KSHV latency, but they do not appear to provide a long-term latent reservoir for the virus. Nonetheless, infected dermal endothelium spindle cells may release progeny virus that can subsequently infect local keratinocytes and the eccrine epithelium in the tumour. Lytic reactivation from latently KSHV-infected cells that results in the release of progeny virions is a critical pathogenic step in multiple human diseases. In immunocompetent KSHV carriers, the immune system plays an essential role in tempering lytic reactivation of the virus (see Lukac & Yuan, 2007 for a detailed review).

1.2 Epidemiology of infection

In the previous IARC monograph (IARC, 1997), preliminary epidemiological data were presented based largely on Polymerase Chain Reaction (PCR) and initial serological studies. Subsequently, substantial additional data have become available with the advent of new serological techniques and large-scale studies.

1.2.1 Prevalence, geographic distribution

(a) Laboratory methods in epidemiological studies of KSHV

Difficulties have arisen in developing reliable serological tests to assess KSHV infection, and the interpretation of some published KSHV prevalence data is therefore challenging. KSHV encodes multiple antigenic proteins that may be expressed in the latent or lytic phase of the virus life cycle (Chandran et al., 1998). The major antigenic proteins are the LANAs encoded by ORF 73, and the lytically expressed K8.1 encoded by ORF 65. First-generation serological assays were developed based on the immunofluorescence of latently infected primary effusion lymphoma cells (Gao et al., 1996; Kedes et al., 1996; Simpson et al., 1996) or primary effusion lymphoma cells induced by treatment with tetradecanoyl phorbol ester acetate (TPA) to produce lytic antigens (Lennette et al., 1996). More recently, enzyme-linked immunosorbent assays (ELISAs) have been developed using recombinant proteins or peptides.

Concordance between assays detecting antibodies to these antigens has improved but remains moderate. Infected subjects may have antibodies to only lytic or latent antigens, and may develop antibody responses to lytic antigens years before they develop antibodies to LANA, and the reverse can also occur (Biggar et al., 2003, Minhas et al., 2008). In addition, antibody titres are very high in Kaposi sarcoma patients but very low in asymptomatic subjects (Biggar et al., 2003, Minhas et al., 2008). [The Working Group concluded that if the serum samples of Kaposi sarcoma patients are used as “gold standard” positive controls then the sensitivity of an assay is likely to be overestimated. At the other end of the spectrum, antibody levels in asymptomatic subjects are so low that establishing a clear assay cut-off is difficult. For these reasons, current assays for KSHV antibody detection, while suitable for comparisons between populations,
are inadequate for diagnostic purposes in low-risk populations. Comparisons between studies using different assays or even different cut-offs are often problematic.]

(b) Seroprevalence estimates and geographic distribution

Despite the difficulties discussed above in estimating the precise prevalence of KSHV, prevalence is in general low (<10%) in northern Europe, the USA and Asia, elevated in the Mediterranean region (10–30%), and high in sub-Saharan Africa (>50%) (Enbom et al., 2002; Dukers & Rezza, 2003). A study of 1000 blood donors in the USA tested by six independent laboratories reported estimates ranging from 0.5–5% (Pellett et al., 2003). More recently, a study of approximately 14000 adults recruited for the National Health and Nutrition Examination survey (NHANES) III, designed to be representative of the general population, estimated KSHV prevalence to be around 7% (Engels et al., 2007). In northern Europe, KSHV is reported to be 2–3% in adults (Simpson et al., 1996; Marcelin et al., 1998; Preiser et al., 2001). Prevalence in Asia is similar to that in the USA and northern Europe. A large international study recently reported a prevalence of 5% for the Republic of Korea, 8–10% for Thailand, and 11–15% for Viet Nam (de Sanjosé et al., 2009).

KSHV prevalence is higher in adults in Mediterranean countries such as Italy than in northern Europe or the USA (Gao et al., 1996; Whitby et al., 1998), with prevalence higher in Southern Italy and in the Po Valley (13–20%) than in most of Northern Italy (Calabrò et al., 1998; Whitby et al., 1998; Cattani et al., 2003; Serraino et al., 2006). The prevalence in Spain is reported to be 3.65% (de Sanjosé et al., 2009), in Greece 7.6% (Zavitsanou et al., 2007), and in Israel 10% (Davidovici et al., 2001). In South America, a high prevalence of KSHV infection is reported in Amerindians but not in the general population (Biggar et al., 2000; Whitby et al., 2004; Cunha et al., 2005).

The prevalence of KSHV in Uganda is 40–50% (Gao et al., 1996; Wawer et al., 2001; Hladik et al., 2003; Newton et al., 2003a). Similar estimates are reported for Kenya (~43%) (Baeten et al., 2002; Lavreys et al., 2003), Zambia (40%) (Klaskala et al., 2005), and the United Republic of Tanzania (~50%) (Mbulaiteye et al., 2003a). KSHV prevalence appears to be higher in Malawi (54–67%), Botswana (76%), and The Demographic Republic of Congo (82%) (Engels et al., 2000; DeSantis et al., 2002; Whitby et al., 2004).

KSHV prevalence is somewhat lower in South Africa (30%) (Sitas et al., 1999; Dedicoat et al., 2004; Malope et al., 2007), and in West African countries such as Burkino Faso (~12.5%) and Cameroon (~25%) (Volpi et al., 2004; Collenberg et al., 2006). The Gambia had a low incidence of Kaposi sarcoma before and during the AIDS epidemic but has a high KSHV prevalence (~75%) (Ariyoshi et al., 1998). The prevalence of KSHV in Nigeria is intermediate (~45%) (de Sanjosé et al., 2009).

1.2.2 Transmission and risk factors for infection

KSHV is primarily transmitted via saliva. In countries where KSHV is highly prevalent, infection occurs during childhood and increases with age (Whitby et al., 2000; Dedicoat et al., 2004; Malope et al., 2007). The peak age of acquisition is generally between 6–10 years (Whitby et al., 2000; Mbulaiteye et al., 2004), and the risk of infection is increased if family members, especially mothers, are infected (Plancoulaine et al., 2000; Dedicoat et al., 2004; Malope et al., 2007; Minhas et al., 2008). Other reported risk factors for infection in childhood include human immunodeficiency virus (HIV) infection (Malope et al., 2007; Minhas et al., 2008), environmental factors such as source of water (Mbulaiteye et al., 2005), and insect bites (Coluzzi et al., 2003).
the USA, Europe and Australia, KSHV prevalence is elevated in homosexual men, especially those infected by HIV (Martin et al., 1998; Smith et al., 1999; Grulich et al., 2005). There is considerable evidence that the risk of infection with KSHV is associated with the number of sexual partners of an individual, and other sexual risk factors (Martin et al., 1998; Smith et al., 1999; Grulich et al., 2005). It is likely that the transmission of KSHV between homosexual men is also via saliva (Martin, 2003; Martró et al., 2007). KSHV is not generally associated with sexual risk factors in heterosexuals (Smith et al., 1999; Engels et al., 2007; Malope et al., 2008; de Sanjose et al., 2009).

KSHV can be detected in peripheral blood suggesting that blood-borne transmission is possible. Some studies have suggested that injecting drug use was not associated with a risk of KSHV infection (Renwick et al., 2002; Bernstein et al., 2003), but others have shown an increased risk of KSHV infection in injecting drug users, especially with prolonged use (Cannon et al., 2001; Atkinson et al., 2003). KSHV transmission via blood transfusion is also likely to be rare, but evidence of both risk and actual transmission has been reported (Mbulaiteye et al., 2003b; Dollard et al., 2005; Hladik et al., 2006). KSHV transmission by organ donation has also been reported (Parravicini et al., 1997; Concato et al., 2008).

2. Cancer in Humans

2.1 Kaposi sarcoma

At the time of the previous IARC Monograph (IARC, 1997), KSHV was classified as ‘probably carcinogenic to humans’ (Group 2A) on the basis ‘that the evidence was compelling but as yet limited’. Since then, a large number of studies have assessed the association between KSHV and Kaposi sarcoma (see Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-04-Table2.1.pdf, and Table 2.2 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-04-Table2.2.pdf).

To date, data on the association between KSHV and Kaposi sarcoma come from 22 cohort studies and 80 case–control studies – all show broadly consistent evidence of an association between KSHV infection and Kaposi sarcoma. In most studies, the relative risks for the association between KSHV infection and Kaposi sarcoma are typically greater than 10. Of the 22 cohort studies, conducted in nine countries across three continents, 13 were among cohorts of HIV-infected people and included data on 561 cases. The largest study included 189 cases (Newton et al., 2006). Nine cohorts of transplant recipients yielded a total of 48 cases (Parravicini et al., 1997; Regamey et al., 1998; Francès et al., 1999, 2000; Rabkin et al., 1999; Cattani et al., 2001; Emond et al., 2002; Marcelin et al., 2004; García-Astudillo & Leyva-Cobián, 2006). No data were available from cohort studies on “classical” or “endemic” Kaposi sarcoma; to date, all studies have included only immunosuppressed subjects. Two studies used PCR to identify evidence of KSHV in peripheral blood mononuclear cells (PBMC) (Whitby et al, 1995; Moore et al., 1996b), most studies used serology, and one study used both (Engels et al., 2003). One study demonstrated a statistically significant increasing risk of Kaposi sarcoma with increasing titre of antibodies against KSHV, before diagnosis, both for a lytic and a latent assay (Newton et al., 2006).

Of the 80 case–control studies, nearly half included data on cases not infected by HIV – i.e. “classical,” “endemic,” and transplant-associated Kaposi sarcoma. A variety of assays were used to detect evidence of infection, including PCR of tumour lesions, semen, prostate tissue, saliva, and bronchial alveolar lavage. Serological studies used assays against lytic or latent antigens, or both. Two studies (Sitas et al., 1999; Newton et al., 2003b) had substantial numbers of Kaposi sarcoma cases among HIV-uninfected...
individuals and together with one other study (Albrecht et al., 2004) were able to demonstrate increasing risks of Kaposi sarcoma associated with increasing titres of anti-KSHV antibodies (Brown et al., 2006a, b). In addition, among KSHV-seropositive people, the presence of KSHV DNA in PBMC was associated both with an increased risk of Kaposi sarcoma, and with an increased risk of disease progression (Laney et al., 2007). Broadly, results were consistent across all studies, demonstrating a clear association between infection with KSHV and Kaposi sarcoma.

2.2 Primary effusion lymphoma

Primary effusion lymphoma is a very rare subgroup of B-cell non-Hodgkin lymphomas presenting as pleural, peritoneal, and pericardial (body cavity) lymphomatous effusions. These comprise less than 2% of HIV-related lymphomas (Sullivan et al., 2008). There is already strong evidence that KSHV is a causal agent of primary effusion lymphoma (IARC, 1997). Because of the rarity of primary effusion lymphoma, much of the information comes from case reports, with a few studies that have examined biopsy tissues, aspirates or cell lines. Primary effusion lymphomas were described in HIV-immunosuppressed individuals by Cesarman et al. (1995a) in association with KSHV, and by Nador et al. (1996) as a distinct disease entity, and almost all occurred in association with HIV (Gaidano et al., 2000). Because of the identification of KSHV in all of the cases presenting as primary lymphomatous effusions in early studies, the presence of this virus has been incorporated as a diagnostic criterion for primary effusion lymphoma. However, it has since been recognized that other lymphoma subtypes such as Burkitt or diffuse large B-cell lymphoma can also present in body cavities, and these lack the presence of KSHV. In addition, some KSHV-positive lymphomas occur as solid tumour masses with or without accompanying effusions. However, KSHV-associated lymphomas have a multitude of morphological and immunophenotypical features that are characteristic, and so, have been grouped as a single clinicopathological entity. Cases without an effusion are considered to be extracavitary variants of primary effusion lymphoma (Said & Cesarman, 2008).

Some primary effusion lymphomas occur in association with post-transplant immunosuppression (Kapelushnik et al., 2001). Many case reports show primary effusion lymphoma to be in association with Kaposi sarcoma or multicentric Castleman disease, both known to be caused by KSHV. Ascoli et al. (2001), for example, also identified four patients with multicentric Castleman disease and primary effusion lymphoma, all of which had evidence of KSHV DNA in pleural effusions. In a prospective cohort study of 60 HIV-positive patients, three developed primary effusion lymphoma in association with multicentric Castleman disease and/or Kaposi sarcoma, the latter conditions appear to be risk factors for the development of this disease (Okohenhilder et al., 2002). Komanduri et al. (1996) and numerous case reports (e.g. Ascoli et al., 1999a, b) described HIV-positive cases of primary effusion lymphoma that had evidence of KSHV infection. Likewise, four patients with primary effusion lymphoma and five cell lines were all reported to be KSHV-positive (Jude et al., 2000). Boulanger et al. (2005) identified 15 HIV-positive patients with primary effusion lymphoma, all of which were KSHV-positive. Of interest, in this study, six primary effusion lymphoma patients had neither Kaposi sarcoma nor multicentric Castleman disease. Asou et al. (2000) found KSHV to be present in 21/21 patients with primary effusion lymphoma, compared with 0/139 patients with other AIDS- and non-AIDS-related lymphomas,
and mucosa-associated lymphoid tissue (MALT) lymphomas.

During 1996–2008, a total of 95 subjects were identified in the literature as case reports. The majority of these were KSHV-positive and HIV-positive. Nineteen subjects were KSHV-negative and HIV-negative; these were unusual in that all but three were elderly, seven of these were characterized by having had some form of genetic abnormality, three had cirrhosis (two of those were due to infection with the hepatitis C virus), two had some other idiopathic immunodeficiency, and one had a renal transplant. These cases do not fulfill the diagnostic criteria for primary effusion lymphoma (Said & Cesarman, 2008), and probably represent another form of non-Hodgkin lymphoma involving body cavities. Primary effusion lymphoma has also been reported in people with no obvious immune suppression or HIV infection. See Table 2.5 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-04-Table2.5.pdf.

2.3 Multicentric Castleman disease

Strictly speaking, multicentric Castleman disease is not a cancer, but a rare polyclonal lymphoproliferative disease that can progress to plasmablastic lymphoma (Dupin et al., 2000). In addition, Oksenhendler et al. (2002) found that 14/60 HIV-infected patients with multicentric Castleman disease developed non-Hodgkin lymphoma, three of which were primary effusion lymphoma. Several studies have associated multicentric Castleman disease with KSHV. Multicentric Castleman disease was recognized in 1956 (Castleman et al., 1956), but appears to have increased in incidence as a result of the HIV epidemic, although multicentric Castleman disease in association with KSHV has also been documented in HIV-negative patients (Hernández et al., 2005).

Table 2.6 (available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-04-Table2.6.pdf) shows case series that have detected KSHV in patients with multicentric Castleman disease (Soulier et al., 1995; Barozzi et al., 1996; Corbellino et al., 1996; Gessain et al., 1996). Several hospital-based studies have shown an association between KSHV and multicentric Castleman disease, irrespective of the way in which KSHV is detected and of differences in tissue type (Table 2.7 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-04-Table2.7.pdf; Soulier et al., 1995; Parravicini et al., 1997; Bélec et al., 1999a; Asou et al., 2000). For example, Soulier et al. (1995) found KSHV to be present in all 14/14 HIV-positive cases and 7/17 HIV-negative cases compared with 1/34 controls. Treatment of three multicentric Castleman disease patients with ganciclovir, an inhibitor of KSHV lytic replication, has been reported to ameliorate multicentric Castleman disease (Casper et al., 2004), while treatment with cidofovir, an inhibitor of KSHV DNA polymerase, was not effective (Corbellino et al., 2001; Berezne et al., 2004).

2.4 Multiple myeloma

A small number of early studies suggested a possible association between KSHV and multiple myeloma (Rettig et al., 1997; Said et al., 1997), but this was not confirmed by subsequent large well designed serology and PCR-based studies (see Table 2.8 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-04-Table2.8.pdf). In addition, the geographic distribution of multiple myeloma is different from that of Kaposi sarcoma and of KSHV, strongly implying different etiologies (Cottoni & Uccini, 1997; Globocan, 2008; Hjalgrim et al., 1998). [The Working Group noted that a map of the global distribution of Kaposi sarcoma was not available from Globocan 2008 because it is only possible to derive estimates for sub-Saharan African countries.]
Several studies containing a clinical series of patients with multiple myeloma and a comparison group of other hospital patients without monoclonal gammopathy of undetermined significance (MGUS, thought to be a precursor of multiple myeloma) or blood donors, have not found an association between markers of KSHV and multiple myeloma (Mackenzie et al., 1997; Marcelin et al., 1997; Masood et al., 1997; Rettig et al., 1997; Whitby et al., 1997; Agbalika et al., 1998; Santarelli et al., 1998; Bélec et al., 1999b, c; Azzi et al., 2001; Beksaç et al., 2001; Patel et al., 2001; Santón Roldán et al., 2002; Zhu et al., 2002; Hermouet et al., 2003; Tsai et al., 2005). Rettig et al. (1997) reported the presence of KSHV sequences using PCR in all 15 of the patients with multiple myeloma, in 2/8 cases with MGUS, and in 0/26 control patients. [The Working Group noted that the majority of studies are based on series of hospital patients which are compared to a series of hospital patients admitted with several unrelated conditions or to blood donors (or both), and therefore the comparison group may not have been adequate. Most studies were typically small in size and did not adjust for age.]

Some of these studies were also reviewed by Tarte et al. (1998, 1999), and many used several serological assays against KSHV LANAs. In two of these studies (Agbalika et al., 1998; Bélec et al., 1999b), 0/25 cases of multiple myeloma was seropositive versus 0/25 in controls. Neither of these studies attempted to account for age (through matching or adjusting the data).

The biological significance of some of the clinical series, especially those using small PCR fragments has been questioned by Zong et al. (2007). Four studies (Schönrich et al., 1998; Sitas et al., 1999; Tedeschi et al., 2001, 2005) had age-matched controls or were based on a case–control/comparison design (Sitas et al., 1999), or had a case–control study design nested within a cohort (Tedeschi et al., 2001, 2005) (see Table 2.9 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-04-Table2.9.pdf).

Schönrich et al. (1998) found KSHV lytic antibodies were present in 4/99 multiple myeloma patients versus 2/67 controls. Sitas et al. (1999) measured the age- and gender-adjusted KSHV immunofluorescence assay seropositivity in 108 black South African incident cancer patients with newly diagnosed multiple myeloma compared with 3185 cancer patients and 85 blood donors. The age- and gender-adjusted KSHV seroprevalence in multiple myeloma patients versus controls was, respectively, 24% versus 32%.

Tedeschi et al. (2001) measured the seroprevalence of KSHV in 47 multiple myeloma cases and 224 age-matched controls from a cohort of 20243 men and 18814 women recruited between 1968–72, and followed until the end of 1991 through the Finnish Cancer Registry. Odds ratios (age-adjusted) for KSHV and multiple myeloma were calculated using IFA lytic antibodies (OR, 2.02; 95%CI: 0.94–4.33; latent OR, 10.0; 95%CI: 0.91–110.3), and Western blot confirmation (any one of ORF 65, 73 and K8.1A; OR, 0.89; 95%CI: 0.25–3.25); none of the associations was found to be statistically significant. In another nested case–control study, Tedeschi et al. (2005) identified 329 cases of multiple myeloma, and matched these to 1631 controls, matched for age and gender. The cases and controls arose from about 1133000 individuals from several Nordic cohorts who had donated blood samples. Seropositivity was 12% in multiple myeloma subjects versus 15% in control subjects. No association was found between multiple myeloma and KSHV lytic (OR, 0.8; 95%CI: 0.5–1.1), or latent antibody levels (OR, 0.6; 95%CI: 0.1–2.7). [The Working Group noted that after stratifying by detection method (PCR, LANA or lytic antibodies), no significant differences in the percent KSHV positivity were observed between multiple myeloma cases and controls when the Working Group used χ² tests for comparison (data not shown).]
2.5 Other cancers

Sitias et al. (1999) measured the seroprevalence of KSHV in 16 major cancer types, including Kaposi sarcoma and multiple myeloma. As expected, the seroprevalence of KSHV among people with Kaposi sarcoma was greater than 80%; among people with all other cancers (oral cavity, oesophagus, lung, stomach, liver, colon/rectum, breast, cervix, prostate, non-Hodgkin lymphoma, Hodgkin disease, leukaemia, myeloma, other minor types [where \( n < 50 \) in each cancer type]), the seroprevalence was similar to the general population (about 20–30%). Several studies have examined the presence of KSHV sequences in various other cancers but the results are inconclusive. These include mesenchymal tumours (1/76 cases KSHV-positive) (Kazakov et al., 2002), and other lymphomas (e.g. Lazzi et al., 1998, 2006; de Sanjosé et al., 2004). A case series was reported suggesting an association with large-cell immunoblastic lymphomas (Hansen et al., 2000), but this has not been confirmed. No associations were found between KSHV and cancer of the prostate in several cohort and case–control studies (Sitias et al., 1999; Huang et al., 2008), however, one case–control study did present a significant inverse association (Sutcliffe et al., 2007). KSHV has not been detected in salivary gland tumours (Atula et al., 1998), despite the fact that KSHV is thought to be transmitted via saliva.

2.6 Kaposi sarcoma and cofactors

Infection with KSHV alone is not sufficient to cause Kaposi sarcoma. The most important cofactor predisposing a KSHV-infected person to Kaposi sarcoma is HIV co-infection or, to a lesser extent, other immunodeficient states such as iatrogenic immune suppression in organ transplant recipients (IARC, 1997). Nonetheless, the incidence of Kaposi sarcoma in specific geographic areas before the HIV epidemic points to a role of as-yet-unknown cofactors in the etiology of this cancer (Dedicoat & Newton, 2003).

2.6.1 Suspected unknown cofactors

Before the HIV epidemic, Kaposi sarcoma had a much greater geographic variation in incidence than most other malignancies. Classic (sporadic) Kaposi sarcoma occurred but was rare in countries around the Mediterranean, particularly in Italy, Greece, and the Middle East, and was almost non-existent elsewhere in Europe and in the USA, except in immigrants from these countries (Grulich et al., 1992; IARC, 1997). In contrast, it represented up to 9% of all cancers in parts of sub-Saharan Africa, such as Uganda, in both men and women (Oettlé, 1962; D’Oliveira & Torres, 1972; Templeton, 1981; Hutt, 1983; Cook-Mozaffari et al., 1998; Dedicoat & Newton, 2003). KSHV infection is prevalent in many African countries, including places where Kaposi sarcoma was almost unknown before HIV, and is as common in women as in men (Dedicoat & Newton, 2003). However, the incidence of classical Kaposi sarcoma varied markedly across the African continent, primarily affecting men (Cook-Mozaffari et al., 1998).

Several exposures have been suggested as possible cofactors for diseases that might explain the geographic variation in incidence before the HIV epidemic, both in Africa and elsewhere. These include malaria and other parasitic infections (Serraino et al., 2003; Lin et al., 2008); fine volcanic soils, which are posited to cause localized immunosuppression in the lower limbs (Ziegler, 1993); and exposure to specific plants, or “onco-weeds” that might increase viral replication (Whitby et al., 2007). Although the existence of cofactors is not disputed, for none of these is the evidence sufficiently strong to conclude that there is a definite increase in risk.
2.6.2 HIV infection as a strong cofactor

In parts of Africa where Kaposi sarcoma was relatively common even before the era of HIV, the HIV epidemic has led to an explosion in the incidence of the disease (Curado et al., 2007). In the mid-1990s, the incidence of Kaposi sarcoma increased about 20-fold in Uganda, Zimbabwe, and other sub-Saharan African countries, such that it is now the most common cancer in men, and the second most common in women (Wabinga et al., 1993, 2000; Bassett et al., 1995; Dedicoat & Newton, 2003). As a result of the HIV epidemic, the incidence of Kaposi sarcoma has also increased in countries where it was previously relatively rare, but where KSHV was prevalent. For example, during 1988–96, the incidence of Kaposi sarcoma increased at least 3-fold in South Africa, and has continued to increase as the HIV epidemic grows. Data from Johannesburg, South Africa, show that incidence rates of Kaposi sarcoma have doubled in men, but have increased 7-fold in women, such that the gender ratio of 7:1 in males versus females in 1988 has now declined to only 2:1 (Sitas & Newton, 2001). [The Working Group noted that this is an artefact of Kaposi sarcoma incidence being higher in men.] Therefore, in the presence of HIV infection, the role of other etiological cofactors may be less relevant for the development of Kaposi sarcoma than before the spread of HIV (Dedicoat & Newton, 2003).

2.6.3 Host genetic susceptibility

Host genetic variation has been investigated in the etiology of Kaposi sarcoma. In particular, emphasis has been placed on genes that may be relevant to the modulation of host immunity against KSHV (Brown et al., 2006a, c; Alkharsah et al., 2007), but data in this area remain sparse. More recent evidence suggests that variations in the viral genome itself may also be of relevance, but currently the findings are inconclusive (Mancuso et al., 2008).

3. Cancer in Experimental Animals

In this volume, the Working Group decided not to include a separate section on “Cancer in Experimental Animals” in the Monographs on viruses but rather to include description of such studies under Section 4 (below). The reasoning for this decision is explained in the General Remarks.

4. Other Relevant Data

4.1 Transforming capacity of KSHV

Transformation is a multistep process and KSHV infection has been shown to induce most steps along this progression: a) KSHV infection of primary human endothelial cells leads to morphological alteration and reduced growth-factor dependence (Ciufo et al., 2001); b) KSHV infection of immortalized human endothelial cells leads to extended survival, loss of contact inhibition, growth-factor and anchorage independence (Flore et al., 1998; Moses et al., 1999; Wang & Damania, 2008), and the outgrowth of fully tumorigenic clones (An et al., 2006); c) KSHV transforms murine endothelial progenitor cells (Mutlu et al., 2007).

KSHV infection cannot transform mature human B cells in culture (Kliche et al., 1998). However, this may be due to low infectivity and/or the absence of susceptible cell populations under routine culture conditions, which do not support the growth of haematopoietic cells. Yet, KSHV is clearly required for continued survival of primary effusion lymphoma cells in culture (Guasparri et al., 2004; Godfrey et al., 2005)

Individual KSHV proteins exhibit transforming capacity in experimental systems (see Sections 4.2 and 4.3), and in transgenic mice (see Section 4.6).
4.2 Biochemical and biological properties of KSHV proteins

Several latent or lytic viral proteins are involved in the carcinogenesis process of KSHV (see Table 4.1).

Five KSHV proteins (K1/VIP, vGPCR, vIRF-1, Kaposin A, LANA) have been reported to have transforming properties in classical transformation assays, others (vCYC, LANA, KbZIP) have been shown to affect cell-cycle regulation or the survival of tumour cells in vivo or in vitro (vFLIP, vIL6, vIRF-3). Because only some of these proteins are expressed during latency and in the majority of tumour cells, not only ‘direct’ transformations (as in classical models of virus-mediated cellular transformation) but also indirect (paracrine) effects are thought to play a role in KSHV-mediated oncogenesis (Ganem, 2007).

4.2.1 Latent KSHV proteins

(a) LANA/ORF 73

LANA, encoded by ORF 73, is expressed during latency and represents the most consistently detected viral protein in KSHV-associated tumour cells (Rainbow et al., 1997; Dupin et al., 1999; Katano et al., 2000; Parravicini et al., 2000). LANA is necessary for replicating the episomal viral DNA; it binds to the latent origin of replication in the TR subunits of the viral genome, and works by recruiting a large variety of cellular interaction partners, among them components of the chromosomal replication machinery such as origin recognition complexes (ORCs), but also cellular proteins linked to transcriptional regulation or proliferation control (see Table 4.1; reviewed in Verma et al., 2007).

Of relevance to a possible direct role of LANA in oncogenesis are the observations that LANA: (i) inactivates p53-dependent transcriptional activation (Friborg et al., 1999); (ii) interacts with pRB and enhances oncogenic ras-mediated transformation of rodent fibroblasts (Radkov et al., 2000); (iii) absorbs GSK-3β and thereby reduces the phosphorylation of, thus stabilizing, β-catenin (Fujimuro et al., 2003); (iv) interacts with Brd2/RING3, a chromatin-binding protein and a lymphomagenic member of the BET protein family (Platt et al., 1999; Viejo-Borbolla et al., 2005); (v) causes B-cell hyperplasia and B-cell lymphoma when expressed in transgenic mice (Fakhari et al., 2006).

(b) vCYC/ORF 72

In-situ hybridization studies indicate that the KSHV CYC/ORF 72 gene is expressed in the majority of tumour cells in vivo (Davis et al., 1997), in keeping with its classification as a latent gene. v-CYC represents another candidate KSHV oncoprotein because of its homology to the human Cyclin-D/Prad oncoprotein. In general, cyclin-D proteins (D1, D2, D3) associate with specific cyclin-dependent kinases (CDKs), and these complexes phosphorylate pRB family members (reviewed in Sherr, 1996). An oncogenic cyclin-D homologue is also present in other gammaherpesviruses (reviewed in Neipel et al., 1997). Ectopic expression of the murine herpesvirus 68 (MHV 68) cyclin in T cells causes T-cell lymphomas in transgenic mice (van Dyk et al., 1999).

The mechanism of transformation by KSHV vCYC is most likely novel and unique, because it phosphorylates pRb but, unexpectedly, also histone H1, p27kip1, and Bcl-2 (Chang et al., 1996; Godden-Kent et al., 1997; Li et al., 1997; Ojala et al., 2000). Unlike human cyclin-D, vCYC/CDK6-mediated phosphorylation of pRB is resistant to inhibition by the cyclin-dependent kinase-inhibitors (CDKIs) p16ink4, p21cip1, and p27kip1 (Swanton et al., 1997). Moreover, vCYC/CDK6 induces the degradation of p27kip1 (Ellis et al., 1999; Mann et al., 1999).
<table>
<thead>
<tr>
<th>KSHV protein</th>
<th>Homology to human protein</th>
<th>Viral Gene</th>
<th>Function in viral life cycle</th>
<th>Biochemical properties</th>
<th>Latent/lytic</th>
<th>Involvement in carcinogenesis</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>K1/VIP&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Related to a family of mammalian complement regulatory proteins</td>
<td>ORF K1</td>
<td>May increase/decrease viral reactivation</td>
<td>Activates several intracellular signalling cascades; Induces angiogenic cytokines; Blocks intracellular transport of BCR-complexes to cell surface</td>
<td>Lytic</td>
<td>Transforming properties Angiogenesis Anti-apoptotic activity</td>
<td>Lee et al. (1998a, b, 2000), Lagunoff et al. (1999, 2001), Prakash et al. (2002), Tomlinson &amp; Damania (2004), Wang et al. (2004a, 2006)</td>
</tr>
<tr>
<td>KCP</td>
<td>Interleukin-6 (IL6) homologue</td>
<td>ORF 4</td>
<td>Inhibits complement activation by virions or virus-infected cells</td>
<td>Cofactor for complement factor I; Accelerates the decay of C3 convertases</td>
<td>Lytic</td>
<td></td>
<td>Mullick et al. (2003), Spiller et al. (2003), Mark et al. (2004)</td>
</tr>
<tr>
<td>vIL6</td>
<td>Viral IL6</td>
<td>ORF K2</td>
<td>Induces proliferation of PEL cell lines</td>
<td>Induces VEGF Induces STAT3 phosphorylation</td>
<td>Lytic</td>
<td>Tumour cell survival in vivo and in vitro Angiogenesis Haematopoiesis</td>
<td>Moore et al. (1996a), Burger et al. (1998), Aoki et al. (1999), Aoki &amp; Tosato (1999), Hoischen et al. (2000), Kovaleva et al. (2006)</td>
</tr>
<tr>
<td>vCCL-2/ vMIP-II</td>
<td>Some homology to chemokines TARC and eotaxin</td>
<td>ORF K4</td>
<td>Viral chemokine</td>
<td>Agonist for CCR3, CCR5, CCR8; Induces monocyte chemotaxis</td>
<td>Lytic</td>
<td>Angiogenic properties</td>
<td>Boshoff et al. (1997), Dairaghi et al. (1999), Endres et al. (1999), Nakano et al. (2003)</td>
</tr>
<tr>
<td>vCCL-3/ vMIP-III</td>
<td>Part of a family of membrane-bound-E3-ubiquitin ligases</td>
<td>ORF K4.1</td>
<td>Viral chemokine</td>
<td>CCR4, XCR1 agonist</td>
<td>Lytic</td>
<td>Angiogenic properties</td>
<td>Nicholas et al. (1997), Stine et al. (2000), Lüttichau et al. (2007)</td>
</tr>
<tr>
<td>KSHV protein</td>
<td>Homology to human protein</td>
<td>Viral Gene</td>
<td>Function in viral life cycle</td>
<td>Biochemical properties</td>
<td>Latent/lytic</td>
<td>Involvement in carcinogenesis</td>
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<tr>
<td>vCCL-1/vMIP-I</td>
<td>Chemokine homologue related to macrophage inflammatory protein (MIP)-1α</td>
<td>ORF K6</td>
<td>Viral chemokine</td>
<td>CCR8 agonist; Induces monocyte chemotaxis (VEGF production)</td>
<td>Lytic</td>
<td>Angiogenic properties Anti-apoptotic activity</td>
<td>Nicholas et al. (1997), Boshoff et al. (1997), Nakano et al. (2003)</td>
</tr>
<tr>
<td>K7/ vIAP</td>
<td>Structurally related to a splice variant of survivin</td>
<td>ORF K7</td>
<td>Apoptosis inhibitor</td>
<td>Binds to and inhibits several proteins involved in apoptosis (see Table 4.2) Induces degradation of IκB, p53, vGCR</td>
<td>Lytic</td>
<td>Anti-apoptotic activity</td>
<td>Feng et al. (2002, 2004, 2008), Wang et al. (2002)</td>
</tr>
<tr>
<td>vBCL-2</td>
<td>Bcl-2 homologue</td>
<td>ORF 16</td>
<td>Viral Bcl-2</td>
<td>Heterodimerizes with human Bcl-2</td>
<td>Lytic</td>
<td>Anti-apoptotic activity</td>
<td>Sarid et al. (1997)</td>
</tr>
<tr>
<td>ORF 36</td>
<td></td>
<td>ORF 36</td>
<td>Viral Cdk2-like kinase</td>
<td>Phosphorylates K-bZIP; Activates JNK pathway</td>
<td>Lytic</td>
<td></td>
<td>Polson et al. (2001), Hamza et al. (2004), Izumiya et al. (2007)</td>
</tr>
<tr>
<td>ORF 45</td>
<td></td>
<td>ORF 45</td>
<td>Virion protein important for lytic replication Inhibits virus-mediated induction of type 1 interferon</td>
<td>Binds to and inhibits phosphorylation of IRF-7</td>
<td>Lytic</td>
<td>Viron infectivity Immune evasion</td>
<td>Zhu et al. (2002a, 2006), Zhu &amp; Yuan (2003)</td>
</tr>
<tr>
<td>K-RTA</td>
<td></td>
<td>ORF 50</td>
<td>Immediate-early transactivator Ubiquitin E3 ligase</td>
<td>Binds to and activates several lytic viral promoters directly or by interacting with RBPJk Promotes ubiquitination and degradation of IRF7 Represses p53</td>
<td>Lytic</td>
<td>Reactivation of lytic viral replication from latency Immune evasion Anti-apoptotic activity</td>
<td>Sun et al. (1998), Gradoville et al. (2000), Gwack et al. (2001), Lukac et al. (2001), Liang &amp; Ganem (2003), Yu et al. (2005)</td>
</tr>
<tr>
<td>K-bZIP</td>
<td></td>
<td>ORF K8</td>
<td>Modulates cell cycle and lytic reactivation</td>
<td>EBV Zta homologue; Binds to lytic replication origin; Binds to, antagonizes, and recruits p53 to ND10/PML bodies; Inhibits G1/S transition; Co-regulator of K-RTA</td>
<td>Lytic</td>
<td>Deregulation of cell cycle</td>
<td>Lin et al. (1999, 2003), Park et al. (2000), Katao et al. (2001), Izumiya et al. (2003a, b)</td>
</tr>
<tr>
<td>KSHV protein</td>
<td>Homology to human protein</td>
<td>Viral Gene function in viral life cycle</td>
<td>Biochemical properties</td>
<td>Latent/lytic</td>
<td>Involvement in carcinogenesis</td>
<td>References</td>
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<tr>
<td>ORF 57/MTA</td>
<td>ORF 57</td>
<td>Exports intronless viral RNAs from nucleus and promotes their translation; Required for the formation of viral progeny</td>
<td>Binds to intronless viral mRNA; Recruits hTREX complex</td>
<td>Lytic</td>
<td></td>
<td>Malik et al. (2004), Nishimura et al. (2004), Majerciak et al. (2007), Boyne et al. (2008)</td>
<td></td>
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<tr>
<td>Kaposin A*</td>
<td>ORF K12</td>
<td>Interacts with cytohesin-1</td>
<td>Latent/lytic</td>
<td>Transforming properties in cultured cells</td>
<td>Zhong et al. (1996), Muralidhar et al. (1998), Sadler et al. (1999), Kliche et al. (2001), Tomkowicz et al. (2005)</td>
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<tr>
<td>KSHV protein</td>
<td>Homology to human protein</td>
<td>Viral Gene</td>
<td>Function in viral life cycle</td>
<td>Biochemical properties</td>
<td>Latent/lytic</td>
<td>Involvement in carcinogenesis</td>
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<tr>
<td>miRs</td>
<td></td>
<td></td>
<td></td>
<td>mIRK-11 regulates similar genes as cellular miR-155</td>
<td>Latent</td>
<td>Potential involvement in: Angiogenesis Immune modulation Anti-apoptotic activity</td>
<td>Cai et al. (2005), Pfeffer et al. (2005), Samols et al. (2005, 2007), Grundhoff et al. (2006), Skalsky et al. (2007), Gottwein et al. (2007)</td>
</tr>
<tr>
<td>vFLIP</td>
<td>Homologue of FLICE (caspase-8)-inhibitory proteins</td>
<td>ORF K13/ ORF 71</td>
<td>Viral persistence; Spindle cell formation and lymphomagenesis; Inhibits lytic viral replication</td>
<td>Activates NF-κB; Inhibits CD95/Fas-induced apoptosis, anoikis, superoxide-induced cell death; Modulates MHC-I expression</td>
<td>Latent</td>
<td>Cell survival Anti-apoptotic activity Immune evasion</td>
<td>Keller et al. (2000), Grundhoff &amp; Ganem (2001), An et al. (2003), Field et al. (2003), Sun et al. (2003), Guasparri et al. (2004, 2006), Grossmann et al. (2006), Lagos et al. (2007), Matta et al. (2007), Ye et al. (2008), Efklidou et al. (2008), Thurau et al. (2009)</td>
</tr>
<tr>
<td>vCyclin/ vCYC</td>
<td>D-type cyclin homologue</td>
<td>ORF 72</td>
<td>Viral cyclin; Strongly activates CDK6 protein kinase activity</td>
<td>Phosphorylates H1, pRB, BCL-2, p27KIP1 in tandem with CDK6</td>
<td>Latent</td>
<td>Deregulation of cell cycle</td>
<td>Chang et al. (1996), Golden-Kent et al. (1997), Li et al. (1997), Swanton et al. (1997), Ellis et al. (1999), Mann et al. (1999), Ojala et al. (2000), Sarek et al. (2006), Koopal et al. (2007)</td>
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<tr>
<td>KSHV protein</td>
<td>Homology to human protein</td>
<td>Viral Gene</td>
<td>Function in viral life cycle</td>
<td>Biochemical properties</td>
<td>Latent/lytic</td>
<td>Involvement in carcinogenesis</td>
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<tr>
<td>LANA</td>
<td>ORF 73</td>
<td>Replication and maintenance of latent viral episome; Partition of episomes to daughter cells</td>
<td>Interacts with histones, p53, pRB, BET proteins, GSK-3β and others; Induces S-phase entry; Activates hTERT transcription; Recruits origin-binding proteins</td>
<td>Latent</td>
<td>Anti-apoptotic activity; Cell survival; Derepression of cell cycle; Transforming properties in cells; Tumorigenicity in mice</td>
<td>Rainbow et al. (1997), Ballestas et al. (1999), Friborg et al. (1999), Platt et al. (1999), Radkov et al. (2000), Ballestas &amp; Kaye (2001), Hu et al. (2002), Fujimuro et al. (2003), Watanabe et al. (2003), Verma et al. (2004), Hu &amp; Renne (2005), Viejo-Borbolla et al. (2005), Fakhari et al. (2006), Ottinger et al. (2006)</td>
<td></td>
</tr>
<tr>
<td>vOX2</td>
<td>OX2 homologue</td>
<td>ORF K14</td>
<td>Modulates inflammatory and T-cell responses</td>
<td>Activates or downregulates myeloid lineage cells in a CD200-like manner</td>
<td>Lytic</td>
<td></td>
<td>Chung et al. (2002), Foster-Cuevas et al. (2004)</td>
</tr>
<tr>
<td>vGCR</td>
<td>Homologue of G-protein-coupled receptor</td>
<td>ORF 74</td>
<td>Stimulates cellular proliferation</td>
<td>Activates Akt, MEK/Erk, JNK, p38; Induces angiogenic cytokines</td>
<td>Lytic</td>
<td>Transforming properties in cells; Tumorigenicity in mice; Angiogenesis; Anti-apoptotic activity</td>
<td>Arvanitakis et al. (1997), Bais et al. (1998), Yang et al. (2000), Holst et al. (2001), Montaner et al. (2001, 2003), Guo et al. (2003), Mutlu et al. (2007), Nicholas (2007) (review)</td>
</tr>
<tr>
<td>K15 protein&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ORF K15</td>
<td>Recruits endothelial cells to infected cells</td>
<td>Activates NF-κB, MEK/Erk; Induces inflammatory cytokines; Interacts with proteins involved in signal transduction (e.g. TRAFs 1, 2, 3), with members of src family of PTK, and with an apoptotic regulatory protein HAX-1</td>
<td>Lytic (possibly latent in B-cells)</td>
<td>Possibly anti-apoptotic activity; Possibly angiogenesis</td>
<td>Glenn et al. (1999), Choi et al. (2000), Sharp et al. (2002), Brinkmann et al. (2003, 2007), Wang et al. (2007)</td>
<td></td>
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</table>

<sup>a</sup> protein unique to KSHV
Compiled by the Working Group
vFLIP/ORF 71 is transcribed from the LANA promoter, and translated from an internal ribosome entry site located within the v-cyclin coding region (Grundhoff & Ganem, 2001; Low et al., 2001). It is therefore thought to be expressed during latency and in all tumour cells. The vFLIP protein is an adhesion molecule, a homologue of cellular FLICE (caspase-8)-inhibitory protein (FLIP) (Hu et al., 1997). It inhibits CD95/FAS-induced apoptosis in vitro by blocking caspase-3, -8 and -9 (Djerbi et al., 1999).

vFLIP directly binds IKKγ and TRAF2; this leads to a constitutive activation of NF-κB signalling (Field et al., 2003; Guasparri et al., 2006). In addition, vFLIP induces MHC-I expression through NF-κB in KSHV-infected lymphatic endothelial cells (Lagos et al., 2007), which underscores the physiological importance of the vFLIP–NF-κB interaction. Moreover, vFLIP transgenic mice develop lymphoma (Chugh et al., 2005). Eliminating either vFLIP or NF-κB activity from primary effusion lymphoma cells induces apoptosis (Keller et al., 2000; Guasparri et al., 2004), demonstrating that this pathway is essential for lymphomagenesis.

The many transcripts spanning the predicted K12 ORF (originally called T0.7) are translated in different reading frames, giving rise to the proteins kaposin A, B, C (Zhong et al., 1996; Sadler et al., 1999). In addition, a long transcript extending through ORF K12 represents the precursor RNA for the KSHV microRNAs (Cai et al., 2005; Pfeffer et al., 2005; Samols et al., 2005). One of these microRNAs, miRK-10, is located within the kaposin A sequence. These transcripts are expressed in all Kaposi sarcoma spindle cells (Staskus et al., 1997; Stürzl et al., 1997), and increase after activation of the lytic replication cycle.

4.2.2 Lytic KSHV proteins

K1/VIP (variable, ITAM-containing protein)

K1/VIP is a viral type I transmembrane protein, featuring two hypervariable domains in its extracellular region, and an immunoglobulin transactivation motif (ITAM) in its C-terminal, cytoplasmic region (Lee et al., 1998b). The K1 protein is expressed during the lytic (productive) replication cycle (Jenner et al., 1998).
while expression of K1 is not a consistent feature of Kaposi sarcoma, some Kaposi sarcoma biopsies show a marked K1-expression both at the transcript and protein level. K1-expression was also documented by immunohistochemistry on a small subpopulation of mantle zone lymphocytes of KSHV-positive multicentric Castleman disease, and in primary effusion lymphoma cell lines (Lee et al., 2003).

Lee et al. (1998a) showed that transfection of a K1-expression vector into rodent fibroblasts induced focus formation, and that K1-transfectants induced lymphoma in the common marmoset. Prakash et al. (2002) reported the emergence of sarcomatoid tumours and plasmablastic lymphoma in transgenic mice expressing the K1 protein under the control of an SV40 promoter. These mice showed an increased expression of bFGF; in transgenic B cells, a constitutive activation of NF-κB and increased c-Lyn activity was noted. Wang et al. (2006) showed that retroviral transduction of primary endothelial cells extended their life span.

K1 activates several intracellular signalling cascades leading to increased Ca-influx, increased phosphorylation of Syk, Vav, Cbl, and the p85 subunit of PI3K, increased NF-κB activity, and activation of NFAT and AP1 (Lee et al., 1998b; Lagunoff et al., 1999, 2001). The activation of PI3K leads to the activation of AKT by K1 (Tomlinson & Damania, 2004). K1 induces the expression of angiogenic cytokines, including vascular endothelial growth factor (VEGF), and may therefore play a paracrine role in the pathogenesis of Kaposi sarcoma or primary effusion lymphoma (Wang et al., 2004a, 2006).

(b) vIRF-1/ORF K9

vIRF-1 belongs to a group of four viral homologues of interferon regulatory factors (Russo et al., 1996; Cunningham et al., 2003). Stable vIRF-1 transfectants in murine NIH 3T3 cells show signs of transformation (loss of contact inhibition, growth in soft agar), and cause tumours in nude mice (Gao et al., 1997). The main function of vIRF-1 appears to be the inhibition of interferon-β-regulated genes such as p21CIP1 (Gao et al., 1997); it also inhibits the induction phase of the interferon response by binding to cellular IRFs (IRF-3, IRF-7), and to the transcriptional co-activators p300 and CBP, and inhibits the formation of functional IRF-3/CBP/p300 complexes and the induction of interferon β transcription (Gao et al., 1997; Burýsek et al., 1999; Seo et al., 2000; Lin et al., 2001). Unlike cellular IRFs, vIRF-1 does not bind directly to cellular DNA.

vIRF-1 is expressed during the lytic (productive) replication in tissue culture and is directly transactivated by K-RTA, the central regulator of the viral lytic programme (Gao et al., 1997; Chen et al., 2000).

(c) vGPCR/ORF 74

ORF 74 encodes a homologue of a G-protein-coupled chemokine receptor, and is constitutively active (reviewed in Nicholas, 2007). It activates a broad range of signalling pathways, including MEK/Erk, JNK, p38, Akt, NFAT, CREB, NF-κB, AP-1, and HIF-1α; these are relevant to the promotion of cell proliferation, cell survival, and angiogenic responses via cytokine gene induction (reviewed in Nicholas, 2007; Hartmann, 2008). Although constitutive, the activity of vGPCR can be modulated both positively and negatively by several cellular chemokines (Groα, IL8, IP-10, SDF-1α), and one viral (vCCL-2) chemokine (reviewed in Hartmann, 2008).

Multiple lines of evidence point to a role of vGPCR in KSHV-induced neoplasia, in particular Kaposi sarcoma. Early studies showed the proliferation-enhancing, constitutive signalling, and transforming properties of vGPCR (Arvanitakis et al., 1997; Bais et al., 1998). Subsequently, vGPCR was shown to cause Kaposi-sarcoma-like tumours in transgenic mice (Yang et al., 1997;
Kaposi sarcoma herpesvirus

In this model, vGPCR was only expressed in a few scattered cells, consistent with a paracrine model involving secretion of angiogenic cytokines (Holst et al., 2001; Guo et al., 2003; Montaner et al., 2003).

In a xenograft model, vGPCR involving a KSHV-transfected murine endothelial cell line was found to be required for tumorigenicity (Mutlu et al., 2007). The relevance of these results to KSHV-associated tumours in humans remains to be determined.

4.2.3 Genomic instability

Evidence of genomic instability has been noted in primary effusion lymphoma cells (microsatellite instability, chromosomal imbalances) (Gaidano et al., 1997; Nair et al., 2006), and late Kaposi sarcoma (Pyakurel et al., 2006). Experimentally, genomic instability has been noted in KSHV-infected primary endothelial cells (Pan et al., 2004), as well as in cell lines stably transfected with LANA (Si & Robertson, 2006), and vCYC-transgenic mice (Verschuren et al., 2004). Abnormal chromosome segregation in KSHV-infected cells was shown to be the consequence of nucleophosmin (NPM1) phosphorylation by CDK6 in concert with vCYC (Cuomo et al., 2008).

4.2.4 DNA-damage response

Transduction of vCYC into primary endothelial cells by a retroviral vector induces a DNA damage response, resulting in the increased phosphorylation of γH2AX (a variant form of histone H2A), which is an early response to DNA double-strand breaks. Increase of γH2AX phosphorylation was also shown in KSHV-infected primary endothelial cells, albeit only after 2 weeks of culture (in spite of vCYC being expressed early on) (Koopal et al., 2007). Other KSHV proteins might therefore interfere with the triggering of the DNA-damage response.

Shin et al. (2006) reported that vIRF-1 prevents the DNA-damage response and γH2AX and p53 phosphorylation by binding to and inhibiting ATM kinase, thereby promoting p53 turnover. As noted above, vIRF-1 and LANA interact with p53 and antagonize the transcription of p53-dependent cellular genes, including p21CIP1 (Gao et al., 1997; Friborg et al., 1999). In KSHV-infected primary effusion lymphoma cells, vIRF-3 also binds to p53 and inhibits the activation of the p53 promoter (Rivas et al., 2001). In primary effusion lymphoma cells, LANA, p53 and Hdm2 form a trimeric complex (Sarek et al., 2007), and the restoration of the p53 function by treatment with an inhibitor of the p53-Hdm2 interaction – Nutlin-3a – induces apoptosis in primary effusion lymphoma cells (Petre et al., 2007; Sarek et al., 2007).

Most reports have concluded that the inhibition of p53-activated cellular genes by LANA, vIRF-1, and vIRF-3 involves other mechanisms than the degradation or increased turnover of p53. However, one report showed that the recruitment of the Cul5-Elongin BC E3 ligase complex by LANA resulted in the degradation of p53 (Cai et al., 2006).

4.2.5 Cell proliferation and differentiation

KSHV-infected primary endothelial cells undergo spindle cell formation, which express markers of the lymphatic endothelium. Gene expression array studies have shown that KSHV can alter the transcriptome profile of vascular endothelial cells towards a profile that is typical for lymphatic endothelial cells (Carroll et al., 2004; Hong et al., 2004; Wang et al., 2004b). This involves the activation of Prox-1, a transcription factor determining lymphatic endothelial cell differentiation, followed by the increased expression of podoplanin and VEGFR-3 – markers for the lymphatic endothelial cell lineage (Carroll et al., 2004; Hong et al., 2004). The signalling pathways gp130 (the β-chain of the IL6 receptor
used by vIL6) as well as PI3K/Akt and JAK2/STAT3 have been reported to be involved in the induction of Prox-1 in KSHV-infected endothelial cells (Morris et al., 2008).

These data raise the possibility that KSHV infects blood or circulating endothelial cells, and drives them to differentiate into the lymphatic endothelium as they become spindle cells. This may be of major importance to Kaposi sarcoma tumour formation (Morris et al., 2008).

The adoption of a spindle morphology in KSHV-infected cells is thought to be due to vFLIP, a homologue of cellular FLIPs, and potent NF-κB inducer; vFLIP is expressed in latently infected endothelial cells, and NF-κB activation appears to be required for the formation of spindle cells (Grossmann et al., 2006; Sun et al., 2006). vFLIP is also required essentially for primary effusion lymphoma cell survival (see Section 4.2.1).

The viral IL6 homologue, vIL6, is expressed in vivo in a subpopulation of primary effusion lymphoma cells and in many KSHV-infected B cells in multicentric Castleman disease lymphoid follicles (Moore et al., 1996c; Katano et al., 2000; Parravicini et al., 2000). It induces proliferation, angiogenesis, and haematopoiesis in IL6-dependent cell lineages (Burger et al., 1998; Aoki et al., 1999; Hoischen et al., 2000), and serves as an essential autocrine factor in primary effusion lymphoma cell lines (Jones et al., 1999). It also induces VEGF, which has been implicated in the pathogenesis of primary effusion lymphoma and of Kaposi sarcoma (Aoki & Tosato, 1999). A single-chain antibody to vIL6, blocking its interaction with the IL6 receptor complex, was found to inhibit the proliferation of a primary effusion lymphoma cell line and to inhibit vIL6-induced STAT3 phosphorylation in vIL6-transfected cells (Kovaleva et al., 2006). Therefore, vIL6 may contribute to primary effusion lymphoma cell proliferation and to the angiogenesis noted in patients with this lymphoma.

Also, the viral D-type cyclin homologue vCYC and LANA each contribute to cell proliferation (see Section 4.2.1).

One of the viral latent transcripts in primary effusion lymphoma cells, miRNA-K12–11, has been found to target the same cellular micro-RNAs as miRNA-155, a cellular microRNA regulating the germinal centre reaction during B-cell maturation (Gottwein et al., 2007; Skalsky et al., 2007; Thai et al., 2007). Both miRNA-K12–11 and miR-155 downregulate several pro-apoptotic cellular genes (see Table 4.2). miRNA-K12–11 may therefore be involved in blocking terminal B-cell differentiation that contributes to the plasmablastic phenotype of primary effusion lymphoma cells or play a role in the protection of primary effusion lymphoma cells against apoptosis.

These findings highlight how KSHV can affect the differentiation of endothelial cells and of B cells.

vIRF3, an interferon regulation factor homologue, is required for primary effusion lymphoma cell survival (Wies et al., 2008).

Ablation of the human cytokines IL-6, IL-10, and VEGF or of VEGFR inhibits the growth of primary effusion lymphoma and Kaposi sarcoma (Masood et al., 1997; Nakamura et al., 1997; Aoki & Tosato, 1999; Arora et al., 1999; Jones et al., 1999; Sin et al., 2007). IFN-α inhibits KSHV reactivation and Kaposi sarcoma tumour growth (Krown et al., 1986; Chang et al., 2000).

**4.2.6 Apoptosis**

Several KSHV proteins have been shown to protect against apoptosis when transfected individually. Among them are some of the proteins already discussed above, as well as a viral homologue of cellular Bcl2. Table 4.2 shows a summary of their mode of actions.
### Table 4.2 Mode of action of KSHV proteins involved in the protection against apoptosis

<table>
<thead>
<tr>
<th>KSHV protein</th>
<th>Mode of action</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1/VIP</td>
<td>Activation of PI3K/Akt; Inhibition of FKHR-mediated apoptosis; Inhibition of Fas-induced apoptosis</td>
<td>Tomlinson &amp; Damania (2004), Wang et al. (2004a), Uddin et al. (2005), Wang et al. (2004a)</td>
</tr>
<tr>
<td>vBcl-2</td>
<td>Inhibition of Bax-mediated apoptosis; Selective interaction with BH3-only proteins</td>
<td>Cheng et al. (1997), Sarid et al. (1997), Flanagan &amp; Letai (2008)</td>
</tr>
<tr>
<td>vCCL-1/vMIP-I; vCCL-2/vMIP-II</td>
<td>VEGF-independent anti-apoptotic effect</td>
<td>Liu et al. (2001)</td>
</tr>
<tr>
<td>K7/vIAP</td>
<td>Inhibition of apoptosis by binding to Bcl-2 and caspase-3; Promotes p53 and IκB degradation by interacting with Ubiquilin/PLIC1; Binds to cellular CAML (calcium-modulating cyclophilin ligand); Increases cytosolic Ca²⁺ response to an apoptotic stimulus</td>
<td>Feng et al. (2002, 2004), Wang et al. (2002)</td>
</tr>
<tr>
<td>K-RTA</td>
<td>Represses p53-dependent transcription and apoptosis through interaction with CBP</td>
<td>Gwack et al. (2001)</td>
</tr>
<tr>
<td>vIRF-2</td>
<td>Inhibits induction of CD95L; Binds to and inhibits the activation of the IFN-induced ds-RNA-activated kinase (PKR)</td>
<td>Burýsek &amp; Pitha (2001), Kirchhoff et al. (2002)</td>
</tr>
<tr>
<td>vIRF-3/LANA-2</td>
<td>Inhibits apoptosis in PEL cells; Binds to p53 and inhibits p53-induced transcription and apoptosis; Inhibits apoptosis triggered by PKR</td>
<td>Rivas et al. (2001), Esteban et al. (2003), Wies et al. (2008)</td>
</tr>
<tr>
<td>miRNA K12–11</td>
<td>Downregulation of proapoptotic cellular genes, e.g. LDOC1, Bim, BCLAF1 (Bcl2-associated transcription factor 1), BAZF (NF-κB regulator)</td>
<td>Gottwein et al. (2007), Skalsky et al. (2007)</td>
</tr>
<tr>
<td>LANA</td>
<td>Inhibits p53-induced apoptosis; Counteracts pro-apoptotic effects of simultaneously expressed vCyc; Stabilizes and activates c-Myc</td>
<td>Friborg et al. (1999), Curreli et al. (2005), Bubman et al. (2007), Liu et al. (2007a), Petre et al. (2007), Sarek et al. (2007), Cuomo et al. (2008)</td>
</tr>
<tr>
<td>vFLIP</td>
<td>Binds to FLICE complex; Inhibits CD95/Fas-induced apoptosis; Persistent activation of NF-κB</td>
<td>Thome et al. (1997), Chaudhary et al. (1999), Dierbi et al. (1999), Stürzl et al. (1999), Liu et al. (2002), An et al. (2003), Field et al. (2003), Sun et al. (2003), Guasparri et al. (2004), Godfrey et al. (2005)</td>
</tr>
<tr>
<td>vGCR</td>
<td>Promotes endothelial cell survival by activating PI3K/Akt pathway</td>
<td>Montaner et al. (2001)</td>
</tr>
<tr>
<td>K15</td>
<td>Interacts with HAX-1 (an anti-apoptotic regulatory protein)</td>
<td>Sharp et al. (2002)</td>
</tr>
</tbody>
</table>

**Compiled by the Working Group**
4.3 Evidence for a role of KSHV in malignant conversion

4.3.1 Kaposi sarcoma

(a) Requirement of KSHV expression for cell growth invasion

In vitro, KSHV alters the transcriptional programme in infected primary endothelial cells, leading to a redifferentiation of vascular endothelial cells into lymphatic endothelial cells; this results in the formation of spindle cells that are similar to spindle cells in Kaposi sarcoma (see Section 4.2.4). These infected cells, however, cannot be maintained in long-term culture as they show evidence of spontaneous lytic reactivation (Ciufò et al., 2001; Lagunoff et al., 2002).

Owing to a lack of an easily tractable in vivo model, not many published studies have addressed the question of whether or not KSHV is required for cell growth and invasion in vivo. Mutlu et al. (2007) reported that transfection of a bacterial artificial chromosome vector carrying a KSHV genome into murine endothelial cells derived from bone marrow generated a cell that would induce a Kaposi-sarcoma-like tumour when transplanted into mice. Sublines that had lost the KSHV genome in the absence of drug selection lost their tumour-inducing potential, as did KSHV-genome carrying cells, in which the expression of vGPCR had been silenced by siRNA (Mutlu et al., 2007).

(b) Persistence of the KSHV genome

Similarly to the Epstein-Barr virus (EBV), KSHV is capable of replicating its latent episomal genome synchronously with the host cell cycle. However, latent genomes do not appear to persist efficiently in infected primary endothelial cells, nor in epithelial, endothelial or fibroblast cell lines (Foreman et al., 1997; Renne et al., 1998; Blackbourn et al., 2000; Grundhoff & Ganem, 2004). In several cell lines, only a small subpopulation of cells are able to retain the virus in a stable manner following acute infection (Grundhoff & Ganem, 2004; An et al., 2006).

(c) Chromosomal abnormalities, and alterations of specific proto-oncogenes

Most cases of Kaposi sarcoma are cytogenically normal. However, in some cases, the loss of Y-chromosomal sequences and the gain at 11q13 with an amplification of two oncogenes FGF4 and INT2, as detected by comparative genome hybridization, have been noted (Kiuru-Kuhlefelt et al., 2000; Pyakurel et al., 2006). In short-term cultures of Kaposi sarcoma cells, numerical chromosomal abnormalities have been noted (Delli Bovi et al., 1986; Scappaticci et al., 1986; Saikevych et al., 1988). Defined chromosomal abnormalities (loss of copies of chromosomes 14 and 21; deletions in the short arm of chromosome 3 at 3p14) were also noted in two permanent cell lines, KS-Y1 and KS-SLK, established from Kaposi sarcoma biopsies (Popescu et al., 1996). Abnormalities at 3p14 were also noted in another permanent Kaposi sarcoma cell line, KS-IMM, in addition to further chromosomal changes (Casalone et al., 2001).

No p53 mutations have been reported for the majority of Kaposi sarcoma. Host cell tumour-suppressor mRNAs are dysregulated in KSHV-associated-tumours.

4.3.2 Primary effusion lymphoma

(a) Requirement of KSHV expression for cell growth and invasion

In primary effusion lymphoma cell lines, siRNA- and shRNA-mediated knockdown of several latent genes, in particular vFLIP and vIRF-3, induces apoptotic death (Godfrey et al., 2005; Guasparri et al., 2006; Wies et al., 2008). This suggests that these viral genes need to be continuously expressed to ensure the survival of primary effusion lymphoma cells.
(b) Persistence of the KHSV genome

B-Lymphoma cell lines derived from primary effusion lymphoma, contrary to most KSHV-infected cells, retain a stable latent viral genome in high copy numbers (50–100 copies/cell) (Cesarman et al., 1995a, b; Boshoff et al., 1998; Katano et al., 1999; Morand et al., 1999).

Knockdown of LANA in primary effusion lymphoma cell lines leads to a reduction in the viral genome copy numbers, this in keeping with the accepted role of LANA in episome replication (Godfrey et al., 2005).

(c) Chromosomal abnormalities

In primary effusion lymphoma cells, complete or partial trisomy 12, trisomy 7, and abnormalities of bands Iq21–25 were noted frequently in addition to mutations in the 5′ untranslated region of the BCL-6 gene (Gaidano et al., 1999; Wilson et al., 2002). Additional chromosomal changes were noted in the studies by Wilson et al. (2002) and by Nair et al. (2006).

No p53 mutations have been reported for the majority of primary effusion lymphoma.

4.3.3 Multicentric Castleman disease

Multicentric Castleman disease is a polyclonal lymphoproliferative disease that can be a precursor to frank lymphoma. In addition to LANA, vIL6 is expressed in multicentric Castleman disease B cells (see Section 4.2.4). Because vIL6 is a potent stimulator of B-cell growth, it is likely that this protein plays an important role in the B-cell proliferation seen in multicentric Castleman disease.

4.4 Interaction between KSHV and environmental agents

Many agents have an impact on the biology of KSHV:

1. EBV is present in 70–90% of primary effusion lymphomas. EBV-positive and EBV-negative primary effusion lymphomas can be distinguished from each other on the basis of host gene transcription (Fan et al., 2005). However, no differences in clinical appearance, tumorigenicity in mice or response to therapy have been observed between EBV-positive and EBV-negative primary effusion lymphomas (Keller et al., 2000; Petre et al., 2007).

2. Cytomegalovirus (CMV) can reactivate KSHV, and the suppression of CMV has been shown to suppress KSHV viral loads (Martin et al., 1999; Vieira et al., 2001). However, at the time of writing, no evidence for a direct role for CMV in Kaposi sarcoma or primary effusion lymphoma exists.

3. HIV type 1 can reactivate KSHV and enhance KSHV infectivity (Mercader et al., 2000; Merat et al., 2002; Aoki & Tosato, 2004; Zeng et al., 2007). These phenotypes are likely to be mediated by cell-derived cytokines. HIV tat protein can cause endothelial cell proliferation in experimental models (Ensoli et al., 1990, 1994). However, Kaposi sarcoma and primary effusion lymphoma develop in the absence of HIV (Cesarman et al., 1996). Even in HIV-infected patients, these two viruses have never been found in the same cells (Delli Bovi et al., 1986).

To explain the well known high incidence of endemic KSHV in certain parts of Africa, it has been postulated that environmental agents might affect KSHV reactivation. In fact, natural, chemical and environmental products can reactivate KSHV from latency, most notably sodium butyrate and phorbol esters or plant extracts (Renne et al., 1996; Zhong et al., 1996; Miller et al., 1997; Zoeteweij et al., 1999; Whitby et al., 2007).
4.5 Animal models

Following injection to experimental animals, KSHV can infect non-human primates (Renne et al., 2004), NOD-SCID mice (Parsons et al., 2006), and humanized SCID mice (Dittmer et al., 1999; Foreman et al., 2001; Wu et al., 2006). These infections do not result in the formation of tumours. Nevertheless, they confirm the viral tropism (B cells and endothelial cells), and drug susceptibility (ganciclovir) in vivo. KSHV homologous viruses exist in the bank vole-mouse (MHV-68), and virtually in all non-human primates (Ensser & Fleckenstein, 2007). The infection of macaques with rhesus rhadinovirus in the context of Simian immunodeficiency virus (SIV) induces B-cell lymphoma and endothelial-cell hyperplasia (Mansfield et al., 1999; Wong et al., 1999).

Multiple tumourgraft models of Kaposi sarcoma and primary effusion lymphoma have been established (Boshoff et al., 1998; Staudt et al., 2004; Wu et al., 2005; An et al., 2006; Mutlu et al., 2007; Sin et al., 2007).

4.6 Transgenic mice models

An alternative approach to infection studies is to use transgenic mice where individual KSHV proteins are expressed in the hope of replicating selected aspects of KSHV pathogenesis. There are some limitations to single transgenic models. Whereas lymphoproliferative lesions and lymphomas in mice are easily classified on the basis of histology and marker–gene expression, this is not the case for endothelial cell tumours. They are referred to as Kaposi-sarcoma-like lesions, but can easily be mistaken for fibrosarcomas (Table 4.3).

4.6.1 Transgenic mice for KSHV latent genes

(a) LANA/ORF 73

The KSHV latent promoter (LANAp) showed B-cell lineage specificity in transgenic mice (Jeong et al., 2002). KSHV LANA expression in transgenic mice resulted in 100% B-cell hyperplasias and lymphomas at about twice the rate of background in the C57/BL6 strain of mice (Fakhari et al., 2006).

(b) vCYC/ORF 72

Whereas vCYC single transgenic mice did not develop tumours, lymphomas developed rapidly in a p53-null background (Verschuren et al., 2002, 2004). [The Working Group noted that, presumably, loss of p53 counteracted the pro-apoptotic signals that are associated with forced vCYC expression.]

(c) vFLIP/ORF 71

The vFLIP transgenic mice exhibited an increased incidence of lymphoma (Chugh et al., 2005).

4.6.2 Transgenic mice for KSHV lytic genes

(a) vGPCR/ORF 74

vGPCR transgenic mice activated the same signalling pathways as predicted from human culture studies, and exhibited Kaposi-sarcoma-like lesions (Yang et al., 2000; Holst et al., 2001; Guo et al., 2003; Montaner et al., 2003; Jensen et al., 2005; Grisotto et al., 2006). Tumour formation required the chemokine binding as well as the constitutive signalling activities of vGPCR (Holst et al., 2001). vGPCR was required for lesion initiation, though it was not essential once a fully malignant tumour had formed (Grisotto et al., 2006).

(b) K1/VIP

K1 transgenic mice also exhibited Kaposi-sarcoma-like lesions and lymphomas (Prakash et al., 2002, 2005).
4.7 Synthesis

The available mechanistic data strongly support an oncogenic role of KSHV in human cancer.

KSHV alters the growth properties of endothelial cells in culture, and induces Kaposi-sarcoma-cell-like morphology (spindle cells).

One or several KSHV gene products are expressed in all KSHV-associated cancers in all KSHV-infected tumour cells.

At the molecular level, KSHV-encoded gene products associated with latent viral infection induce cell proliferation, block apoptosis, induce genomic instability or modulate cell migration and tumour progression.

Mechanistic data strongly support an oncogenic role of KSHV in primary effusion lymphoma and in Kaposi sarcoma in immunocompromised (post-transplant patients, AIDS patients), as well as in immunocompetent individuals.

KSHV proteins like vIL6 induce B-cell proliferation, and are expressed in KSHV-associated multicentric Castleman disease, strongly suggesting that infection with KSHV is causally associated with this lymphoproliferative disease.

5. Evaluation

There is sufficient evidence in humans for the carcinogenicity of KSHV. KSHV causes Kaposi sarcoma and primary effusion lymphoma. Also, a positive association has been observed between exposure to KSHV and multicentric Castleman disease.

For multiple myeloma, there is evidence suggesting lack of carcinogenicity.

KSHV is carcinogenic to humans (Group 1).
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