National Toxicology Program
U.S. Department of Health and Human Services

Peer-Review Draft:
Report on Carcinogens Monograph on Kaposi Sarcoma Herpesvirus

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Office of the Report on Carcinogens
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Foreword

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Report on Carcinogens (RoC) is prepared in response to Section 301 of the Public Health Service Act as amended. The RoC contains a list of identified substances (i) that either are *known to be human carcinogens* or are *reasonably anticipated to be human carcinogens* and (ii) to which a significant number of persons residing in the United States are exposed. The Secretary, Department of HHS, has delegated responsibility for preparation of the RoC to the NTP, which prepares the report with assistance from other Federal health and regulatory agencies and nongovernmental institutions. The most recent RoC, the 13th Edition (2014), is available at http://ntp.niehs.nih.gov/go/roc.

Nominations for (1) listing a new substance, (2) reclassifying the listing status for a substance already listed, or (3) removing a substance already listed in the RoC are evaluated in a scientific review process (http://ntp.niehs.nih.gov/go/rocprocess) with multiple opportunities for scientific and public input and using established listing criteria (http://ntp.niehs.nih.gov/go/15209). A list of candidate substances under consideration for listing in (or delisting from) the RoC can be obtained by accessing http://ntp.niehs.nih.gov/go/37893.

Overview and Introduction

This collection of monographs on selected viruses provide cancer hazard evaluations for the following human viruses: Epstein-Barr virus, Kaposi sarcoma herpesvirus, human immunodeficiency virus-1, human T-cell lymphotropic virus-1, and Merkel cell polyomavirus for potential listing in the Report on Carcinogens (RoC). Currently, there are three human oncogenic viruses listed in the RoC: human papillomaviruses: some genital-mucosal types (HPV), hepatitis B virus (HBV), and hepatitis C virus (HCV). The five viruses covered in these monographs were selected for review for the RoC based on a large database of information on these agents, including authoritative reviews, and public health concerns for disease mortality and morbidity both in the United States and worldwide because of significant numbers of infected people.

This section provides background information on the preparation of the monographs as well as a discussion of overarching issues related to evaluating the evidence for cancer from human epidemiology studies and evaluating the causation by viruses.

Background

The RoC draft monograph for each virus consists of the following components: (Part 1) the cancer evaluation component that reviews the relevant scientific information and assesses its quality, applies the RoC listing criteria to the scientific information, and recommends an RoC listing status, and (Part 2) the draft substance profile containing the NTP's preliminary listing recommendation, a summary of the scientific evidence considered key to reaching that recommendation, and information on properties, exposure, and Federal regulations and guidelines. Information reviewed in the monographs, with the exception of information on properties and exposure, comes from publicly available and peer-reviewed sources. All sections of the monographs underwent scientific and quality assurance review by independent reviewers.

The cancer evaluation component provides the following information relevant to a RoC listing recommendation: Properties and Detection (Section 1), Exposure (Section 2), Human Cancer Hazard Evaluation for specific cancer endpoints (Section 3), Mechanistic and Other Relevant Data (Section 4), and Preliminary Listing Recommendation (Section 5). Because these viruses are primarily species-specific for humans and similar to the approach used by IARC, we are including information on studies in experimental animals in the Mechanistic and Other Relevant Data section of the monographs. Also, specific details about the strains of the viruses are given only if needed to provide context, such as in the viral Properties and Detection section. The monographs relied on the information and data provided in previous IARC monographs on these five viruses in addition to newer key studies or reviews published since the IARC monographs; it is an independent assessment of available data through August 17, 2015. Literature search strategies to obtain information relevant to the cancer evaluation are in Appendix A of each virus monograph; search terms were developed in collaboration with a reference librarian.

Issues related to evaluating the evidence from human epidemiological studies

The available studies of specific cancer endpoints in the human virus studies present several challenges with respect to the evaluation of methodological strengths and limitations of the body of evidence. Large prospective cohort studies, particularly those that follow individuals for whom infection status is documented prior to follow-up or cancer diagnosis, have several

potential methodological strengths, including evidence that infection precedes cancer diagnosis, adequate statistical power and, in some studies, the ability to analyze dose-response relationships. However, there is the potential for misclassification of exposure in studies that measure the virus once, but with a long follow-up period as they may miss new infections. For most cancer endpoints, only cross-sectional or retrospective cohort studies or hospital or clinic-based case-control studies are available, which lack direct evidence of temporality and may lack power or adequate data on, e.g., viral load. However, molecular evidence from human studies and mechanistic data can be used in the evaluation of temporality, distinguishing latent infections caused by the tumor virus and causality. For some (typically rare) outcomes (e.g., cutaneous T-cell lymphoma and human T-cell lymphotropic virus type 1, or lymphoepithelial carcinoma of the salivary gland and Epstein-Barr virus), only case-comparison studies, in which selection of comparison groups may be biased, unmatched, or inadequately described, or case series, are available.

In addition, for several rare endpoints, e.g., adult T-cell leukemia/lymphoma and human T-cell lymphotropic virus type 1, or primary effusion lymphoma and Kaposi sarcoma herpesvirus, the presence of the virus in the tumor cells is used as a diagnostic criterion to define the cancer, and thus evidence of causality relies on cases defined by this criterion and molecular evidence from human studies rather than on epidemiological population-based studies of the association of the virus with a level of cancer risk.

For several viruses, e.g., Epstein-Barr virus, the population prevalence may exceed 90%, so that cohort and case-control studies must rely on the evaluation of cancer risk using measures such as Epstein-Barr virus titer or antibody levels rather than exposed and non-exposed categories of study participants, allowing for the possibility that past or current viral level could be misclassified. In addition, for a number of these viruses, e.g., Kaposi sarcoma herpesvirus, the presence of the virus may be necessary but not sufficient to increase the risk for a specific cancer endpoint and more than one virus may be associated with risk. Thus, methodologically adequate studies should include measurement of such cofactors and consider potentially confounding factors; however, relatively few studies have measured a panel of other viruses or taken into account other cofactors. In addition, while studies comparing cancer risk in treated vs. untreated populations may provide indirect evidence of the role of human immunodeficiency virus-1, these studies, in particular calendar-period analyses, may not adequately account for changes in risk attributable to improved survival rates or changes in other risk factors.

Issues related to evaluating causality of viruses

Approximately 12% of all human cancers have been attributed to viral infections; however, viruses are rarely fully oncogenic themselves and only a small percentage of infected individuals develop cancer, often decades after the initial infection (Mesri *et al.* 2014). Therefore, oncogenic viruses are generally considered necessary but not sufficient to cause cancer. Additional cofactors, such as infective organisms, chemicals, or environmental agents in conjunction with risk modifiers such as immune dysfunction or chronic inflammation can contribute to malignant transformation. Severe immunosuppression, as seen with congenital immunodeficiency syndromes, chronic human immunodeficiency virus type 1 infection, or as a result of tissue anti-rejection medication, can severely compromise the immune surveillance capabilities of the patient. In addition, some cofactors produced by other organisms or agents have been shown to activate the oncogenic potential of some of these viruses. There are also other challenges that are

somewhat unique to the evaluation of the epidemiological studies (discussed below) and thus molecular evidence is often considered in the evaluation of causality.

In light of these issues, IARC monographs and several other publications have discussed paths to evaluate causality, which are discussed below and incorporated into the NTP approach for evaluating causality of the viruses. What is important for public health in determination of causation of a health effect, such as risk for cancer, is whether that health effect is eliminated or mitigated by removal of the substance.

There have been a number of attempts to develop criteria that address causal associations. However, all of them have limitations, especially when applied to infectious agents (Moore and Chang 2010). The following sections identify factors to consider for evaluating causality, some of the limitations associated with strict application of the criteria in the context of virally induced cancers, some alternative approaches, and the NTP's approach for evaluating the role of select viral agents in human cancer.

Hill's characteristics for evaluation of epidemiological studies

Hill proposed nine characteristics to consider when evaluating causality, primarily for epidemiological studies, although they have been expanded for evaluating mechanistic and other types of data (Table 1). Several considerations—strength of the association, consistency across studies, evidence of an exposure-response gradient, and temporality of exposure (Hill 1965)—are used to help guide the RoC evaluations of the human epidemiological data (see RoC Handbook, NTP 2015). However, it should be noted that these are not criteria; with the exception of temporality, each and every element is not required in order to demonstrate causality (Rothman and Greenland 2005). Hill (1965) avoided discussing the meaning of "causation" noting that the "cause" of an illness could be immediate and direct or remote and indirect. The primary question addressed by Hill was "whether the frequency of the undesirable event B will be influenced by a change in the environmental feature A."

Table 1. Hill's epidemiological characteristics for causality

Criterion	Description
1. Strength of association	A strong association between a virus and a cancer is most consistent with causality unless confounded by some other exposure. However, a weak association does not give evidence against causality.
2. Consistency	Consistent findings observed by different persons, in different places, circumstances and times.
3. Specificity	A viral exposure is limited to specific types of cancer (considered a weak factor because there are well-established examples in which multiple types of disease are caused by one type of exposure). However, the more specific the association, the higher the probability of a causal relationship.
4. Temporality	Exposure to the virus must occur prior to the onset of the cancer, in contrast to a "passenger infection."
5. Biologic gradient	The virus is more likely to be found at the tumor site than at non-tumor sites.
6. Plausibility	Should be applied with caution because it is limited by current medical knowledge (e.g., an implausible mechanism may gain acceptance with increased understanding of the underlying biology).
7. Coherence	A virus-cancer association should not seriously conflict with known facts on the

	cancer's natural history and biology.
8. Experiment	Changing either exposure or continued infection in a randomized clinical trial should change the measure of clinical outcome (e.g., vaccination programs for HPV and HBV).
9. Analogy	Are related viruses clearly established to cause cancers in animals or humans?

Source: Moore and Chang 2010.

Consideration of mechanistic data from studies in humans

In their evaluation of the evidence for Epstein-Barr virus, the IARC working group noted that the large majority of people are latently infected with Epstein-Barr virus, thus epidemiological studies may be limited in determining whether the presence of Epstein-Barr virus in tumor tissue is a cause of the cancer or an effect of the tumor. Thus, in addition to the Hill characteristics, IARC (1997) also considered the following in their evaluation of Epstein-Barr virus, which are applicable to other viruses:

- the proportion of Epstein-Barr virus-positive cases in a given tumor entity,
- the proportion of tumor cells that carry the virus,
- the monoclonality of Epstein-Barr virus in the tumor, and
- the expression of Epstein-Barr virus proteins.

zur Hausen (2001, 1994) also noted the difficulty of applying stringent criteria to identify human tumor viruses and proposed the following:

- the regular presence and persistence of the respective viral DNA in tumor biopsies and cell lines derived from the same tumor type,
- the demonstration of growth-promoting activity of specific viral genes or of virus-modified host cell genes in tissue culture systems or in suitable animal systems,
- the demonstration that the malignant phenotype depends on the continuous expression of viral oncogenes or on the modification of host cell genes containing viral sequences,
- epidemiological evidence that the respective virus infection represents a major risk factor for cancer development.

It is difficult to prove that a virus causes cancer, and such determinations almost always generate considerable controversy and debate (Moore and Chang 2010). Viral cancers employ various mechanisms that involve both direct and indirect modes of interaction (Table 2) (zur Hausen and de Villiers 2014). Understanding and managing viral-induced cancers in humans has been hampered by a lack of suitable animal models, the disparate nature of tumor types, a long latency period between primary infection and cancer development, the different types of oncogenic viruses, and the complex nature of the virus-host cell interactions leading to cancer (Mesri *et al.* 2014, zur Hausen and de Villiers 2014).

Table 2. Direct and indirect modes of interaction of viral infections

Type	Description
Direct carcinogenesis	• Continued presence and expression of viral oncogenes usually after viral genome integration into host cell DNA • Insertional gene activation or suppression • Continued episomal presence of viral nucleic acid and suppression or activation of cellular genes (e.g., by viral microRNA)
Indirect carcinogenesis	• Induction of immunomodulation, activation of latent tumor virus genomes • Induction of oxygen and nitrogen radicals • Amplification of latent tumor virus DNA • Induction of mutations and/or translocations • Prevention of apoptosis

Source: zur Hausen and de Villiers 2014.

Multicausality issues

Although thousands of viruses are known to cause infection, only a few have been shown to cause cancer in humans (Moore and Chang 2010). An agent that is both necessary and sufficient for a disease to occur describes a complete causal effect. However, this is not a practical definition for infectious diseases that emerge from complex interactions of multiple factors and may be caused by more than a single agent. An important consideration regarding multicausality is that most of the identified causes are neither necessary nor sufficient in the absence of other factors to produce the disease; however, a cause does not have to be either necessary or sufficient for its removal to result in disease prevention (Rothman 1976, zur Hausen and de Villiers 2014). Although the known oncogenic viruses belong to different virus families, they share several common traits: (1) they are often necessary but not sufficient for tumor development; (2) viral cancers appear in the context of persistent infections and occur many years to decades after acute infection; and (3) the immune system can play a deleterious or a protective role (Mesri *et al.* 2014).

Application of causality criteria and alternative approaches

Moore and Chang (2010) investigated the difficulties associated with strict application of the Hill characteristics for two of the most recently discovered oncogenic viruses: Kaposi sarcoma herpesvirus and Merkel cell polyomavirus. Kaposi sarcoma herpesvirus was shown to fulfill Hill's characteristics for causality of Kaposi sarcoma; however, the application of the characteristics was problematic in the case of Merkel cell polyomavirus and Merkel cell carcinoma (see the monographs for Kaposi sarcoma herpesvirus and Merkel cell polyomavirus). These two examples illustrate the diversity in the patterns of tumor virus epidemiology. Some of the reasons Hill's characteristics worked for Kaposi sarcoma herpesvirus but not Merkel cell polyomavirus is that all clinical forms of Kaposi sarcoma require Kaposi sarcoma herpesvirus while most studies indicate that all forms of Merkel cell carcinoma do not require Merkel cell polyomavirus infection. Further, Kaposi sarcoma herpesvirus infection is uncommon in most parts of the world but was confirmed to be present in nearly all AIDS-associated Kaposi sarcoma cases, while widespread Merkel cell polyomavirus infection rate implies that it cannot be a specific causal factor for a rare cancer like Merkel cell carcinoma. In the case of Merkel cell polyomavirus, additional considerations, as suggested by IARC (1997) and zur Hausen (2001,

1994), provide molecular evidence of the association between Merkel cell polyomavirus and Merkel cell carcinoma, such as the tumor-causing form of the virus is mutated and monoclonally integrated into the tumor genome and that tumor cells require the presence of viral oncoproteins for cell survival and proliferation.

While causal criteria can be helpful, there are flaws and practical limitations that restrict their use in cancer biology (Moore and Chang 2010). Therefore, a more probabilistic approach may be more useful for determining whether or not certain viruses cause human cancers. For example, instead of trying to determine if virus A causes cancer B, the probabilistic approach examines if cancer B is more probable in the presence of virus A. Although a correlation does not imply causation, it can be argued that correlations that are strong, reproducible, and predictive have a similar value as a causative conclusion. In a similar fashion, zur Hausen and de Villiers (2014) also expressed concern over all attempts to summarize criteria for "causality" of infectious agents in cancer development and proposed replacing "causal factor" with "risk factor" and grading them according to their contribution to an individual's cancer risk. This will require a greater understanding of the complexity of factors involved and their mechanistic contribution to individual cancers.

NTP's approach

For each virus, the NTP applied the RoC listing criteria (see text box) to the body of literature to reach the preliminary listing recommendation. The level of evidence conclusion from studies in humans considers the evidence from epidemiological studies as well as clinical and molecular studies of tissues from exposed (i.e., infected) individuals. In evaluating the

RoC Listing Criteria

Known To Be Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans*, which indicates a causal relationship between exposure to the agent, substance, or mixture, and human cancer.

Reasonably Anticipated To Be Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans*, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded, OR

there is sufficient evidence of carcinogenicity from studies in experimental animals, which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or type of tumor, or age at onset, OR

there is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance, or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either known to be a human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to, dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub-populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals, but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

*This evidence can include traditional cancer epidemiology studies, data from clinical studies, and/or data derived from the study of tissues or cells from humans exposed to the substance in question that can be useful for evaluating whether a relevant cancer mechanism is operating in people.

mechanistic data and determining the preliminary recommendations for its level of evidence conclusion and overall listing recommendation, the NTP considered the principles outlined by Hill, IARC, zur Hausen, and Rothman in its assessment of causality for the five viruses reviewed. However, these factors were not used as a strict checklist to either prove or disprove a causal association but rather as guidance to assess the level of epidemiological or molecular evidence that a virus contributes to a carcinogenic effect.

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Part 1

Draft Cancer Hazard Evaluation

Properties and Detection

Exposure

Human Cancer Studies

Mechanisms and Other Relevant Data

Preliminary Listing Recommendation

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1 Properties and Detection

This section reviews the biological properties (Section 1.1) and detection methods (Section 1.2) of the Kaposi sarcoma herpesvirus (KSHV). The information in this section is summarized in Section 1.3.

1.1 Biological properties

1.1.1 Family and type

KSHV, also designated as human herpesvirus 8 (HHV8), was the first member of the gamma-2 herpesviruses identified in humans in 1994 in association with acquired immunodeficiency syndrome (AIDS) (Chang *et al.* 1994, Fukumoto *et al.* 2011, IARC 1997). Rhadinoviruses also include herpesviruses that infect New World monkeys, macaque Old World monkeys, cattle, and rodents (IARC 1997).

KSHV consists of linear double stranded DNA (dsDNA) wrapped around a core protein within a protein capsid, surrounded by a membrane envelope containing glycoproteins (Edelman 2005, Griffin and Damania 2014, IARC 2012, Fukumoto *et al.* 2011, IARC 1997) (see Figure 1-1). The capsid is made up of five major capsid proteins forming hexamer and pentamer proteins and a minor capsid protein that binds to the edges of the hexamer and pentamer junctions (Giffin and Damania 2014, IARC 2012). Between the capsid and the membrane envelope are six tegument proteins and 11 viral RNA strands (Giffin and Damania 2014, Fukumoto *et al.* 2011).

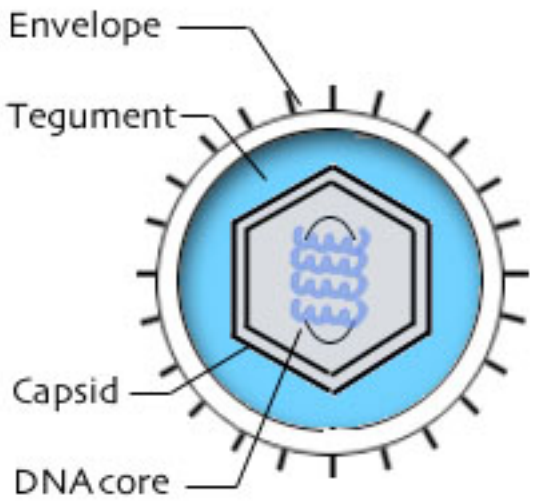

Figure 1-1. Kaposi sarcoma herpes virus particle

Source: Los Alamos National Laboratory

1.1.2 Genome

The KSHV genome, with an overall size of 165 kbp, consists of two main sections, (1) the long unique region, which contains over 80 open reading frames (ORF) for protein and microRNA (miRNA) genes, is 140 kbp long and (2) two flanking terminal repeat regions composed of a variable number of repeating units (see Figure 1-2) (IARC 2012, Fukumoto *et al.* 2011, IARC 1997). The terminal repeats do not contain open reading frames, but function by providing the origin of replication, location of genome circularization, and binding sites to tether the viral genome to host chromosomes.

Most of the genes are conserved among herpesviruses and are named after the genes of the closest related virus, Herpesvirus saimiri, which infects non-human primates (IARC 2012, 1997). These genes are designated by the number identifier of their open reading frame, e.g., ORF 16, and are numbered consecutively from left to right across the genome (Giffin and Damania 2014). Other genes originated from host cellular genes during viral evolution and are designated by their cellular homologue, preceded by "v-" denoting a viral origin, e.g., v-Bcl-2 (IARC 2012, 1997). Some genes are unique to KSHV and are designated by the letter "K" in their open reading frame, e.g., ORF K12. MiRNAs are designated by "miR" and a number identifier, e.g., miR-K5.

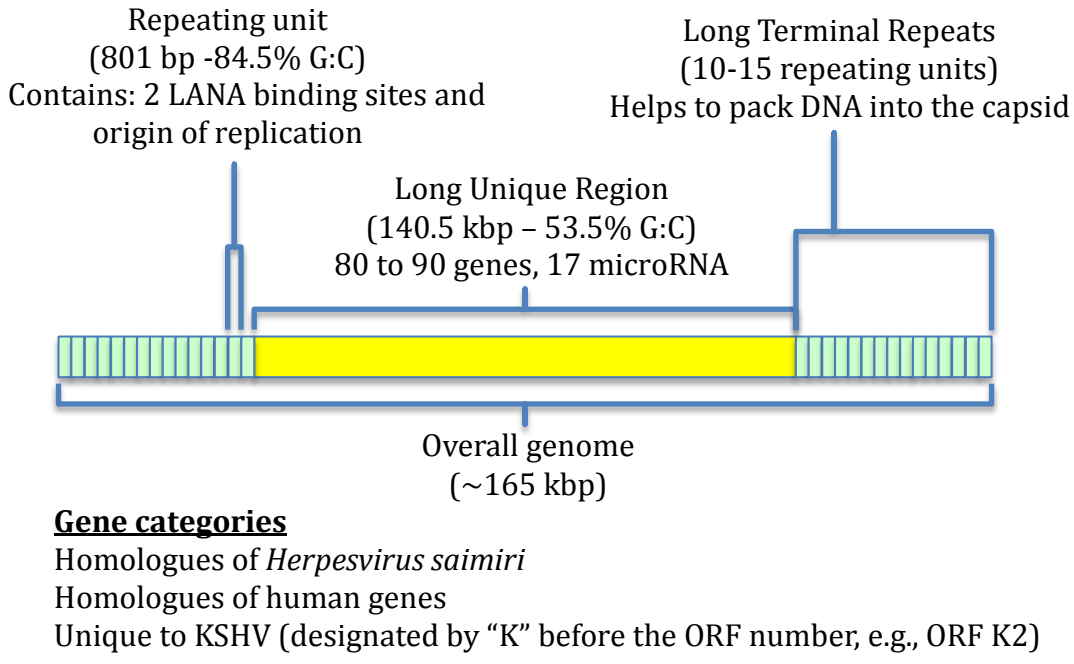

Figure 1-2. Genome schematic

LANA = latency-associated nuclear antigen.

1.1.3 Infection and replication

Like other herpesviruses, the KSHV replication cycle includes a latent phase and a lytic phase, which allow the virus to transition between a quiescent infection and active replication (see Figure 1.3). KSHV has been detected in endothelial cells, epithelial cells, B lymphocytes, and monocytes *in vivo*, while a wider variety of cells can be infected *in vitro* (Giffin and Damania

2014). $CD19^+$ B lymphocytes are a long-term latency reservoir for the virus. KSHV glycoproteins on the capsid surface mediate fusion with several target cell receptors and the replication cycle starts as KSHV enters a cell by a receptor-mediated endocytosis or macropinocytosis process (Campbell *et al.* 2014a, Giffin and Damania 2014). The viral capsid then enters the nucleus and releases dsDNA. Inside the virus particle, the genome is linear, but within the host cell nucleus it circularizes, joining the terminal repeat ends together and attaching to host cell chromosomes (Campbell *et al.* 2014a, Fukumoto *et al.* 2011, IARC 1997). In the latent phase, the viral episome persists and is replicated by host machinery to daughter cells with each cell division. No infectious virus particles are produced during latency and only a small number of genes located in the latency locus are expressed. Only a low copy number of the viral genome is present in a latently infected cell, while a high copy number is produced during a lytic infection.

Most of the time, KSHV will remain in the latent phase but cell stressors, such as phorbol esters, sodium butyrate, some signaling cytokines, cell differentiation, reactive oxygen species, and innate immune signaling by Toll-like receptors, can initiate the lytic phase (Giffin and Damania 2014). At the start of the lytic phase the viral lytic transactivator protein, RTA, causes the expression of many viral genes, beginning with those involved in controlling viral gene expression. The next viral genes expressed are those involved in DNA replication and immunomodulation. Viral DNA replication occurs at multiple origins within the terminal repeat region of the circular genome to produce linear copies of newly formed genomes. The viral capsid is then enveloped by the lipid bilayer membrane as it buds off of the nuclear membrane into the cytoplasm and progeny virions are released from the cell. See Section 4.2.2 for a detailed description of latent and lytic genes.

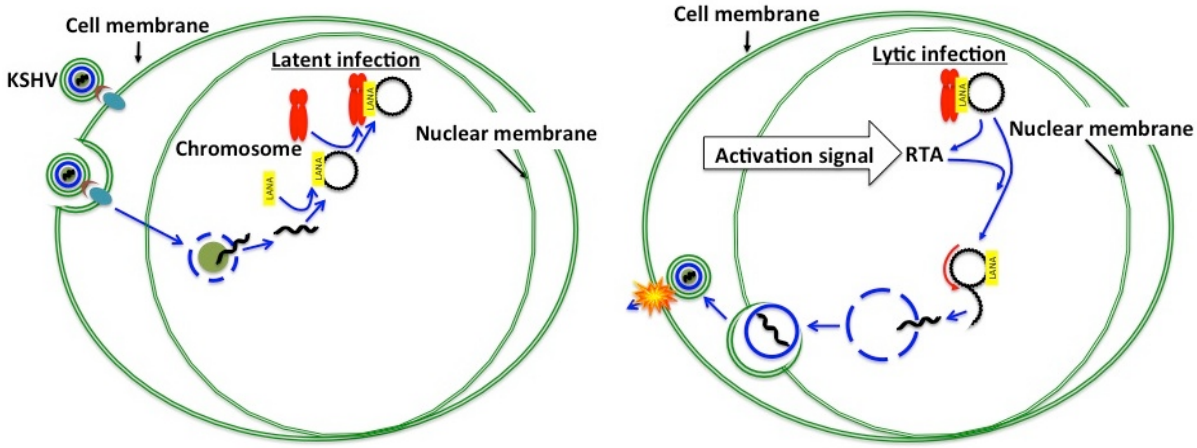

Figure 1-3. KSHV infection and replication cycle

1.2 Detection methods

KSHV infections can be detected by measuring viral protein antigens or viral DNA in tissues and antibodies against the virus in blood. The level of KSHV antibodies in the blood only indicates that a viral infection has occurred (Xu *et al.* 2015, Fukumoto *et al.* 2011). KSHV DNA or antibodies against KSHV-specific antigens in the blood can be used to measure viral load. The nature of the antigens recognized by the antibodies, i.e., either latent or lytic, can help to determine the predominant nature of the infection, but this test alone is not conclusive because of

variations in development of antibody responses among individuals (IARC 2012). Both latent and lytic viral infections are associated with carcinogenesis (IARC 2012). In cases of Kaposi sarcoma or primary effusion lymphoma, KSHV latently infects almost all tumor cells while lytic infection is frequently seen in multicentric Castleman disease (see Mechanisms, Section 4). Antibodies against two main viral proteins have been measured in humans to detect KSHV, though other protein-specific antibodies can be detected as well (see Mechanisms, Section 4) (IARC 2012, Fukumoto *et al.* 2011, IARC 1997). These include the latent phase protein, latency-associated nuclear antigen (LANA) (ORF 73), and the lytic phase protein K 8.1.

1.2.1 Detection in fluids

Antibodies

The level of antibody response varies depending on the phase of the infection (IARC 2012). Antibody responses are low in latently infected individuals and higher in patients with lytic infections or in patients with Kaposi sarcoma (IARC 2012). Latently infected cell lines can be used to immunohistochemically detect anti-KSHV antibodies, which stain in the nucleus only. Infected cell lines can be stimulated with phorbol esters or sodium butyrate to increase the number of cells in the lytic phase and to detect antibodies against lytic antigens, which stain the cytoplasm as well as the nucleus (Fukumoto *et al.* 2011, IARC 1997). Recombinant viral proteins and peptides have also been used in ELISA tests for KSHV antibodies (IARC 2012). Polymerase chain reaction (PCR) techniques can detect viral DNA in saliva and to a lesser extent in vaginal secretions and semen (Campbell *et al.* 2014a); however, these techniques are not used clinically.

Detection in cells

Antigens

KSHV can infect many different cell types including B cells, dendritic cells, monocytes, prostate cells, keratinocytes, fibroblasts, endothelial cells, and epithelial cells, as detected by immunohistochemical staining for KSHV antigens (Campbell *et al.* 2014a, Giffin and Damania 2014, IARC 2012, Fukumoto *et al.* 2011, IARC 1997). KSHV encodes a viral homolog of interleukin-6 (vIL-6), which can be detected in blood and cells of multicentric Castleman disease and primary effusion lymphoma, but not in Kaposi sarcoma cells (Bhutani *et al.* 2015, Fukumoto *et al.* 2011, Parravicini *et al.* 1997). Free vIL-6 can be detected in blood of patients with multicentric Castleman disease and primary effusion lymphoma, but is not found in patients with Kaposi sarcoma (Bhutani *et al.* 2015). vIL-6 is thought to play a role in multicentric Castleman disease pathogenesis by stimulating the proliferation of plasma cells.

DNA

Viral DNA detected in tissues by PCR can indicate KSHV infections in those tissues (IARC 2012, Fukumoto *et al.* 2011, IARC 1997). KSHV DNA can be detected in peripheral blood mononuclear cells, but not all KSHV-infected patients will give positive results (Campbell *et al.* 2014a, Fukumoto *et al.* 2011, IARC 1997). KSHV infection can occur without Kaposi sarcoma or multicentric Castleman disease, so detecting KSHV DNA in peripheral blood mononuclear cells does not necessarily indicate the presence of these diseases. However, those with Kaposi

sarcoma or multicentric Castleman disease tend to have higher viral loads in peripheral blood mononuclear cells.

Table 1-1. Detection methods

Analyte	Method	Lytic marker	Latent marker
Fluids			
Antibodies	Immunohistochemistry – infected primary effusion lymphoma cells	Nuclear and cytoplasmic staining[a]	Only nuclear staining
	ELISA	High levels – suggestive	Low levels – suggestive
DNA	PCR	Genome detection - Not phase specific	
Cells			
Antigen	Immunohistochemistry – tissue	Nuclear and cytoplasm staining	Only nuclear staining
DNA	PCR	Genome detection - Not phase specific	

[a]Phorbol esters are used to induce the lytic phase in primary effusion lymphoma cells.

1.3 Summary

Kaposi sarcoma herpesvirus (KSHV) is an enveloped DNA virus found in Kaposi sarcomas, primary effusion lymphoma, and some cases of multicentric Castleman disease. The genome circularizes and attaches to the host chromosome but does not integrate into the host genome. There are over 80 genes and 17 mRNAs, which include some genes that originated from host cell genes during the evolution of KSHV. The virus can reproduce lytically or can remain latent, tethered to the host chromosome. The lytic phase can be initiated by innate immune signaling, cytokine signaling, or cell differentiation or by exposure to phorbol esters, sodium butyrate, or reactive oxygen. KSHV can be dtected by anti-KSHV antibodies, intracellular viral antigens, and DNA.

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2 Exposure

This section describes prevalence and transmission (Section 2.1), and non-cancer diseases, prevention, and treatment of Kaposi sarcoma herpesvirus (KSHV) (Section 2.2). The material presented in Sections 2.1 and 2.2 is summarized in Section 2.3.

2.1 Prevalence and transmission

Serological tests for detecting the multiple antigens encoded by KSHV vary in sensitivity and specificity, resulting in some uncertainty with respect to comparative prevalence of KSHV in different populations (IARC 2012). KSHV prevalence rates appear to vary widely in different populations; high-level endemic areas (adult general public seroprevalences between 30% and 70%) are found in many parts of Africa (Martin 2007); intermediate-level endemic areas (general public seroprevalences between 10% and 25%) are found primarily in the Mediterranean area (Whitby *et al.* 1998); and non-endemic areas (general public seroprevalences less than 10%) include North America, Central America, South America, northern Europe, and Asia. However, in non-endemic areas, certain population groups exhibit seroprevalences comparable to those in high- and intermediate-level endemic areas (e.g., between 30% and 60% of HIV-1-infected men who have sex with men and between 20% and 30% of HIV-1-uninfected men who have sex with men are infected with KSHV (Martin *et al.* 1998, O'Brien *et al.* 1999).

The first systematic evaluation of KSHV epidemiology in the general public in the United States (based on enzyme immunoassays conducted on serum samples from NHANES III data collection) reported that overall KSHV seroprevalence is approximately 7% and is similar among men and women (Engels *et al.* 2007a). These seroprevalence data indicate that a significant number of people in the United States are exposed to KSHV. A previous study of 1,000 U.S. blood donors (collected in 1994 and 1995) tested by six independent laboratories, using various serological tests ranging from single immunofluorescence assays to decision trees based on results of several individual assays, reported KSHV seropositivity estimates ranging from 0.5% to 5% (IARC 2012, Pellett *et al.* 2003). KSHV seropositivity has been found to be lower among HIV-1-infected women than in HIV-1-infected men who have sex with men, including bisexual men (Kedes *et al.* 1997). Global KSHV seroprevalence has been shown to mirror the geographical distribution of classic or endemic (i.e., non-HIV-1-associated) forms of classic Kaposi sarcoma, e.g., high (15% to 60%) in African and southern Mediterranean regions, and low (1% to 5%) in the United States and northern Europe (Minhas and Wood 2014, Ganem 2010).

Transmission of KSHV appears to be primarily via saliva (IARC 2012). The presence of KSHV in peripheral blood suggests that transmission via blood is also possible, and transmission among some intravenous drug users, transfusion recipients, and organ recipients has been reported. In populations with high endemic KSHV prevalence, both horizontal and vertical transmission appear to occur, primarily in childhood between the ages of 6 and 10 years (particularly if the mother is infected) and infection rates increase with age.

Risk factors for infection may include contact with, e.g., infected family members, sources of water, possibly insect bites (Amodio *et al.* 2011b, Coluzzi *et al.* 2003) and, in particular, HIV-1

infection (IARC 2012), so factors that increase the risk of HIV-1 infection (e.g., number of sexual partners) also increase the risk of infection with KSHV. Further, higher KSHV infection rates in HIV-1-positive cohorts other than those acquiring HIV-1 via parenteral infection were found to reflect sexual activity, for which HIV-1 seropositivity serves as a marker (Kedes *et al.* 1996). Men who have sex with men may have a higher risk of KSHV transmission than heterosexual sex partners even in the absence of HIV-1 infection (Giuliani *et al.* 2007), and orogenital sex has been shown to be significantly correlated with KSHV seroconversion in men who have sex with men (Dukers *et al.* 2000).

KSHV is generally not associated with sexual risk factors in heterosexuals (IARC 2012, Engels *et al.* 2007a, Smith *et al.* 1999); however, uninfected heterosexual partners have been reported to have a higher risk of infection if one of the partners is KSHV positive, than among controls in which both partners are KSHV negative, despite apparent similarities in factors related to frequency and type of sexual activity (Dupuy *et al.* 2009).

2.2 Diseases, prevention, and treatment

Most individuals who are otherwise healthy and infected with KSHV are asymptomatic (ACS 2014, NCI 2014, DHHS 2013). There are very few reports of primary infection with KSHV, but reported symptoms associated with initial KSHV infection include a febrile maculopapular skin rash (as observed in immunocompetent children in Egypt) (Andreoni *et al.* 2002), as well as diarrhea, fatigue, localized rash, and lymphadenopathy (in men who have sex with men without HIV-1 infection) (Wang *et al.* 2001). Other symptoms include fever, splenomegaly, cytopenia, and bone marrow failure with plasmacytosis (in renal transplant recipients) (Luppi *et al.* 2000b). Active KSHV infection may be associated with fever, cutaneous rash, and hepatitis in autologous peripheral blood stem cell infusion recipients (Luppi *et al.* 2000c).

A limited number of studies have suggested associations between KSHV infection and some non-cancer endpoints, including proliferative and nonproliferative skin diseases such as pemphigus vulgaris and pemphigus foliaceus, and actinic keratosis, but other studies have failed to confirm these associations (Ablashi *et al.* 2002). In addition, KSHV has been found in inflammatory cells in isolated cases of interstitial pneumonitis, in sarcoid tissue, and in histocytic necrotic lymphadenitis, indicating lymph node infiltration, but an etiologic role of KSHV in these diseases has not been established (Ablashi *et al.* 2002).

Because KSHV transmission is associated with KSHV shedding in saliva and occasional shedding in genital secretions, avoiding salivary exposure and following safe sex practices should theoretically prevent transmission (DHHS 2014, 2013, Chang-Moore Laboratory 2009).

Some drugs have been reported to reduce or inhibit KSHV shedding (e.g., see Cattamanchi *et al.* 2011, Casper *et al.* 2008); however, currently there are no FDA-approved drugs for treatment of KSHV infection. Currently, there is no vaccine against KSHV; however, limited efforts to develop a vaccine are ongoing (ACS 2014, Wu *et al.* 2012).

2.3 Summary

U.S. seroprevalence study data indicate that a significant number of people living in the United States are exposed to KSHV. Most individuals who are otherwise healthy and infected with KSHV are asymptomatic. Transmission appears to be primarily via saliva. Transmission via

blood is also possible, and transmission among some intravenous drug users, transfusion recipients and organ recipients has been reported. Both horizontal and vertical transmission appears to occur, primarily in childhood (between the ages of 6 and 10 years, with infection rates increasing with age) in populations with high endemic KSHV prevalence, particularly if the mother is infected. Risk factors for infection may include contact with infected family members, sources of water, and possibly insect bites, and particularly HIV-1 infection, so factors that increase the risk of HIV-1 infection also increase the risk of infection with KSHV. Men who have sex with men may have a higher risk of KSHV transmission than heterosexual sex partners even in the absence of HIV-1 infection. A limited number of studies have suggested associations between infection with KSHV and some non-cancer endpoints (e.g., pemphigus vulgaris, pemphigus foliaceus, and actinic keratosis), but other studies have failed to confirm these associations. There is currently no vaccine against KSHV, although limited vaccine development efforts are ongoing.

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3 Human Cancer Studies

3.1 Introduction

The majority of human epidemiological studies for KSHV have focused on Kaposi sarcoma, which was first described by Dr. Mori Kaposi in 1872 (IARC 1997), prior to the discovery of the virus in 1994 (Chang *et al.* 1994). Since 1994, a number of other cancer endpoints have been evaluated, the major ones are certain B-cell non-Hodgkin lymphomas, in particular rare forms of primary effusion lymphoma, multicentric Castleman disease, multiple myeloma, and other lymphohematopoietic cancers. The evidence for these endpoints, which were identified by IARC (IARC 2012, 1997), is assessed in this section. A limited number of studies of solid tumor endpoints (including prostate, bladder, head and neck, angiosarcoma, skin, hepatocellular, and vulvar cancers, and childhood inflammatory myofibroblastoma) have also been conducted, but only in single or small studies for most endpoints, and thus are only revised briefly.

One of the key issues in the cancer hazard evaluation is the extent to which observed associations between KSHV and specific cancer endpoints can be explained by cofactors, including host genetic factors, co-infection with other viruses, or other environmental and/or other risk factors, as distinct from confounding factors. However, while a substantial number of studies have investigated KSHV cancer risk in association with HIV-1 infection, relatively few studies have clearly identified other cofactors, in particular the presence of other oncoviruses (e.g., Epstein-Barr virus) and non-viral cofactors.

In the majority of studies, KSHV exposure is detected based on DNA in the peripheral blood mononuclear cells, tumor, normal tissue, semen, saliva, and other biological samples or via serological measures usually measuring antibodies to lytic or latent viral antigens. Earlier studies included in this evaluation primarily used serological measurements, while later studies generally used more sensitive and specific seroprevalence measures or detected KSHV DNA.

This evaluation of the human cancer hazard associated with KSHV is divided into three major parts. First, a summary of the approach for selection of the studies is provided (Section 3.1). Next, the cancer evaluation for each endpoint is presented (Sections 3.2 to 3.5), and lastly, a summary of the evaluation across cancer endpoints is provided (Section 3.6). The preliminary level of evidence from cancer studies in humans also considers molecular studies of tissues from humans in addition to epidemiological studies and is provided in Section 5.

The NTP used the body of knowledge published in the IARC (2012, 1997) monographs on KSHV as the resource for studies conducted up to and including 2008. The key human studies identified in these monographs, together with new human studies identified from 2008 to 2015, are considered in the RoC monograph and used to evaluate the scientific evidence for specific cancer endpoints independently of IARC's conclusions. Where available, IARC data tables of the effect estimates have informed the cancer hazard assessment.

3.2 Selection of the literature

A literature search of major databases, citations, and other authoritative sources for literature from 2008 to August 2015 was conducted. The literature search strategy (including the databases and search terms, and other sources for identifying literature), procedures for selecting the

literature (systematic screening procedures and inclusion/exclusion criteria) and the literature search is described in Appendix A. In general, single case reports or case series are excluded from further review, although summaries of multiple case reports or case-series studies (e.g., the summary of 75 cases of primary effusion lymphoma identified in IARC [2012]) are noted and may be considered in the overall evaluation. In addition, studies for which risk estimates were not reported or could not be calculated were generally excluded. Cross-sectional or prospective cohort studies, and case-control studies, which may range from broadly defined, non-matched hospital or population case-comparison designs to formal age-, sex, and race-matched case-control designs, are included in the review.

3.3 Cancer evaluation: Kaposi sarcoma

3.3.1 Background information

Studies of Kaposi sarcoma incidence or prevalence have been conducted among four main epidemiological types. Although Kaposi sarcoma is histologically indistinguishable in these four types, the age at diagnosis, clinical features, and progression of the disease vary considerably by type.

- "Epidemic" or "HIV-1/AIDS-related" Kaposi sarcoma occurs among HIV-1-positive, typically immunocompromised individuals;
- "Iatrogenic," "transplant," or "immunosuppressive" Kaposi sarcoma occurs primarily among organ recipients, mostly renal and liver transplant patients, generally in the presence of immune suppression, and also among other patients with immune suppression (e.g., hemodialysis patients Jalilvand *et al.* [2011]);
- "Classic," "sporadic," or "indolent" Kaposi sarcoma is rare, less aggressive in its course than HIV-1-related Kaposi sarcoma (Hiatt *et al.* 2008), predominantly affects the lower extremities, and occurs mostly among older males 50 to 70 years of age in specific populations, e.g., in some Mediterranean countries such as southern Italy, or among East Europeans of Jewish descent with higher (e.g., 10% to 20%) KSHV prevalence rates (IARC 2012, 1997);
- "Endemic" Kaposi sarcoma also occurs mostly among men, but among younger groups than classic Kaposi sarcoma, including children, in regions of sub-Saharan Africa with relatively high KSHV seroprevalence rates, e.g., of 25% to over 50% (IARC 2012). The course of Kaposi sarcoma in children tends to be much more aggressive than that observed in adults (Senba *et al.* 2011). Since the advent of the HIV-1 epidemic, the ratio of Kaposi sarcoma in men to women has decreased from 7:1 to 2:1 in South Africa (Sitas and Newton 2001), despite little evidence that the male to female ratio of KSHV infection differs or has changed over the past two decades (Dedicoat and Newton 2003).

Kaposi sarcoma appears to be rare in immunocompetent individuals among populations with the epidemic type of the cancer, and the expression and course of the cancer may also be affected by compromised immune status in populations with classic or endemic types of Kaposi sarcoma. In the United States, Kaposi sarcoma occurred in less than 1 per 100,000 individuals prior to the HIV-1/AIDS epidemic starting in the early 1980s, peaking at approximately 5 to 6 per 100,000

in the U.S. population by the early1990s, prior to the advent of highly active antiretroviral therapy (HAART) (NCI 2014).

Approximately 12,000 patients were diagnosed with Kaposi sarcoma between 1975 and 2005, according to SEER registry statistics (Armstrong *et al.* 2013). In Europe, the Surveillance of Rare Cancers in Europe (RARECARE) project (Stiller *et al.* 2014) reported 2,667 incident cases of Kaposi sarcoma between 1995 and 2002, an age-standardized rate of 0.3/100,000 across the entire European Union. In the subgroup of southern European countries, where predominantly classic cases have been identified, the average incidence was 0.8/100,000; the incidence rates for other areas in Europe ranged from 0.02 to 0.25. The Kaposi sarcoma incidence rate among U.S. men (approximately 3.3/100,000) (Rouhani *et al.* 2008) is higher than that among European men (0.5/10,000) but comparable among women in the United States and Europe (approximately 0.1/100,000; see Stiller *et al*. 2014).

3.3.2 Descriptive epidemiological studies

A number of case series, descriptive epidemiological, or surveillance studies of Kaposi sarcoma from multiple countries, conducted from 2008 to 2015, were identified (Koski *et al.* 2014 [Tanzania], Mousavi *et al.* 2014 [immigrants to Sweden], Stiller *et al.* 2014 [European cancer surveillance registry], Jalilvand *et al.* 2012 [Iran], Tiussi *et al.* 2012 [Brazil], Jalilvand *et al.* 2011 [Iran], Ogoina *et al.* 2011 [Nigeria], Senba *et al.* 2011 [Kenya], Kanno *et al.* 2010 [Japan], Magri *et al.* 2009 [Brazil], Hiatt *et al.* 2008 [United States], Lanternier *et al.* 2008, Mwakigonja *et al.* 2008 [Tanzania], Nsubuga *et al.* 2008 [Uganda]). As supported by studies reviewed by IARC (2012), descriptive studies that report KSHV seroprevalence (typically LANA) or KSHV-DNA among cases of Kaposi sarcoma indicate ranges from approximately 88% to 98%, irrespective of whether these are HIV-1 positive, or HIV-1 negative, endemic and classic Kaposi sarcoma cases (IARC 2012, Ogoina *et al.* 2011, Kanno *et al.* 2010, Magri *et al.* 2009, Hiatt *et al.* 2008, Lanternier *et al.* 2008, Mwakigonja *et al.* 2008, Nsubuga *et al.* 2008). However, relatively few case studies of KSHV seroprevalence have been conducted among HIV-1-negative populations in regions with endemic or classic Kaposi sarcoma.

3.3.3 Cohort and nested case-control studies

Table 3-1 summarizes findings from 13 cohort or nested case-control studies conducted prior to 2009 and reviewed by IARC (2012) and from two prospective cohort and one nested case-control studies identified since the IARC review (Nawar *et al.* 2008) (Labo *et al.* 2015, Wakeman *et al.* 2015) that reported risk estimates for KSHV seroprevalence or DNA and Kaposi sarcoma. These studies were conducted among HIV-1-positive populations (13 studies) and organ transplant recipients (3 studies). All the cohort studies were of HIV-1-positive or transplant recipient populations and were conducted among U.S., Canadian, or European-based populations, with the exception of two cohort studies of southern Mediterranean populations who have a high prevalence of classic Kaposi sarcoma (Rezza *et al.* 1999, Parravicini *et al.* 1997) and one nested case-control study in Uganda, a country with a high prevalence of endemic Kaposi sarcoma (Labo *et al.* 2015).

Among the studies reviewed by IARC (2012), all of the relative risks were statistically significant, ranging from 2.5 to 75; elevated risks were observed in both HIV-1-positive and organ transplant populations, which provide support for KSHV causing Kaposi sarcoma

independently of HIV-1 infection. Moreover, Newton *et al.* (2006) found that risk of Kaposi sarcoma increased with increasing anti-KSHV titers. The two prospective cohort studies conducted in the United States (Labo *et al.* 2015, Nawar *et al.* 2008), and the small nested case-control study conducted in Uganda (Wakeman *et al.* 2015), published after the IARC review, are consistent with these findings and reported risk estimates ranging between 1.8 to 9.5. One cohort study, the multi-city U.S. cohort study of 1,354 men who have sex with men (Nawar *et al.* 2008), found that acquiring HIV-1 infection prior to KSHV infection was associated with an increase in Kaposi sarcoma risk vs. co-prevalent infection (hazard ratio [HR] = 1.81, 95% CI = 1.32 to 2.48), whereas acquiring KSHV infection prior to HIV-1 did not increase risk in comparison with co-prevalent infection (HR = 1.05, 95% CI = 0.59 to 1.87). The nested case-control study calculated risk for both lytic and latent antigens; relative risks were somewhat higher for antibody titers to K8.1 lytic antigen (RR = 4.14, 95% CI = 3.34 to 4.94) than latent antigen (LANA) (RR = 2.2, 95% CI = 1.16 to 3.25) (Wakeman *et al.* 2015). It is not possible to draw conclusions as to whether risk varies among different countries due to the limited number of studies (and few studies reported in detail on the racial or ethnic composition of their cohorts).

IARC also reported on seven cohort studies of HIV-1-positive populations or organ transplant recipients (Garcia-Astudillo and Leyva-Cobian 2006, Marcelin *et al.* 2004, Frances *et al.* 1999. 2000, Chatlynne *et al.* 1998, Melbye *et al.* 1998, Regamey *et al.* 1998) that did not calculate risk estimates, primarily due to small numbers; three of these publications reported that KSHV infection was significantly higher in Kaposi sarcoma cases than controls with *P*-values ranging from < 0.001 (Garcia-Astudillo and Leyva-Cobian 2006, Frances *et al.* 2000) to < 0.0001 (Frances *et al.* 1999).

Table 3-1. Summary of cohort and nested case-control studies of risk of Kaposi sarcoma among KSHV-seropositive vs. KSHV-negative participants

Reference	Detection Methods	Cohort (comparison group) and cases and controls	RR
HIV-1–positive/AIDS populations			
Cohort studies			
Grulich *et al.* 1999 (USA)	Latent and lytic antigens	AIDS+ MSM	4.4*
O'Brien *et al.* 1999 (USA)	Latent nuclear antigen	HIV-1+ MSM	3.6*
Jacobson *et al.* 2000 (USA)[a]	Lytic antigens	HIV-1+ MSM (seroconversion to KSHV after HIV-1 infection vs. before HIV-1 infection)	2.6*
Rezza *et al.* 1999 (Italy)	Latent nuclear antigen	HIV-1+ (various exposures)	29.6*
Nawar *et al.* 2008 (USA)	Lytic antigens	KSHV and HIV-1 concurrent infections; MSM	1.8*
		KSHV infection prior to HIV-1 infection; MSM	1.1 (NS)
Labo *et al.* 2015	Latent and lytic	HIV-1+ men and women	9.5*

Reference	Detection Methods	Cohort (comparison group) and cases and controls	RR
(USA, Puerto Rico)	antigens		
Range			**1.8–29.6**
Nested case-control studies			
Moore *et al.* 1996 Parry and Moore 1997 (USA)	PCR	KS cases: AIDS+ MSM Controls: HIV-1+ MSM	11.6*
Gao *et al.* 1996 (USA)	Latent nuclear antigen	KS cases: AIDS+ MSM Controls: HIV-1+ MSM	[16.3]*
Engels *et al.* 2003b (USA)	Lytic antigens PBMC DNA	KS cases: HIV-1+ MSM Controls: HIV-1+ MSM	11.7*
Quinlivan *et al.* 2001[b] (Switzerland)	Latent nuclear antigen	KS cases; HIV-1+ M or F Controls HIV-1+ M or F	3.0
Newton *et al.* 2006 (UK)	Latent and lytic antigens	KS cases: HIV-1+ M or F Controls: HIV-1+ M or F Titer (latent antigens) - Min Med Max P_{trend}	4.4* 3.5* 4.1* 8.7* < 0.001
Whitby *et al.* 1995 (UK)	PCR	KS cases: AIDS+ M or F Controls: HIV-1+ M or F	12.7*
Wakeman *et al.* 2015 (Uganda)	Latent and lytic antigens	KS cases: HIV-1+ M or F Controls: HIV-1+ M or F K8.1 LANA	 4.1* 2.2*
Range			**2.2– 16.3**
Organ transplant recipients			
Cohort study			
Cattani *et al.* 2001 (Italy)	Latent and lytic antigens	Transplant recipients tested for KSHV before transplant, follow-up 10 years	34.4*
Nested case-control studies			
Parravicini *et al.* 1997 (Italy)	Latent and lytic antigens	Transplant recipients tested for KSHV before transplant	75.0*
Rabkin *et al.* 1999 (Canada)	Latent antigens, whole virus assay	Transplant recipients tested for KSHV before transplant	2.5 (NS)

Source: IARC 2012, Table 2.2, except for Nawar *et al.* (2008), Lado *et al.* (2015), Wakeman *et al.* (2015).

Studies to 2008 inclusive in which risk estimates were reported or calculated by IARC (2012): IARC also described seven other nested case-control or cohort studies that reported number of Kaposi sarcoma occurring among KSHV seroprevalance among cases and controls but did not report risk estimates.
[ ] RR or OR calculated by NTP.
AIDS = acquired immunodeficiency syndrome; HIV-1 = human immunodeficiency virus type 1; KS = Kaposi sarcoma; KSHV = Kaposi sarcoma herpesvirus; LANA = latent antinuclear antigen; MSM = men who have sex with men; PBMC = peripheral blood mononuclear cells; PCR = polymerase chain reaction; RR = relative risk; NS = lower 95% confidence interval (CI) < 1.0.
*Lower 95% confidence interval (CI) > 1.0.
[a]KSHV- and HIV-1-positive MSM with detectable KSHV viral load 1 year prior to diagnosis vs. KSHV-and HIV-1–positive MSM with no detectable viral load.
[b]Note that membrane antigen RR = 1.7 (95% CI = 0.5 to 6.7) and lytic antigen RR = 0.9 (95% CI = 0.2 to 6.8).

3.3.4 **Case-control studies**

IARC (2012) reviewed 80 case-control studies of Kaposi sarcoma; 68 studies included AIDS cases, 7 included HIV-1-positive or "non-AIDS" cases, and 10 studies included HIV-1-negative cases (plus 4 studies in which HIV-1 status was unclear or not specified); 27 included classic cases, 14 included endemic cases, and 9 included transplant cases (some studies had more than one group). Table 3-2 summarizes data from 39 case-control studies in which risk estimates for KSHV were reported. One new case-control study, of classic cases in a Sicilian population (Amodio *et al.* 2011b, based on an earlier study by [Goedert *et al.* 2002]), was identified after the IARC (2012) review. (Further studies among KSHV-positive patients in this population were conducted by Pelser *et al.* [2010] and Anderson *et al.* [2008a]) and are discussed under cofactors in Section 3.2.5, below). Cases comprising more than one category of Kaposi sarcoma were sometimes combined by study authors for analysis. Comparison groups may be HIV-1-positive or AIDS patients, or blood donors, oncology patients, or patients with other skin diseases or other non-cancer conditions, or combinations of such comparison groups from which a single risk estimate was calculated by authors or IARC working groups.

Among the studies of HIV-1-positive or AIDS-patient populations, odds ratios (ORs), almost all statistically significant, ranged from 1.0 to 1,683 (Table 3-2). The two studies of HIV-1-negative cases and controls, both conducted in African populations with, as noted above, high endemic KSHV prevalence rates, reported ORs of 1.5 to 12.0, depending on the level of KSHV titer (Newton *et al.* 2003b, Sitas *et al.* 1999). The studies designated as grouped populations consist of combinations of HIV-1-positive, HIV-1-negative or undetermined, endemic, classic, or transplant-related Kaposi sarcoma patients in comparison with similar Kaposi sarcoma-negative groups, as noted in the table, and for which only single risk estimates were reported, ORs ranging from 3.8 to 924 were observed. ORs could also be calculated for some constituent groups within these studies, although in some instances, only a single combined control group was used and/or no formal matching of controls was conducted, so that such risk estimates must be interpreted with caution. Nevertheless, for both classic and endemic Kaposi sarcoma, KSHV was observed in > 80% to 100% of cases (Table 3-2). Among the few studies that included transplant patients with Kaposi sarcoma, KSHV was observed in approximately 66% to 100% of cases.

Three studies reviewed by IARC (2012) reported on dose-response relationships between titers of KSHV exposure and risk for Kaposi sarcoma. Sitas *et al.* (1999) and Newton *et al.* (2003b) observed statistically significant trends ($P < 0.001$) among both HIV-1-positive and HIV-1-negative groups with a higher prevalence of endemic Kaposi sarcoma in Africa; Albrecht *et al.* (2004) reported a similar trend among HIV-1-positive AIDS patients in Germany.

Table 3-2. Summary of case-control studies of Kaposi sarcoma and Kaposi sarcoma herpesvirus (KSHV)

Reference	Detection method	OR[a]	Comments
HIV-positive/AIDS populations			
Chang *et al.* 1994 (US)	PCR	68.8	
Whitby *et al.* 1995 (UK)	PCR	29.0	
Monini *et al.* 1996b, Monini *et al.* 1996a (Italy)	PCR	1.7[a]	
Parry and Moore 1997 (US)	PCR	7.3 MSM 21.8 hemophiliacs	
Humphrey *et al.* 1996 (US)	PCR	2.3	
Decker *et al.* 1996 (US)	PCR	1.1[b]	
Gao *et al.* 1996 (US)	LANA, WB	18.9	
Lefrere *et al.* 1996 (France)	PCR	285.0	
Miller *et al.* 1996 (US)	WB, IFA	13.4 (WB)/12.2 (IFA)	
Noel 1995 (Belgium)	PCR	42.2	
Howard *et al.* 1997 (UK)	PCR	10.1	
Sitas *et al.* 1999 (South Africa)	LANA, IFA	10.8–1683	Sig. ↑ inc. KSHV titer
Min and Katzenstein 1999 (US)	PCR	16.3	
Boivin *et al.* 1999 (Canada)	PCR	6.7	
Greenblatt *et al.* 2001 (US)	PCR	148.0	
Keller *et al.* 2001 (Brazil)	PCR	7.4–17.0	
Cannon *et al.* 2003 (US)	PCR	8.6	
Newton *et al.* 2003b (Uganda)	LANA, IFA	4.1 – 10.8	Sig. ↑ inc. KSHV titer
Alagiozoglou *et al.* 2003 (South Africa)	LANA, IFA, PCR,	15.4 – 25.1	
Albrecht *et al.* 2004 (Germany)	IFA	1.04 – 23.2	Sig. ↑ inc. KSHV titer
Szalai *et al.* 2005 (Hungary)	LANA, IFA, ELISA	2.3[b] –23.1	
van der Kuyl *et al.* 2005 (Netherlands)	PCR	6.3	
Martro *et al.* 2007 (Spain)	IFA	4.8	
Widmer *et al.* 2006 (Switzerland)	PCR	1.2 [b]	
Laney *et al.* 2007 (US)	PCR	1.6[b] – 3.4	
Zago *et al.* 2000 (Brazil)	PCR	23.4–104.8	
Range		**1.0 –1683**	
HIV-negative populations			
(Sitas et al. 1999 (South Africa)	LANA, IFA	1.5–12.0	Endemic – sig. trend with ↑ KSHV titer
Newton *et al.* 2003b, Newton et al. 2003a (Uganda)	LANA, IFA	2.3–7.1	Endemic – sig. trend with ↑ KSHV titer
Cattani *et al.* 2001	IFA	34.4	Transplant patients
Amodio *et al.* 2011 (Sicily)	LANA, lytic (K8.1)	5.13[b]	Classic
Grouped populations			
			Case groups
Moore and Chang 1995 (US)	PCR	400 (19–17,300)	Total (HIV-1+ men, HIV-1–neg. MSM, Classic
		[210 (12–3,712]	HIV-1 +
		[∞]	HIV-1 neg. MSM

Reference	Detection method	OR[a]	Comments
		[∞]	Classic
Chang *et al.* 1996 (US)	PCR	49.4 (9.0–328.0)	HIV-1+, endemic
		[66 (5.1–857.7)]	HIV-1 +
		[36 (5.3–253.6)]	Endemic
Luppi *et al.* 1996 (Italy)	PCR	41.6 (7.0–327.1)	HIV-1+, classic
		[∞]	HIV-1+
		[∞]	Classic
Albini *et al.* 1996 (Italy)	PCR	924.3 (125–10,194)	Total: HIV-1+, endemic, classic, transplant
		[∞]	AIDS vs. HIV-1+
		[∞]	Endemic vs. HIV+
		[∞]	Classic vs. HIV-1+
		[198 (10.8–3,617)]	Transplant vs. HIV- 1 neg.
Herman *et al.* 1998 (US)	PCR	336 (14.9–47,561)	HIV-1+, classic (vs. HIV-1+ and HIV-1 neg. controls combined)
Cattani *et al.* 1998 (Italy)	PCR	7.4 (2.4–23.8)	Classic, transplant
		[5.9 (1.8–19.2)]	Classic
		[20.0 (1.7–238.6)]	Transplant
Kazakov *et al.* 2002 (Switzerland)	PCR	543 (52–14,041)	Total: HIV-1+, transplant, classic
		[600 (34.2–10,541.2)]	HIV-1+
		[150 (11.1–2,025)]	Transplant
		1,275 (75.9–21,420)]	Classic
Lager *et al.* 2003 (South Africa)	PCR	572 (26–79,693)	HIV-1 status unknown
Massambu *et al.* 2003 (Tanzania)	PCR	3.8 (0.3–60.9)	HIV-1+, endemic

Source: IARC 2012, Table 2.1, except for *Amodio et al.* (2011).
[ ] = OR calculated by NTP.
CI = 95% confidence interval; ELISA = enzyme-linked immunosorbent assay; HIV-1 = human immunodeficiency virus type 1; IFA = immunofluorescence assay; KS = Kaposi sarcoma; KSHV = Kaposi sarcoma herpesvirus; LANA = latent antinuclear antigen; MSM = men who have sex with men; OR = odds ratio; PCR = polymerase chain reaction; WB = Western blot assay.
[a]Lower 95% CI = 0.0.
[b]Lower 95% CI < 1.0.

3.3.5 Cofactors for Kaposi sarcoma

KSHV has been identified in virtually all Kaposi sarcoma lesions, but KSHV infection alone appears to be insufficient to cause Kaposi sarcoma (IARC 2012, Dedicoat and Newton 2003). The principal cofactor identified for Kaposi sarcoma in the presence of KSHV infection is HIV-1 infection via immune suppression, both among populations in the United States and Europe ("epidemic" Kaposi sarcoma) and those with an increased prevalence of "classic" or "endemic" Kaposi sarcoma (Tables 3-1 and 3-2). Prospective studies, largely among men who have sex with

men and infrequently among HIV-1-positive intravenous drug users or women, suggest that HIV-1 infection significantly increases the risk of Kaposi sarcoma development in the presence of KSHV in a range of geographical populations, particularly among AIDS patients. There is additional evidence that infection with HIV-1 prior to infection with KSHV may increase the risk of Kaposi sarcoma by up to 2.6-fold compared with those infected with KSHV before HIV-1, and increasing duration of HIV-1 infection and decreasing CD4 counts are associated with a more rapid course of development of the cancer (Nawar *et al.* 2008, Jacobson *et al.* 2000, Renwick *et al.* 1998). (See also accompanying HIV-1 monograph.)

Immune suppression among organ recipients, primarily renal and liver transplant patients, has also been identified as a major cofactor among patients who were KSHV positive prior to transplantation or who acquired KSHV during organ transplantation (see Table 3-1). In a review of 38 case series studies by Hosseini-Moghaddam *et al.* (2012), approximately 13% of previously KSHV-seropositive patients reportedly developed Kaposi sarcoma after renal transplantation; approximately 5% of the patients developed the cancer as a result of primary KSHV (donor KSHV-positive, recipient KSHV-negative) infection.

Few studies have investigated the role of recreational drugs with immunomodulatory properties. In one prospective study, prior use of marijuana, amyl nitrate "poppers," cocaine, or amphetamines was weakly associated with Kaposi sarcoma risk among co-KSHV and HIV-1-infected men who have sex with men, although a clear dose-response relationship was lacking (Chao *et al.* 2009).

Never smoking, diabetes, and the use of oral corticosteroids have all been identified as potential risk factors for classic Kaposi sarcoma among KSHV-positive individuals in the absence of HIV-1 infection or iatrogenic or other causes of immune suppression in a well-conducted case-control study of a population in Sicily (Amodio *et al.* 2011a, Anderson *et al.* 2008a, Goedert *et al.* 2002). While this study provides limited evidence that these factors increase the risk of Kaposi sarcoma among KSHV-positive individuals, there are few other data in other populations that have examined these risk factors and no firm conclusions can be drawn about their role, or that of other risk factors.

With respect to co-infection with other viruses, the role of Epstein-Barr virus is uncertain. Although it appears that the prevalence of Epstein-Barr virus infection is similar in Kaposi sarcoma and non-Kaposi sarcoma patients, it does not appear to play a direct role in the development of the sarcoma, at least according one well-conducted case-control study among classic cases of Kaposi sarcoma (Table 3.3) (Pelser *et al.* 2010). Other viruses, such as herpes simplex type 2, human cytomegalovirus, human herpesvirus-6 and 7, and human papillomavirus have also been implicated in the activating lytic replication of KSHV and thus in Kaposi sarcoma pathogenesis (see Section 4 [mechanistic evidence] and review by, e.g., Purushothaman *et al.* 2015). No cohort or case-control studies specifically addressing the risk of Kaposi sarcoma in the presence of these viruses as cofactors among KSHV-positive populations have been identified to date, however.

Among populations with high rates of endemic Kaposi sarcoma, particularly prior to the global HIV-1 epidemic, other potential cofactors, including malaria (see e.g., Nalwoga *et al.* 2015), other parasitic infections, soil types causing immunosuppression, or oncogenic plants have been suggested as increasing the risk of KSHV infection and/or reactivation (IARC 2012, Dedicoat

and Newton 2003). Tuberculosis has been reported as exacerbating the severity of endemic cases (e.g., in Tanzania, Koski *et al.* 2014) but it is not clear if it is a risk factor for the initial development of the sarcoma. Finally, prior to the HIV-1 epidemic, Kaposi sarcoma was more prevalent among men than women in, e.g., populations with endemic cases, despite similar KSHV seroprevalence in both sexes, suggesting that cofactors as not yet clearly established may play a role in the development of the cancer (Dedicoat and Newton 2003).

Table 3-3. Case-control study of co-factors for the development of classic Kaposi sarcoma among a KSHV-positive population in Sicily

Reference	Cofactor	# KS Cases//KSHV+controls	OR (95% CI) for KS
Anderson *et al.* 2008a	Diabetes	Cases (45/142) Controls (15/123)	4.02 (1.73–9.37)
	Never smoking	Cases (23/142) Controls (12/123)	2.66 (1.06–6.64)
	Oral corticosteroid use	Cases (53/142) Controls (26/123)	2.25 (1.16–4.38)
Pelser *et al.* 2010	EBV	Cases (low EBNA-1) (43/119) Controls (low EBNA-1) (34/105)	0.97 (0.52–1.82)[a]
		Cases (high EBV VCA) (45/119) Controls (high EBV VCA) (45/105)	0.87 (0.45–1.69)[a]

CI = confidence interval; EBNA = Epstein-Barr virus nuclear antigen; EBV = Epstein-Barr virus; EBV VCA = EBV viral capsid antigen; KS = Kaposi sarcoma; KSHV = Kaposi sarcoma herpesvirus; OR = odds ratio.
[a]Adjusted for age, sex, smoking, diabetes, and oral corticosteroid use.

3.4 Integration across studies of Kaposi sarcoma

At least 25 cohort or nested case-control studies and 81 case-control studies conducted in populations differing by race or ethnicity and in various geographical locations have demonstrated that KSHV infection causes Kaposi sarcoma. KSHV has been identified in over 80% of cases by serology and in virtually all Kaposi sarcoma tumors, and is considered to be the diagnostic prerequisite for a diagnosis of Kaposi sarcoma. The histopathology of all four epidemiological types of Kaposi sarcoma (epidemic, iatrogenic, classic, and endemic) is identical. Case-control studies conducted among populations exhibiting the different types showed statistically significant increased risks for Kaposi sarcoma generally exceeding 10 and up to 300 or more (using serology and/or DNA in the tumor to identify KSHV infection). Most cohort studies report risks from approximately 2 to 16 although a few studies, primarily among transplant patients, reported risks up to 75. In addition, statistically significant dose-response relationships for measures of KSHV infection and Kaposi sarcoma risk among both HIV-1-positive and/or HIV-1-negative populations have been reported (Newton *et al.* 2006, Albrecht *et al.* 2004, Newton *et al.* 2003b, Newton *et al.* 2003a, Sitas *et al.* 1999).

The heterogeneity in risk observed particularly in case-control studies may be at least partly due to methodological limitations. A majority of the case-control or case-comparison studies have small numbers of cases, and a lack of formal methods of selection or matching of controls or

inadequate reporting. In addition, temporality (evidence that KSHV infection preceded the development of Kaposi sarcoma) cannot generally be clearly established in these studies. The majority of cohort or nested case-control studies have somewhat limited statistical power, and the sensitivity and specificity of KSHV detection methods in earlier studies is generally more limited than in later studies. However, several prospective cohort studies and nested case-control studies (e.g., Gao *et al.* 1996, Moore *et al.* 1996, Whitby *et al.* 1995, Nawar *et al.* 2008, Lado *et al.* 2015, Wakeman *et al.* 2015) provide evidence of temporality. Overall, confounding by other factors can be ruled out with confidence because of the findings of highly statistically significant increased risks across studies with different designs and conducted in different population, and evidence of a dose-response relationship. .

With respect to cofactors, immune suppression – primarily resulting from HIV-1 infection or iatrogenically in, for example, transplant cases – is the principal cofactor; HIV-1 infection prior to KSHV infection may increase the risk of epidemic Kaposi sarcoma between 50% and 100% compared with HIV-1 infection during or after KSHV infection. While a recent well-conducted case-control study in Sicily (Anderson *et al.* 2008a) provides limited evidence that diabetes, corticosteroid use, and never smoking increase the risk of Kaposi sarcoma among KSHV-positive individuals, there are few other data in other populations that have examined these risk factors and no firm conclusions can be drawn. Clear evidence for a modulatory role of other specific cofactors, such as genetic factors, malaria, hypoxic stress, or environmental and dietary factors, particularly among endemic and classic cases, is lacking. Co-infection with other viruses, such as Epstein-Barr virus, cannot be completely ruled out as cofactors in the development of Kaposi sarcoma in KSHV-infected individuals; although one case-control study (Pelser *et al.* 2010) suggests no association with co-infection with Epstein-Barr virus, there are few other data to evaluate the role of this or other viruses.

3.5 Lymphohematopoietic cancers

3.5.1 Primary effusion lymphoma

Background information

Primary effusion lymphoma, a monoclonal lymphoproliferative disorder also called body cavity-based B-cell lymphoma, is a rare type of B-cell non-Hodgkin lymphoma that presents primarily as pleural, peritoneal, or pericardial lymphomatous effusions and makes up approximately 2% to 4% of HIV-1-related non-Hodgkin lymphomas (Sullivan *et al.* 2008, Simonelli *et al.* 2003). In addition to lymphomatous effusions, diagnosis of primary effusion lymphoma includes KSHV infection; large-cell immunoblastic or anaplastic large-cell lymphomas; expression of CD45, clonal immunoglobulin gene rearrangements; and lack of c-*myc*, *bcl-2*, *ras*, and p53 gene alterations (Abiashi *et al.* 2002, Nador *et al.* 1996). Primary effusion lymphomas are thought to arise from post-germinal center B cells because they have mutations in their immunoglobin genes. Because a large number of cases of primary effusion lymphoma identified in association with KSHV have distinctive morphological, immunophenotypical, and molecular genetic characteristics, they have been grouped as a single distinct pathological entity (IARC 2012, 1997). Such entities should be distinguished from other large body cavity-based B-cell lymphomas or lymphoproliferative disorders observed in KSHV-negative patients and may also have a different prognosis (see e.g., Xiao *et al.* 2013, Chen *et al.* 2009c, Kishimoto *et al.* 2009).

Epidemiological studies

Epidemiological evidence of an association between primary effusion lymphoma and KSHV relies primarily on a large number of case reports, case series and case-comparison studies rather than formal case-control or cohort studies, due to the rarity of the condition, and the fact that the presence of KSHV is a diagnostic criterion for the specific pathological entity that constitutes primary effusion lymphoma. A majority of reported KSHV-positive cases of primary effusion lymphoma are also HIV-1 positive and approximately half of the cases have Kaposi sarcoma. IARC (2012, Table 2.5) reviewed 97 individual cases reported in the literature to be primary effusion lymphoma; 44 cases were reported as both KSHV and HIV-1 positive, 31 cases were KSHV-positive but HIV-1 negative, and three case were uncertain as to KSHV (N=2)) or HIV-1 status (N=1). The remaining 19 cases were KSHV negative and HIV-1 negative, and associated with genetic or other pre-existing conditions and/or observed among patients 67 years of age or older; however, IARC noted that the KSHV-negative cases probably represented another form of non-Hodgkin lymphoma because they do not met the diagnostic criteria of primary effusion lymphoma. Among 47 KSHV-positive cases of primary effusion lymphoma with known HIV-1 status, approximately half of the cases in both HIV-1-positive (14/30) and HIV-1-negative patients (8/17) also had Kaposi sarcoma; some also presented with multicentric Castleman disease (reviewed below). The large majority of primary effusion lymphoma cases occur among men, irrespective of HIV-1 or Kaposi sarcoma status, presumably reflecting a higher prevalence of KSHV infection among men.

In other studies, including several case-series studies (Oksenhendler *et al.* 2002, Aoki *et al.* 2001, Judde *et al.* 2000), and a case-comparison study (Parravicini *et al.* 2000), a total of 12 cases of KSHV-positive primary effusion lymphoma were reported, 9 of which (75%) were from HIV-1-positive patients. In addition, a larger case-comparison study in which a range of lymphoproliferative disorders were investigated for viral KSHV DNA among 191 patients (Asou *et al.* 2000), 21 patients were identified with primary effusion lymphoma, of whom all were KSHV positive, in contrast to 1 of 170 patients with AIDS-related non-Hodgkin lymphoma or with non-AIDS-related lymphomas (Castleman disease, reactive lymphoadenopathy, mucosa-associated lymphoid tissue lymphoma, and non-AIDS non-Hodgkin lymphoma). (In this study, the HIV-1 status of primary effusion lymphoma cases is unclear.) No cohort or case-control studies have been subsequently identified (see review by Zhang *et al.* 2010).

Some cases of primary effusion lymphoma have been reported in HIV-1-negative but KSHV-positive populations that are known to have a high risk of endemic or classic epidemiological Kaposi sarcoma, leading to the proposition that a “classic” form of primary effusion lymphoma can be identified (Yiakoumis *et al.* 2010, Ascoli *et al.* 2002). Primary effusion lymphoma among HIV-1-positive patients exhibits a rapid progression with short survival times, whereas in HIV-1-negative and immunocompetent patients, progression appears to be much slower (IARC 1997).

Cofactors for primary effusion lymphoma

Immune suppression resulting from HIV-1 infection is a cofactor for primary effusion lymphoma development among KSHV-positive cases. Due in part to its rarity, there are few data on primary effusion lymphoma cases among other immunosuppressed groups although one study reviewed by IARC (2012), Kapelushnik *et al.* (2001), reported a case in a KSHV-positive renal transplant patient, but no other case studies were identified for the current review. A substantial proportion

of KSHV-related primary effusion lymphoma cases also have evidence of Epstein-Barr virus infection (IARC 2012, 1997). The role of Epstein-Barr virus has also been postulated as a cofactor for the expression of primary effusion lymphoma with KSHV (Gloghini *et al.* 2013, Bryant-Greenwood *et al.* 2003, Boulanger *et al.* 2005), but several earlier studies reported that cell lines derived from primary effusion lymphoma lymphomas were latently infected with KSHV, not Epstein-Barr virus (IARC 1997).

3.5.2 Multicentric Castleman disease

Background and epidemiological evidence

Multicentric Castleman disease is a rare polyclonal lymphoproliferative B-cell lymphoma that can develop into plasmablastic lymphomas, and occurs in individuals with and without HIV-1 infection. Multicentric Castleman disease is a subset (20%) (Talat and Schulte 2011) of Castleman disease, first identified by Castleman (1956) and characterized by inflammatory symptoms, plasmacytic lymphadenopathy, splenomegaly, and cytopenia with polyclonal hyperimmunoglobulinemia and high serum levels of IL-6 (Oksenhendler *et al.* 2013, Fukumoto *et al.* 2011). In contrast to primary effusion lymphoma, in which cells predominantly express latent KSHV antigens, KSHV-associated multicentric Castleman disease cells appear to express lytic KSHV antigens, including a virally encoded IL-6 (Burbelo *et al.* 2010). Multicentric Castleman disease has an aggressive course and poor prognosis (IARC 1997).

KSHV has been identified in some but not all cases of multicentric Castleman disease, primarily in mantle-cell large immunoblastic B cells (Fukumoto *et al.* 2011, Parravicini *et al.* 2000, Ascoli *et al.* 1999, Dupin *et al.* 1999). Evidence of a positive association between multicentric Castleman disease and KSHV has been reported in several early case reports and case series reviewed by IARC (2012) (Barozzi *et al.* 1996, Corbellino *et al.* 1996, Gessain *et al.* 1996). Four case-comparison studies were reviewed by IARC (2012), including a total of 63 cases of multicentric Castleman disease and 213 controls, and which report a higher frequency of KSHV, primarily in HIV-1-positive and infrequently in HIV-1-negative groups, in comparison with controls (Table 3-4).

Table 3-4. Summary of case series and case-control/comparison studies of KSHV and multicentric Castleman disease

Reference	KSHV detection method	HIV-1 status of MCD cases/controls	KSHV-exposed cases/total cases or OR (95% CI)[a] exposed cases vs. exposed controls/
Case series			
Corbellino *et al.* 1996	PCR	HIV-1 negative	4/6
Barozzi *et al.* 1996	PCR	HIV-1 negative	1/16
Gessain *et al.* 1996	PCR	HIV-1 positive HIV-1 negative	3/4 1/6
Case-comparison studies			
Belec *et al.* 1999a France	IFA antibodies	?/healthy controls	[∞ (6/8 vs. 0/15)]

Reference	KSHV detection method	HIV-1 status of MCD cases/controls	KSHV-exposed cases/total cases or OR (95% CI)[a] exposed cases vs. exposed controls/
Parravicini et al. 1997 Italy	PCR	All HIV-1 negative	[∞ (6/14 vs. 0/25)]
Soulier *et al.* 1995 France	PCR	HIV-1 positive HIV-1 negative Total	14/14 vs. 1/34 7/17 [69.3. (8.3–581.5)] (21/31 vs. 1/34)
Asou *et al.* 2000 Japan	PCR	Unclear	[∞ (1/10 vs. 0/139)]

Source: IARC 2012, Table 2.6 and Table 2.7.
CI = confidence interval; IFA = immunofluorescence assay; HIV-1 = human immunodeficiency virus type 1; OR = odds ratio; PCR = polymerase chain reaction.
[a][ ] ORs calculated by NTP.

Cofactors for multicentric Castleman disease

HIV-1 co-infection substantially increases the risk of multicentric Castleman disease among KSHV-positive patients (Table 3-5; also see Section 4), and KSHV appears to be found in almost all cases of HIV-1-related multicentric Castleman disease and approximately half of HIV-1-negative cases (Oksenhendler *et al.* 2013, Oksenhendler *et al.* 2002, Parravicini *et al.* 1997). The role of KSHV in multicentric Castleman disease among HIV-1-negative individuals is not clear, however (van Rhee *et al.* 2010).

In a U.K. study of 41 HIV-1-positive patients with KSHV-related Kaposi sarcoma or multicentric Castleman disease, Westrop *et al.* (2012) reported that KSHV-infected HIV-1-positive people of African ancestry were significantly more likely to develop multicentric Castleman disease than their European counterparts (relative risk [RR] = 2.42, 95% CI = 1.28 to 4.55). An analysis of the frequency of A299G, a TLR4 single nucleotide polymorphism associated with increased multicentric Castleman disease incidence, showed the polymorphism to be three-fold higher in patients of native African descent compared to those of European descent. Finally, the role of Epstein-Barr virus in KSHV-associated multicentric Castleman disease has not been extensively investigated, but the mantle zone plasmablastic cells predominantly infected with KSHV do not appear to be co-infected with Epstein-Barr virus (Oksenhendler *et al.* 2002, Dupin *et al.* 2000).

3.5.3 Multiple myeloma

Multiple myeloma is a comparatively rare type of lymphohematopoietic cancer, making up less than 2% of incident cancers in the United States. The annual (2008 to 2011) U.S. incidence and death rate has been stable for the past two decades, at approximately 6.3/100,000 in men and 3.3/100,000 in women with an approximately 47% 5-year survival rate (SEER 2015).

Twenty hospital-based case-control/comparison studies and two nested case-control studies of the association between multiple myeloma and KSHV were reviewed by IARC (2012). One large case-control study, including 254 cases of multiple myeloma, has been identified since 2008 (Benavente *et al.* 2011), together with two case series of multiple myeloma patients that

reported a higher frequency of KSHV in bone marrow tissue (Ismail *et al.* 2011, Sadeghian *et al.* 2008) than among healthy controls. Excluding five studies using a case-control or case-comparison design (Benavente *et al.* 2011, Sitas *et al.* 1999, Schonrich *et al.* 1998) or nested case-control design (Tedeschi *et al.* 2005, Tedeschi *et al.* 2001) with age-matched controls (Table 3-6), the majority of studies were clinic based and did not report methods for the selection of cases and controls or comparison groups and few appear to have controlled for age, sex, or other variables. Among these lower quality studies, a total of 118 cases of multiple myeloma were associated with KSHV (22%) compared with 233 of 1,524 patients without multiple myeloma (23%) in 17 of 18 studies where rates among controls were reported. The five case-control or nested case-control studies also do not support a positive association between KSHV infection and multiple myeloma risk (Table 3-5). In the nested case-control studies (Tedeschi *et al.* 2005, Tedeschi *et al.* 2001), increased risks were observed for KSHV detected by latent antibodies; however, IARC (2012) reported that after stratifying by detection method, no statistically significant differences in KSHV positivity between cases and controls were observed by the Working Group.

Table 3-5. Summary of five case-control/comparison and nested case-control studies of KSHV and multiple myeloma[a]

Reference Study type	KSHV detection method	HIV-1 status	OR or RR (95% CI) (KSHV-exposed cases vs. exposed controls)
Schonrich *et al.* 1998 Germany Case-comparison	IFA lytic KS-1	HIV-1 negative	[1.39 (0.15–13.3)] (4/99 cases vs. 1/34 healthy controls[b])
Sitas *et al.* 1999 S Africa Combined 2 case-control studies	IFA latent Titer 1:100 1:>20,000	Not specified	Adjusted prevalence rate[c] (108 cases and 108 controls) 24% cases vs. 32% blood-donor controls 1.5% cases vs. 3% blood- donor controls
Tedeschi *et al.* 2001 Finland nested case-control study	IFA lytic Latent (LANA) Western blot (ORF 65, 73, or K8.1A)	Not specified	2.02 (0.94–4.33) (13/47 cases vs. 36/224 controls) 10.0 (0.91–110.3) (2/47 cases vs. 1/224 controls) 0.89 (0.25–3.25) (3/47 cases vs. 16/224 controls)
Tedeschi *et al.* 2005 Scandinavia 5 cohorts nested case-control studies	IFA Lytic IFA Latent	Not specified	1.08 (0.5–1.1) (39/329 cases vs. 238/1393 controls[d]) 0.6 (0.1–2.7) (2/329 cases vs. 16/1615 controls[d])
Benavente *et al.*	Latent	Not specified	0.31 (0.11–0.85)

Reference Study type	KSHV detection method	HIV-1 status	OR or RR (95% CI) (KSHV-exposed cases vs. exposed controls)
2011	(ORF 73 antigen)		(4/254 cases; NR controls[e])
Europe EpiLymph multisite case-control study	Lytic (K8.1)		0.43 (0.15–1.2) (4 cases; NR controls[e])
Reference Study type	**KSHV detection method**	**HIV-1 status**	**OR or RR (95% CI) (KSHV-exposed cases vs. exposed controls)**

Source: IARC 2012, Table 2.8 and Table 2.9.) except for Benavente *et al.* (2011); [ ] = OR calculated by NTP.
CI = confidence interval, IFA = immunofluorescence assay; LANA = latent nuclear antigen; KS = Kaposi sarcoma; KSHV = Kaposi sarcoma herpesevirus; MM = multiple myeloma, NR=not reported, OR = odds ratio, RR = relative risk.
[a] Studies with unclear selection of cases or controls or no adjustment for age or other variables were excluded.
[b]Age and sex matched controls.
[c]Adjusted for age, sex, and education and sex partner.
[d]Age, sex, and area-matched controls; OR adjusted for age.
[e]Age, sex, sampling date, length of follow-up and area-matched controls.

3.5.4 **Other lymphohematopoietic cancers**

Several case-series and case-control studies have been conducted of other lymphohematopoietic cancers, including other lymphomas. An increased incidence of non-Hodgkin lymphoma has been reported in association with KSHV-related multicentric Castleman disease or primary effusion lymphoma in some patients (Oksenhendler *et al.* 2013, Oksenhendler *et al.* 2002). Pan *et al.* (2012) recently described a rare KSHV-associated large B-cell lymphoma in lymph nodes or extranodal sites that do not exhibit lymphomatous effusions and appear to have a different immunological profile but which may form a continuum with classic primary effusion lymphoma. Deloose *et al.* (2005) also reported that 38% of HIV-1-positive cases of solid immunoblastic/plasmablastic non-Hodgkin lymphoma were KSHV positive among 99 cases of non-Hodgkin lymphoma without evidence of primary effusion lymphoma or multicentric Castleman disease. Burbelo *et al.* (2012) also reported a higher frequency of KSHV among a small series of HIV-1-positive non-Hodgkin lymphoma cases than among healthy controls, but no differences in KSHV frequency were reported in a series of HIV-1-positive non-Hodgkin lymphoma cases compared according to duration of HIV-1 immunosuppression (Gerard *et al.* 2009).

A nested case-control study of 155 incident cases of non-Hodgkin lymphoma among HIV-1-positive men who have sex with men vs. 155 non-cancer controls (Beachler *et al.* 2011) did not detect a difference in serum KSHV after adjustment for age and CD4 status. The multisite EpiLymph study in Europe (Benavente *et al.* 2011), comprising 2,083 cases of lymphomas and 2,048 age-, sex-, and country-matched controls with KSHV serotyping, also reported no associations between KSHV seropositivity and B-cell lymphoma, T-cell lymphoma or Hodgkin lymphomas. Positive associations for splenic marginal zone lymphoma (OR = 4.1, 95% CI = 1.57 to 10.83; 6 cases, using the lytic K 8.1 antigen) and mantle-cell lymphoma (OR = 2.63, 95% CI = 0.88 to 7.86; 4 cases, using lytic K 8.1 antigen). Although this was a large study, exposure prevalence was low resulting in few exposed cases for some subtypes of B-cell lymphomas, T-cell lymphomas, and Hodgkin lymphomas. KSHV-related non-Hodgkin lymphoma is

uncommon in sub-Saharan African populations despite a high prevalence of endemic KSHV (Engels *et al.* 2007b).

3.6 Integration across studies

3.6.1 Lymphohematopoietic cancers

Approximately 109 KSHV-positive cases of primary effusion lymphoma have been reported in the literature, primarily in case reports or case series, and have demonstrated a form of KSHV-related primary effusion lymphoma with distinguishing clinical, morphological, and immunophenotypic features that are uniquely associated with KSHV infection; the majority of cases occur in HIV-1-positive patients although cases also occur in HIV-1-negative patients (IARC 2012). Based on a limited number of case series and case-comparison studies, KSHV has also been associated with some forms of multicentric Castleman disease, where it is found primarily in mantle-cell large immunoblastic B cells (see also Section 4). A majority of KSHV-related cases occur in HIV-1-positive patients (IARC 2012). To date it is unclear if other viruses, including Epstein-Barr virus, or other cofactors, play a role in the development of these conditions, although their rarity has precluded large-scale studies of these factors. No association of multiple myeloma with KSHV infection was found in several studies. Data are insufficient to evaluate other lymphomas.

3.6.2 Other tumor sites

Few studies have been conducted on other tumor sites, with the partial exception of prostate cancer. Case-control or nested case-control studies of prostate cancer identified since 2008 have reported no or small increases in risk in association with KSHV (Sutcliffe *et al.* 2015, McDonald *et al.* 2011, Huang *et al.* 2008). In the most recent nested case-control study within a prospective clinical cancer prevention trial, Sutcliffe *et al.* reported no difference in KSHV-positive serostatus (11.6% in cases, 11.1% in controls, $P = 0.81$). These findings are consistent with those reported in earlier studies and reviewed by IARC (2012). IARC (1997) reviewed six case series studies of angiosarcoma, four of which were reported as showing no association with KSHV; five case studies of skin cancer were reviewed, four of which showed no association with KSHV (IARC 1997). In addition, IARC (1997) reviewed approximately 28 case series and small case-control studies comparing KSHV DNA in skin and other tissue samples from patients with Kaposi sarcoma lesions with patients with other skin tumors or tissue lesions resembling Kaposi sarcoma and found, overall, only 3% of non-Kaposi sarcoma tissues contained KSHV DNA.

Since 2008, single studies have been identified for KSHV and hepatocellular (Su *et al.* 2014), vulvar (Simbiri *et al.* 2014), and bladder cancers (Panagiotakis *et al.* 2013), head and neck squamous cell carcinoma (McLemore *et al.* 2010), laryngeal cancer (Mohamadian Roshan *et al.* 2014, Guvenc *et al.* 2008), and childhood inflammatory myofibroblastoma (Alaggio *et al.* 2010). The number of studies on specific solid tumors is insufficient to evaluate these endpoints, with the possible exception of prostate cancer, for which no associations with KSHV serostatus have been reported to date.

3.7 Synthesis across cancer endpoints

A summary of the evidence for KSHV infection and the different cancer endpoints form epidemiological studies is provided in Table 3-6. The preliminary level of evidence from cancer

studies in human also considers studies of tissues from humans in addition to epidemiological studies and is provided in Section 5.

Table 3-6. Summary of KSHV cancer endpoints and strength of the epidemiological evidence

Cancer endpoint	Strength of Evidence
Kaposi sarcoma	• Consistent evidence of highly increased, statistically significant risk in multiple prospective cohort and case-control studies. • KSHV found in > 80% of Kaposi sarcoma tumors in biopsies from Kaposi sarcoma case series. • Dose-response relationships observed in several studies.
Primary effusion lymphoma	• Consistent evidence of KSHV seropositivity or KSHV DNA in morphologically and immunophenotypically distinct primary effusion lymphoma in multiple case series and case-comparison studies.
Multicentric Castleman disease	• Evidence of KSHV seropositivity in some forms of MCD (primarily in mantle-cell large immunoblastic B cells) in several case series and case-comparison studies. • Most cases observed in HIV-1-positive individuals.
Multiple myeloma	• Inconsistent evidence, the majority with no or non-significant modest positive associations, from several case-control or case-comparison studies.
Other lymphohematopoietic cancers and solid tumor sites	• Inconsistent evidence for prostate cancer risk based on few studies. • Inadequate number of epidemiological studies to evaluate other endpoints.

4 Mechanisms and Other Relevant Data

KSHV-associated oncogenesis is a complex process that involves interactions among various viral, host, and environmental cofactors (Mesri *et al.* 2014, Fukumoto *et al.* 2011). Although many of the mechanisms by which KSHV is able to establish and maintain infection are known, and many KSHV-encoded proteins and their functions have been characterized *in vitro*, some aspects of infection and transformation are still not well understood (Giffin and Damania 2014, Fukumoto *et al.* 2011). This section reviews the characteristics of KSHV-associated neoplasms and risk factors (Section 4.1), evaluates the mode of action and evidence that KSHV is an oncogenic virus (Section 4.2), and provides a synthesis of the information (Section 4.3).

As discussed in Section 3, three neoplasms have been associated with KSHV in humans, although the level of evidence for each endpoint varies: (1) Kaposi sarcoma, (2) primary effusion lymphoma, and (3) some cases of multicentric Castleman disease. These cancers occur most frequently in the context of immunosuppression and are the focus of this section (Giffin and Damania 2014, Mesri *et al.* 2014). Although other lymphohematopoietic neoplasms and several solid tumor sites have been investigated, the available data were insufficient to establish a causal relationship with KSHV (see Section 3).

4.1 Characteristics and risk factors

Some basic characteristics of the neoplasms associated with KSHV are shown in Table 4-1. The epidemiological data reviewed in Section 3 indicated that HIV-1 infection and immunosuppression are important host-related cofactors that increase the risk of developing KSHV-associated neoplasms. However, it is also clear that not all host and environmental cofactors have been identified. Further, KSHV latent and lytic transcripts include genes and non-coding RNAs that promote acquisition of various cancer hallmarks (e.g., insensitivity to antigrowth signals, avoiding cell-cycle arrest, immune evasion, genetic instability, cell proliferation, resisting apoptosis, and angiogenesis) (Mesri *et al.* 2014). This section reviews the role of immunosuppression, viral proteins, and non-coding RNAs, and other possible cofactors in KSHV-induced neoplasms.

Table 4-1. Characteristics of KSHV-associated neoplasms

Neoplasm	Presentation	Lineage and primary tumor cell	Clonality	KSHV genomes
KS	Highly angiogenic lesions found on skin, visceral organs or mucosal surfaces; leaky vasculature	Endothelial cell origin; tumor cells are spindle cells with mixed blood and lymphatic endothelial cell markers	Oligoclonal or monoclonal	> 99% of tumor cells contain single copy of KSHV genome
PEL	Non-Hodgkin lymphoma; B-cell expansion (usually serous effusion without detectable tumor masses) in body cavity	B cells; CD20−; markers resemble partially differentiated plasma cell	Monoclonal	Tumor cells contain 50 to 100 copies of KSHV genome
MCD	Plasmablastic variant; disseminated lymphoadenopathy	B cell; IgM γ-restricted plasmablasts	Typically Polyclonal	Unknown, detected in most HIV-1+ MCD cases

Source: Adapted from (Giffin and Damania 2014).
KS = Kaposi sarcoma, MCD = multicentric Castleman disease, PEL = Primary effusion lymphoma.

4.1.1 HIV-1 and immunosuppression

Although KSHV encodes oncogenic genes that could potentially induce all Kaposi sarcoma phenotypes, the risk of malignancy is greatly enhanced in the presence of cofactors, particularly immunosuppression by HIV-1 or iatrogenically during organ transplantation (Mesri *et al.* 2010). The importance of HIV-1 infection as a potent cofactor for KSHV oncogenesis is demonstrated by the dramatic increase in Kaposi sarcoma incidence in HIV-1-infected individuals (see Section 3). The risk for developing Kaposi sarcoma is strongly associated with defects in both cellular and humoral immunity. Decreasing CD4 cell counts are associated with an increasing risk of Kaposi sarcoma in both AIDS-associated and classic Kaposi sarcoma cases, and decreased CD19 cell counts are associated with an increased risk of Kaposi sarcoma in HIV-1-positive and HIV-1-negative individuals (Uldrick and Whitby 2011). Iatrogenic immunosuppression also is associated with an increased risk (primarily in kidney allograft patients), but it is not nearly as great as that seen with HIV-1 infection (Mesri *et al.* 2010). This also may reflect differences in KSHV infection rates rather than HIV-1-specific causes, or differences in immune dysfunction. Further, HIV-1 infection causes changes in cytokine profiles and produces several viral proteins (HIV-1 transactivating protein [tat], HIV-1 negative factor protein [Nef]) that are proven to be potent cofactors that can activate KSHV lytic replication and influence pathogenesis (Purushothaman *et al.* 2015, Mesri *et al.* 2014, Zhu *et al.* 2014). The biological basis for the role of immunosuppression in KSHV oncogenesis also involves the interactions of latent and lytic infection and is discussed in the following sections.

4.1.2 KSHV transcripts

The KSHV genome contains 87 open reading frames including many genes found in other herpesviruses, several unique open reading frames (designated K1 to K15) not found in other herpesviruses, and at least 14 viral genes that are homologues of human cellular genes (Mesri *et al.* 2010, Wen and Damania 2010). Viral transcripts dysregulate several cellular signaling pathways including those involved in cell-cycle progression (e.g., vCyclin, LANA), angiogenesis

(e.g., LANA, K1, vCCL, vGPCR, vIL6), apoptosis (e.g., LANA, K1, vFLIP, vBCL-2), immune modulation/evasion (e.g., vFLIP, K3, K5, vIRFs), signal transduction (e.g., K1, K15), and antiviral responses (IARC 2012, Mesri *et al.* 2010, Wen and Damania 2010). While latent infection is dominant in KSHV-infected cells *in vivo* and *in vitro*, both latent and lytic genes contribute to the malignant phenotype (Fukumoto *et al.* 2011, Mesri *et al.* 2010). A large portion of the KSHV genome is devoted toward evading the innate immune response of the host (e.g., interferon production, interferon regulatory factor activation, NK cell activity, complement activation, inflammasome activation, and chemokine activity), thus facilitating life-long infection (Giffin and Damania 2014, Fukumoto *et al.* 2011). In addition, KSHV evades adaptive immune responses by repressing viral antigen presentation, T-cell activation, BCR-mediated B-cell activation and B-cell differentiation (Giffin and Damania 2014). Professional antigen-presenting cells, including B cells, dendritic cells, and macrophages, are primary targets for KSHV infection (Campbell *et al.* 2014a, Knowlton *et al.* 2013). Cytokine profiles, surface expression of MHC molecules, and T-cell activation are altered in infected antigen-presenting cells. This likely explains why T-cell responses to KSHV antigens are not very robust. Both complete and abortive virus replication cycles in antigen-presenting cells (APCs) can affect viral pathogenesis and progression to Kaposi sarcoma and B-cell cancers. Host cell transformation and oncogenesis are thought to result inadvertently from the survival mechanisms used by KSHV and other oncogenic viruses (Wen and Damania 2010).

In a healthy host, KSHV infection is not oncogenic because it leads to latent infection that cannot transform cells (Cavallin *et al.* 2014). Although lytic infection leads to expression of oncogenic and angiogenic genes, it is cytopathic and immunogenic in immunocompetent hosts and does not transform cells. However, in an immune-compromised host, lytic infected cells escape immunosurveillance and are able to express the full oncogenic repertoire, including genes that induce angiogenesis and inflammation (Cavallin *et al.* 2014, Mesri *et al.* 2014). In addition, some latently infected cells also express some of the early lytic genes but do not complete the lytic cycle. These cells may become progressively transformed and acquire oncogenic alterations that allow them to switch back to a less immunogenic latent form. Latent proteins and miRNAs play an important role in KSHV-associated neoplasms through inhibition of apoptosis and maintenance of latency while KSHV lytic proteins mimic or disrupt host cytokine signals, resulting in microenvironments that promote tumor growth through mechanisms called "paracrine neoplasia" (Cavallin *et al.* 2014, Fukumoto *et al.* 2011, Mesri *et al.* 2010). This section briefly reviews the role of latent proteins, lytic proteins, non-coding RNAs and their interactions in KSHV pathogenesis.

Latent proteins

Latent KSHV infection is characterized by a circularized, extra-chromosomal viral genome (episome) that is tethered to the host chromosomes by latency-associated nuclear antigen (LANA-1) (Giffin and Damania 2014). All KSHV-infected cells express LANA-1, and antibody-based detection methods are the most powerful tool for diagnosis of pathological samples of KSHV infection (Fukumoto *et al.* 2011). Only a small portion of the viral genome is actively transcribed during latency and no functional or infectious viral particles are produced (Giffin and Damania 2014). LANA-1 is the most consistently detected viral protein in KSHV-associated tumor cells and is necessary for replicating the episomal viral DNA (IARC 2012). In addition to LANA-1, latent transcripts include vCyclin, viral FLICE-inhibitory protein (vFLIP), kaposin A

and B, viral interferon regulatory factors (vIRF), LANA-2 (expressed only in primary effusion lymphoma) and viral miRNAs. Although the primary function of LANA-1 is to maintain the viral episome, it also interferes with several anti-tumorigenic pathways, including p53 and anti-proliferative transforming growth factor-β (TGFβ), and might contribute to angiogenesis by stabilizing hypoxia-inducible factor-1α (HIF-1α) and by targeting von Hippel Lindau for degradation (Mesri *et al.* 2010). Recent evidence also suggests that caspase cleavage sites in LANA-1 have an important role in delaying apoptosis and blunting the caspase-1-mediated inflammasome, thus inhibiting key cellular defense mechanisms (Davis *et al.* 2015). vCyclin is a constitutive activator of cyclin-dependent kinase 6 (CDK6), a gene that is overexpressed in lymphomas and leukemias, induces cytokinesis defects and polyploidy, and promotes viral replication (Kollmann *et al.* 2013, Mesri *et al.* 2010). vFLIP and kaposin A and B contribute to the inflammatory microenvironment. Thus, these transcripts are associated with growth and proliferative signals, evasion of apoptosis, angiogenic and inflammatory signals, and a limitless replicative potential that support tumor growth and progression (Mesri *et al.* 2010). Transgenic mice expressing some or all of the KSHV latency genes develop phenotypes characteristic of KSHV malignancies (e.g., B-cell hyperplasia and lymphoma) (Sin and Dittmer 2013, Fakhari *et al.* 2006). Some biochemical and biological properties, including their role in carcinogenesis, of KSHV latent proteins are shown in Table 4-2.

Table 4-2. Biochemical and biological properties of latent KSHV proteins

KSHV gene/protein	Function in viral life cycle	Homology to human protein	Biochemical properties	Role in carcinogenesis
ORF 73/LANA-1	Replication and maintenance of latent viral episome; partition of episomes to daughter cells	None	Interacts with histones, pRB, BET proteins, GSK-3β and others; inactivates p53-dependent transcriptional activation; induces S-phase entry and activates hTERT transcription; recruits origin-binding proteins	Anti-apoptotic activity, cell survival, deregulation of cell cycle, enhances *ras*-mediated transforming properties *in vitro*, tumorigenic in transgenic mice
ORF 71, K13/ vFLIP	Viral persistence; spindle cell formation and lymphomagenesis; inhibition of lytic viral replication	FLICE (caspase-8)-inhibitory proteins	Activates NF-κB; inhibits CD95/Fas-induced apoptosis, anoikis, superoxide-induced cell death; modulates MHC-1 expression	Anti-apoptotic activity, cell survival, immune evasion
ORF 72/vCYC	Viral cyclin; strongly activates CDK6 protein kinase activity	D-type cyclin	Phosphorylates H1, pRB, BCL-2, $p27^{KIP1}$ in tandem with CDK6	Deregulation of cell cycle
K12/Kaposin A[a], Kaposin B[a]	Modulation of cytokine mRNAs regulated by the p38 pathway	None	Interacts with cytohesin-1 Modulates p38/MK2	Transforming properties in cultured cells
K10.5/vIRF3, LANA-2[b]	Modulates viral interferon response	Interferon regulatory factor	Modulates p53 function; activates IRF-3, IRF-7, c-myc; inhibits IRF-5	Anti-apoptotic activity, immune evasion, cell survival
K15/K15 protein[b]	Recruits endothelial cells to infected cells	None	Activates NF-κB, MEK/Erk; induces inflammatory cytokines; interacts with proteins involved in signal transduction, with members of src family of PTK, and with an apoptotic regulatory protein HAX-1	Possible anti-apoptotic and angiogenic activity

Source: (IARC 2012).

BET = bromodomain and extra-terminal; CDK6 = cyclin-dependent kinase 6; FLICE = FADD-like interleukin-1 beta-converting enzyme; HAX-1 = HS1-associated protein X1; hTERT – human telomerase reverse transcriptase; IRF = interferon regulatory factor; LANA = latency associated nuclear antigen; MHC = major histocompatibility complex; NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells; pRB = retinoblastma protein; PTK = protein tyrosine kinase; vCYC = viral cyclin; vIRF3 = viral interferon regulatory factor-3.

[a]Expression increases after activation of the lytic cycle.

[b]Lytic in endothelial cells; latent in B cells.

Lytic KSHV proteins

The lytic cycle is characterized by expression of many transcripts in an orchestrated temporal order, replication of the viral genome, viral assembly, and viral egress (Wen and Damania 2010). Lytic reactivation requires expression of the replication and transcription activator (RTA) protein and is under epigenetic control (Giffin and Damania 2014, Li *et al.* 2014, Pantry and Medveczky 2009). RTA plays an important role as both an initiator and controller of KSHV lytic DNA replication (Purushothaman *et al.* 2015). Many of the lytic viral proteins, including K1, viral interferon response factors (vIRFs), vIL-6, viral-encoded chemokines (vCCLs), viral G protein-coupled receptor (vGPCR) and K15 contribute to the angiogenic and inflammatory phenotype of oncogenic lesions (Purushothaman *et al.* 2015, Sodhi *et al.* 2000). However, lytic infection generally results in cellular lysis and is unlikely to have a direct role in endothelial cell growth, transformation, or immortalization (Mesri *et al.* 2010). Two hypotheses (paracrine oncogenesis and abortive lytic replication) have been proposed to explain how the interactions between latent and lytic KSHV-infected cells contribute to a malignant phenotype (Cavallin *et al.* 2014, Mesri *et al.* 2010, Bais *et al.* 2003).

The paracrine hypothesis recognizes that host and viral growth factors can enhance latent genes and cytokines supplied by a minority of lytically infected cells or latently infected cells expressing early lytic genes (Cavallin *et al.* 2014, Mesri *et al.* 2010). Lytic genes induce a number of paracrine-acting factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), IL-6, angiopoietin 2 (ANGP2), and others that could drive latently infected cell proliferation, angiogenesis, and inflammation and support immune escape and survival. Experimental evidence supporting the role of lytic infection in oncogenesis include the following: (1) lytic viral proteins are expressed and virions are present in a minority of cells within Kaposi sarcoma lesions, (2) immunosuppression increases KSHV re-activation and lytic replication, (3) interruption of lytic replication by immune reconstitution or anti-lytic herpes anti-virals inhibits or prevents tumor development, (4) lytic infection is necessary to support viral episomal maintenance by recruitment of new cells to latency, and (5) endothelial cells expressing latent genes were tumorigenic in nude mice when co-injected with a few vGPCR-expressing cells (at a ratio that approximated the proportion of vGPCR-expressing and latent gene-expressing tumor cells found in human Kaposi sarcoma) but lost their tumorigenic potential in the absence of paracrine secretions from vGPCR-expressing cells (Mesri *et al.* 2010, Montaner *et al.* 2006). In addition, controlling KSHV lytic infection decreases the risk of developing Kaposi sarcoma in patients with AIDs (Uldrick and Whitby 2011) and transgenic mice expressing vGPCR develop angiogenic lesions that resemble Kaposi sarcoma (Guo *et al.* 2003, Yang *et al.* 2000).

The abortive lytic hypothesis recognizes that not all cells undergoing lytic reactivation complete the lytic cycle (i.e., abortive lytic replication). Such cells express only the oncogenic early lytic genes and can be transformed by genetic or epigenetic alterations, including vGPCR-mediated immortalization, and switch back to a less immunogenic latent status (Cavallin *et al.* 2014, Bais *et al.* 2003). Lytically infected cells can stimulate these cells through paracrine mechanisms.

Biochemical and biological properties of some KSHV lytic proteins are shown in Table 4-3.

Table 4-3. Lytic KSHV proteins and their role in carcinogenesis

KSHV Gene/Protein	Function in viral lifecycle	Homology to human protein	Biochemical properties	Role in carcinogenesis
K1/VIP	May increase/decrease viral reactivation	None	Activates intracellular signaling cascades; induces angiogenic cytokines; blocks intracellular transport of BCR-complexes to cell surface	Transforming properties, angiogenesis, anti-apoptotic activity
ORF 74/vGPCR	Stimulates cellular proliferation	G-protein-coupled receptor	Activates Akt, MEK/Erk, JNK, p38; induces secretion of proinflammatory cytokines and angiogenic growth factors	Transforming properties in cells, tumorigenicity in mice, angiogenesis, anti-apoptotic activity
K2/vIL6	Viral IL6	Interleukin-6	Induces proliferation of PEL cell lines; induces VEGF and STAT3 phosphorylation	Tumor cell survival, angiogenesis, hematopoiesis
K3/miR-1, ZMP-B	Downmodulates HLA, ICAM-1, B7-2	None	E3 ubiquitin ligase	Immune evasion
K5/miR-2	Downmodulates HLA-A, HLA-B, ICAM-1, CD86, CD1d	Part of a family of membrane-bound-E3-ubiquitin ligases	E3 ubiquitin ligase	Immune evasion
K6/vCCL-1, vMIP-I	Viral chemokine	Chemokine homologue related to macrophage inflammatory protein (MIP)-1α	CCR8 agonist; induces monocyte chemotaxis (VEGF production)	Angiogenic properties, anti-apoptotic activity
K4/vCCL-2, vMIP-II	Viral chemokine	Chemokine homologue related to macrophage inflammatory protein (MIP)-1α	CCR3, CCR5, CCR8 agonist; induces monocyte chemotaxis	Angiogenic properties
K4.1/vCCL-3, vMIP-III	Viral chemokine	Some homology to chemokines TARC and eotaxin	CCR4, XCR1 agonist	Angiogenic properties
K7/vIAP	Apoptosis inhibitor	Structurally related to a splice variant of survivin	Binds to and inhibits several proteins involved in apoptosis; induces degradation of IkB, p53, vGCR	Anti-apoptotic activity
ORF 16/vBCL-2	Viral Bcl-2	Bcl-2	Heterodimerizes with human Bcl-2	Anti-apoptotic activity

KSHV Gene/Protein	Function in viral lifecycle	Homology to human protein	Biochemical properties	Role in carcinogenesis
ORF 45/ORF 45	Virion protein important for lytic replication; inhibits virus-mediated induction of type 1 interferon	None	Binds to and inhibits phosphorylation of IRF-7	Virion infectivity, immune evasion
ORF 50/K-RTA	Immediate-early transactivator; ubiquitin E3 ligase	None	Binds to and activates several lytic viral promoters directly or by interacting with RBPJk; promotes ubiquitination and degradation of IRF-7; represses p53	Reactivation of lytic viral replication from latency, immune evasion, anti-apoptotic activity
K8/K-bZIP	Modulates cell cycle and lytic reactivation	None	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	Deregulation of cell cycle
K9/vIRF-1	Modulates viral interferon responses	Interferon regulatory factor	Prevents IRF-3-mediated transcription; inhibits p53-transcriptional activity and prevents p53-dependent apoptosis	Transformation of rodent fibroblasts, immune evasion, anti-apoptotic activity
K11/vIRF-2	Modulates viral interferon responses	Interferon regulatory factor	Inhibits interferon induction; inhibits induction of CD95L	Immune evasion, anti-apoptotic activity
K10.5/vIRF3, LANA-2[a]	Modulates viral interferon responses	Interferon regulatory factor	Modulates p53 function; activates IRF-3, IRF-7, c-myc; inhibits IRF-5	Anti-apoptotic activity, immune evasion, cell survival
K15/K15 protein[a]	Recruits endothelial cells to infected cells	None	Activates NF-κB, MEK/Erk; induces inflammatory cytokines; interacts with proteins involved in signal transduction, with members of src family of protein tyrosine kinases, and with an apoptotic regulatory protein HAX-1	Possible anti-apoptotic and angiogenic activity

Sources: (Purushothaman *et al.* 2015, IARC 2012).
[a]Lytic in endothelial cells; latent in B cells.

Non-coding RNAs

KSHV-encoded miRNAs are expressed in latently infected cells and are involved with suppressing lytic reactivation by activating NF-κB, endothelial cell differentiation, and angiogenesis (Moody *et al.* 2013, Mesri *et al.* 2010). KSHV infection also induces cellular miRNAs, particularly miR-132. miR-132 inhibits anti-viral innate immune responses and induces abnormal endothelial cell proliferation, thus, linking viral immune escape with angiogenesis, two important features of Kaposi sarcoma. Moody *et al.* (2013) demonstrated that KSHV miRNAs mediated cellular transformation and tumorigenesis using a KSHV-induced cellular transformation system of primary rat mesenchymal precursor cells. A mutant virus with a cluster of precursor miRNAs deleted failed to transform primary cells and caused cell-cycle arrest and apoptosis. The oncogenicity of the mutant virus was restored by complementation with the miRNA cluster or several individual precursor miRNAs. Qin *et al.* (2010) also demonstrated that KSHV miRNAs induce IL-6 and IL-10 secretion in murine macrophages and human myelomonocytic cells and support a role in programming macrophage cytokine responses that favor KSHV-associated tumor progression.

In addition to the miRNAs, KSHV also encodes a long, non-coding RNA known as polyadenylated nuclear (PAN) RNA during the early lytic stage (Campbell *et al.* 2014b). PAN RNA has been implicated in KSHV gene expression, replication, and immune modulation and is required for optimal expression of all KSHV lytic genes. It also facilitates LANA-episomal dissociation during lytic replication.

4.1.3 Other possible cofactors

It is likely that other cofactors are involved in KSHV-associated neoplasms as evidenced by the geographic variation in incidence before the HIV-1 epidemic (see Section 3.2.1). Co-infection with other human herpesviruses (e.g., Epstein-Barr virus; see monograph on Epstein-Barr virus), human cytomegalovirus, or human papillomavirus can activate KSHV lytic replication and influence its pathogenesis (Purushothaman *et al.* 2015). While other cofactors have been proposed (see Section 3), their mode of action and role as risk factors for specific cancers have not been established.

4.2 Mode of action and evidence for cancer causation

As discussed in the Overview and Introduction Section, it is often difficult to apply stringent criteria, such as Koch's postulates or Hill's considerations, for determining that a human tumor virus is oncogenic (Moore and Chang 2010, zur Hausen 2001). Therefore, in addition to the usual criteria used by epidemiologists to determine causality, other factors should be considered as proposed by IARC (1997) and zur Hausen (2001, 1994). Unlike other oncogenic viruses, Hill's epidemiological considerations for causality are met for KSHV and Kaposi sarcoma (Moore and Chang 2010). Thus, application of various criteria applicable to oncogenic viruses and mechanistic data provide strong evidence that KSHV is oncogenic in humans and are briefly reviewed below by tumor type.

4.2.1 **Kaposi sarcoma**

Chang *et al.* (1994) was the first to report KSHV in Kaposi sarcoma tissues obtained from AIDS patients. There is now substantial evidence that KSHV is the etiological agent of Kaposi sarcoma. KSHV is present in the vast majority of Kaposi sarcoma tumor cells, thus meeting Hill's consideration for strength and consistency (each cell contains one copy of the KSHV genome and its seroprevalence was correlated with the global incidence of the disease) (Fukumoto *et al.* 2011, Mesri *et al.* 2010). In addition, the most difficult of the Hill considerations, experimental epidemiological evidence in a randomized clinical trial, was fulfilled when AIDS patients who were treated with an antiherpesvirus drug were protected from new occurrences of Kaposi sarcoma but not against established tumors (Moore and Chang 2010). Other lines of evidence linking KSHV and Kaposi sarcoma include the following (Cavallin *et al.* 2014):

- KSHV is associated with all four clinical forms of the disease,
- KSHV is found in spindle cells,
- KSHV infection precedes the onset of Kaposi sarcoma,
- KSHV seroprevalence is highest in areas of high Kaposi sarcoma incidence,
- KSHV encodes many viral oncogenes, and
- KSHV transforms and induces tumorigenesis in endothelial cells.
- Monoclonal expansion of KSHV tumors supports an etiologic role for the virus (Duprez *et al.* 2007, Judde *et al.* 2000).

In addition, *in vitro* models and animal models have been developed that accurately reproduce many features observed in KSHV-associated neoplasms (Ashlock *et al.* 2014, Mesri and Cesarman 2011, Mutlu *et al.* 2007).

4.2.2 **Primary effusion lymphoma**

Primary effusion lymphoma is extremely rare. It occurs most frequently in HIV-1-infected homosexual males but has been described in association with post-transplant immunosuppression and in HIV-1-negative elderly patients of Eastern European/Mediterranean and Jewish descent, two populations with high seropositivity for KSHV and elevated incidence of classic Kaposi sarcoma (IARC 2012, Fukumoto *et al.* 2011, Dourmishev *et al.* 2003). KSHV DNA sequences were first identified in eight body-cavity-based lymphomas (later identified as primary effusion lymphoma) taken from patients with AIDS, but they were not detected in 185 other lymphomas examined from patients with or without AIDS (Cesarman *et al.* 1995). Asou *et al.* (2000) reported similar findings – KSHV was present in 21 of 21 primary effusion lymphoma patients but was not detected in 139 patients with other AIDS- and non-AIDS-related lymphomas. KSHV infection in primary effusion lymphoma cells is predominantly latent with only a small subpopulation of cells that stain for markers of lytic reactivation (Fukumoto *et al.* 2011). vIL-6 is the most frequently detected lytic protein and induces VEGF expression and cytokine signals in a broad range of cell types (Fukumoto *et al.* 2011, Aoki *et al.* 2001). Further evidence that supports KSHV infection as a causal agent in primary effusion lymphoma is as follows:

- Primary effusion lymphoma neoplasms always carry KSHV and are commonly co-infected with Epstein-Barr virus (Fukumoto *et al.* 2011, Cesarman *et al.* 1995);

- KSHV has been incorporated as a diagnostic criterion for primary effusion lymphoma (IARC 2012);
- About half of primary effusion lymphoma patients also have Kaposi sarcoma, and a previous Kaposi sarcoma diagnosis confers an increased risk of primary effusion lymphoma relative to all other AIDs-associated non-Hodgkin lymphomas (Fukumoto *et al.* 2011, Dourmishev *et al.* 2003);
- Primary effusion lymphoma cells contain 50 to 100 copies per cell of KSHV DNA, and the expression pattern of KSHV-encoded proteins is almost identical in primary effusion lymphoma and Kaposi sarcoma (Giffin and Damania 2014, IARC 2012, Fukumoto *et al.* 2011);
- Several KSHV-infected cell lines have been established from primary effusion lymphoma cells that retain a stable latent viral genome including a KSHV+/EBV− cell line established from KSHV+ and Epstein-Barr virus+ primary effusion lymphoma cases, suggesting that KSHV plays an essential role in primary effusion lymphoma pathogenesis (Fukumoto *et al.* 2011, Katano *et al.* 1999);
- Expression of several KSHV viral genes was required for survival of primary effusion lymphoma cells in culture (IARC 2012); and
- Classic primary effusion lymphoma patients show a high incidence of congestive heart failure, a condition shared with KSHV infection and classic Kaposi sarcoma patients (Dourmishev *et al.* 2003).

4.2.3 **Multicentric Castleman disease**

Castleman disease is an uncommon lymphoproliferative disorder with several pathologic variants and two clinical forms; unicentric (or localized) and multicentric (Al-Maghrabi 2011, Bonekamp *et al.* 2011, El-Osta and Kurzrock 2011). Soulier *et al.* (1995) reported the first association of KSHV with multicentric Castleman disease. All 14 cases of HIV-1-positive multicentric Castleman disease patients were infected with KSHV; however, KSHV was detected in only 7 of 17 HIV-1-negative multicentric Castleman disease cases. KSHV-associated multicentric Castleman disease is now recognized as a distinct entity from other forms of multicentric Castleman disease and is characterized by severe inflammatory symptoms (attributed primarily to elevated levels of IL-6) an aggressive course, and a poor prognosis (Fajgenbaum *et al.* 2014, Venkataraman *et al.* 2013, Polizzotto *et al.* 2012, Bonekamp *et al.* 2011). Dysregulated IL-6 levels are thought to contribute to multicentric Castleman disease development (Cai *et al.* 2010b). Fewer studies have investigated the association of multicentric Castleman disease and KSHV compared to Kaposi sarcoma or primary effusion lymphoma; however, additional supporting evidence is as follows:

- Several hospital studies have reported an association between KSHV and multicentric Castleman disease (IARC 2012);
- Cases of Kaposi sarcoma and multicentric Castleman disease frequently occur together in the HIV-1+ population (Reddy and Mitsuyasu 2011, Cronin and Warnke 2009);
- The level of KSHV in peripheral blood monocytes or plasma corresponds with symptoms during multicentric Castleman disease flares in HIV-1+ individuals (Reedy and Mitsuyasu 2011);

- KSHV+ multicentric Castleman disease cases are associated with KSHV lytic infection, and high levels of vIL-6 have been detected in infected B cells and sera and likely are associated with B-cell proliferation seen in multicentric Castleman disease (IARC 2012, Fukumoto *et al.* 2011, Aoki *et al.* 2001);
- vIL-6, a frequently detected lytic protein, enhances cytokine signaling, increases human IL-6 and induces VEGF expression, and likely exacerbates inflammation and disease progression and may be a mechanism leading to multicentric Castleman disease (Giffin and Damania 2014, Al-Maghrabi 2011, Fukumoto *et al.* 2011);
- Treatment of multicentric Castleman disease cases with an inhibitor of KSHV infection has shown some success (Casper *et al.* 2004); and
- A rare case of cutaneous Castleman disease responded well to anti-IL-6 treatment (Ahmed *et al.* 2007).

4.3 Synthesis

Human viral oncogenesis is a complex process that involves interactions among many viral, host, and environmental factors. As with most other oncoviruses, KSHV infection is necessary but not sufficient for cancer development. Immunosuppression is an important cofactor based on the dramatic increase in Kaposi sarcoma incidence among HIV-1-infected individuals. KSHV infection has been clearly linked with all clinical forms of Kaposi sarcoma, as well as primary effusion lymphoma and HIV-1-positive multicentric Castleman disease, and biologically plausible modes–of–action have been described. One or more KSHV transcripts are expressed in all KSHV-associated tumors and are required for survival of tumor cells in culture. Latent transcripts drive viral persistence and replication, promote host cell proliferation, and prevent apoptosis while lytic transcripts dysregulate cell signaling pathways that contribute to the angiogenic and inflammatory phenotype of oncogenic lesions via paracrine mechanisms.

5 Preliminary Listing Recommendation

Kaposi sarcoma virus (KSHV) is known to be a human carcinogen based on sufficient evidence from studies in humans. This conclusion is based on epidemiological and molecular studies showing that it causes Kaposi sarcoma (Table 5-1) and primary effusion lymphoma (Table 5-2) in humans, together with supporting evidence from mechanistic studies demonstrating the biological plausibility of its carcinogenicity in humans. There is limited evidence of a causal association for multicentric Castleman disease from studies in humans (Table 5-3).

Data are inadequate to evaluate the association between KSHV and multiple myeloma, which has inconsistent evidence from epidemiological studies and no available evidence from mechanistic studies.

The following tables provide the preliminary level of evidence recommendations for the carcinogenicity of KSHV for each endpoint from studies in humans, including the key data from both epidemiological and molecular studies in humans.

Table 5-1. Evidence for KSHV and Kaposi sarcoma from human studies

Types of studies	Kaposi sarcoma
Epidemiological	
Positive associations	38/39[a] case-control and all 16[a] cohort/nested case-control; ORs/RRs – mostly statistically significant and some very high (10- to > 100-fold) Elevated RR in both HIV-1-negative and HIV-1-positive populations and in all types of Kaposi sarcoma. Dose response in several studies
Molecular (human tissue)	
Clonality	Mono- or oligoclonal
% KSHV-infected tumors	> 99% (~1 copy/cell)
KSHV protein expression[b]	
Latent	LANA-1, vCyclin, vFLIP, Kaposin A and B
Lytic	RTA, K1, vIRFs, vIL-6, vGPCR, vCCLs, K15
Level of evidence	**Sufficient**

HIV-1 = human immunodeficiency virus type 1; K1 = unique KSHV protein 1; K15 = unique KSHV protein 15; KSHV = Kaposi sarcoma herpes virus; LANA = latency-associated nuclear antigen; OR = odds ratio; RR = risk ratio.; RTA = replicaton and transactivation protein; vCCLs = viral-encoded chemokines; vFLIP = viral FLICE-inhibitory protein; vGPCR = viral G protein-coupled receptor; vIL-6 = viral homolog of interleukin-6; vIRF = viral interferon regulatory factors.
[a]The number of studies is based on those reporting risk estimates.
[b]See Section 4.1.2 for more details.

Table 5-2. Evidence for KSHV and primary effusion lymphoma from human studies

Types of studies	Primary effusion lymphoma (PEL)
Epidemiological	
Positive associations	Found in 109 cases; ~76 individual case reports and 31 cases in three case-series and two comparison studies PEL is a single distinct pathological entity Found in both HIV-1-negative and + cases Approximately 50% cases also have Kaposi sarcoma
Molecular (human tissue)	
Clonality	Monoclonal
% KSHV-infected tumors	100% (high copy number)
KSHV protein expression	Similar to Kaposi sarcoma
Other	KSHV is part of diagnostic criteria
Level of evidence	**Sufficient**

HIV-1 = human immunodeficiency virus type 1; KSHV = Kaposi sarcoma herpes virus; PEL = primary effusion lymphoma.

Table 5-3. Evidence for KSHV and multicentric Castleman disease from human studies

Types of studies	Multicentric Castleman disease
Epidemiological	
Positive associations	4/4 case comparison studies; very high ORs
Molecular (human tissue)	
Clonality	Typically Polyclonal[b]
% KSHV-infected tumors	100% of cancerous lymph nodes KSHV+ with HIV-1+ and 50% KSHV+ with HIV-1-
KSHV protein expression[a]	LANA-1, vIL-6
Level of evidence	**Limited**

HIV-1 = human immunodeficiency virus type 1; KSHV = Kaposi sarcoma herpes virus; LANA = latency-associated nuclear antigen; OR = odds ratio; vIL-6 = viral homolog of interleukin-6.

[a]Multicentric Castleman disease is associated with lytic infection.

[b]Monoclonal B-cell expansion have been reported.

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Glossary

Case report: Detailed descriptions of a few patients or clinical cases (frequently, just one sick person) with an unusual disease or complication, uncommon combinations of diseases, an unusual or misleading semiology, cause, or outcome (maybe a surprising recovery). They often are preliminary observations that are later refuted. They cannot estimate disease frequency or risk (e.g., for lack of a valid denominator).

Case series: A collection of subjects (usually, patients) with common characteristics used to describe some clinical, pathophysiological, or operational aspect of a disease, treatment, exposure, or diagnostic procedure. A case series does not include a comparison group and is often based on prevalent cases and on a sample of convenience. Common selection biases and confounding severely limit their power to make causal inferences.

Case-comparison study (case-control study, case referent study): The observational epidemiological study of persons with the disease (or another outcome variable) of interest and a suitable control group of persons without the disease (comparison group, reference group). The potential relationship of a suspected risk factor or an attribute to the disease is examined by comparing the diseased and non-diseased subjects with regard to how frequently the factor or attribute is present (or, if quantitative, the levels of the attribute) in each of the groups (diseased and non-diseased).

Cellular immunity: immunity independent of antibody but dependent on the recognition of antigen by T cells and their subsequent destruction of cells bearing the antigen or on the secretion by T cells of lymphokines that enhance the ability of phagocytes to eliminate the antigen.

Cofactor: A factor that activates or enhances the action of another entity such as a disease-causing agent. Cofactors may influence the progression of a disease or the likelihood of becoming ill.

Diagnostic criteria: The specific combination of signs, symptoms, and test results that a clinician uses to identify a person as representing a case of a particular disease or condition.

Enzyme immunoassay: An assay that uses an enzyme-bound antibody to detect antigen. The enzyme catalyzes a color reaction when exposed to substrate.

Highly active antiretroviral therapy: Treatment regimens that stop or slow the HIV virus from reproducing and keep HIV disease from progressing. The usual HAART regimen combines 3 or more HIV drugs from at least 2 different classes.

Horizontal transmission: The spread of an infectious agent from one individual to another, usually through contact with bodily excretions or fluids, such as sputum or blood, which contains the agent.

Humoral immunity: The component of the immune system involving antibodies that are secreted by B-cells and circulate as soluble proteins in blood.

Immunohistochemical staining: A method to detect specific antigens in cells based on an

antigen-antibody reaction, which can be recognized at the light microscopic level.

Latent phase: A phase of the virus life cycle during which the virus is not replicating.

Lytic phase: A phase of the virus life cycle during which the virus replicates within the host cell, releasing a new generation of viruses when the infected cell lyses.

Lytic reactivation: The biological events beginning with emergence of a virus from latency and ending with lysis of the host cell and release of progeny virions.

microRNA: small, non-coding RNA molecules approximately 22 nucleotides in length that act post translationally in a regulatory role to target messenger RNAs for cleavage or translational expression.

Monoclonal: Pertaining to or designating a group of identical cells or organisms derived from a single cell or organism.

Non-coding RNAs: Functional RNA molecules that are transcribed from DNA but are not translated into proteins.

Oligoclonal: A few different clones, or the product of a few different clones.

Paracrine neoplasia: A process whereby KSHV-infected cells induce neighboring uninfected cells to produce cytokines and growth factors that are necessary as autocrine and paracrine factors for driving tumor production.

Parenteral: By some other means than through the gastrointestinal tract; the parenteral route of infection involves breaks in the skin such as cuts and scrapes, puncture wounds, bites and burns.

Peripheral blood monocytes: Circulating precursors of macrophages and dendritic cells that migrate from the blood stream across vascular endothelium for immunological surveillance, as well as respond to inflammation.

Polyclonal: Pertaining to or designating a group of cells or organisms derived from several cells.

Polymerase chain reaction: A laboratory technique used to produce large amounts of specific DNA fragments. Polymerase chain reaction is used for genetic testing and to diagnose disease.

Titer: A laboratory measurement of the concentration of a substance in a solution (e.g., an antibody titer measures the presence and amount of antibodies in the blood).

Toll-like receptors: A family of pattern recognition receptors involved in the detection of structures associated with pathogens or damaged host tissues.

Vertical transmission: The transmission of infection from one generation to the next (e.g., from mother to infant prenatally, during delivery, or in the postnatal period via breast milk.

Abbreviations

AIDS:	Acquired Immune Deficiency Syndrome
ANGP2:	angiopoietin 2
APC:	antigen-presenting cell
BET:	bromodoman and extra-terminal
CDC:	Centers for Disease Control and Prevention
CDK6:	cyclin-dependent kinase 6
CI:	confidence interval
DNA:	deoxyribonucleic acid
DOT:	Department of Transportation
dsDNA:	double stranded DNA
EBNA:	Epstein-Barr virus nuclear antigen
EBV:	Epstein-Barr virus
EBV VCA:	EPV viral capsid antigen
ELISA:	enzyme-linked immunosorbent assays
F:	female
FDA:	Food and Drug Administration
FLICE:	FADD-like interleukin-1 beta-converting enzyme
HAART:	Highly active antiretroviral therapy
HAX-1:	HS1-associated protein X1
HHV8:	human herpesvirus 8
HIF-1α:	hypoxia-inducible factor-1α
HIV:	human immunodeficiency virus
HR:	hazard ratio
hTERT:	human telomerase reverse transcriptase
IARC:	International Agency for Research on Cancer
IFA:	immunofluorescence assay
HHV8:	human herpesvirus 8
IFA:	immunofluorescence assay
K1:	unique KSHV protein 1
K15:	unique KSHV protein 15
KS:	Kaposi sarcoma

KSHV:	Kaposi sarcoma herpesvirus
LANA:	latency-associated nuclear antigen
MHC:	major histocompatibility complex
miRNA:	microRNA
M:	male
Max:	maximum
MCD:	multicentric Castleman disease
Med:	median
Min:	minimum
MM:	multiple myeloma
MSM:	men who have sex with men
Nef:	negative factor protein
NF-κB:	nuclear factor kappa-light-chain enhancer of activated B cells
NHANES:	National Health and Examination Survey
NR:	not reported
NS:	not significant
NTP:	National Toxicolog Program
OR:	odds ratio
ORF:	open reading frames
OSHA:	Occupational Safety and Health Administration
PAN:	polyadenylated nuclear
PBMC:	peripheral blood mononuclear cells
PCR:	polymerase chain reaction
PDGF:	platelet-derived growth factor
PEL:	primary effusion lymphoma
pRB:	retinoblastoma protein
PTK:	protein tyrosine kinase
RARECARE:	Surveillance of Rare Cancers in Europe
RNA:	ribonucleic acid
RR:	relative risk
RTA:	replication and transcription activator
SEER:	Surveillance, Epidemiology, and End Results Program

TGFBeta:	transforming growth factor-β
U.K. :	United Kingdom
U.S. :	United States
U.S.A.:	United States of America
vCCL:	viral-encoded chemokines
VEGF:	vascular endothelial growth factor
vFLIP:	viral FLICE-inhibitory protein
vGPCR:	viral G protein-coupled receptor
vIL-6:	interleukin-6
vIRF:	viral interferon regulatory factors
WB:	western blot assay

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Appendix A: Literature Search Strategy

The objective of the literature search approach is to identify published literature that is relevant for evaluating the potential carcinogenicity of the Kaposi sarcoma-associated herpes virus (KSHV). As discussed in the Viruses Concept Document (https://ntp.niehs.nih.gov/ntp/roc/concept_docs/2014/virusesconcept_508.pdf), the monograph relies on the IARC monograph and studies published since the monograph (new studies). The literature search strategy was used to identify new human cancer studies and recent reviews of mechanistic data.

General approach

Database searching encompasses selecting databases and search terms and conducting the searches. Searches of several citation databases are generally conducted using search terms for the individual viruses of interest, combined with search terms for cancer and/or specific topics, including epidemiological and mechanistic studies. A critical step in the process involves consultation with an information specialist to develop relevant search terms. These terms are used to search bibliographic databases. IARC used literature found by searching PubMed for KSHV through 12/2008, so PubMed, Web of Science and Scopus were searched for new information about HTLV in from >2008 to August 2015. Table 1 highlights the general concepts searched with selected example terms. To review all the terms used, please refer the to full search strings below.

Table 1. Major topics searched

Topics	Example terms
Kaposi's sarcoma-associated herpes virus	Herpesvirus 8, human(Mesh), Kaposi's sarcoma-associated herpesvirus, human herpesvirus 8, KSHV, HHV8

The literature for KSHV was searched without using narrowing terms within the bibliographic databases. The results were then processed in EndNote to remove duplicates before being transferred to DistillerSR for screening.

The bibliographic database search results (3314) were processed in Endnote then imported into DistillerSR for first and second tier screening. Relevant studies found through the citations of review articles and other secondary searched were also included. Tagging in DistillerSR categorized the useful articles into Human Epidemiologic literature (102) or Mechanistic literature (369).

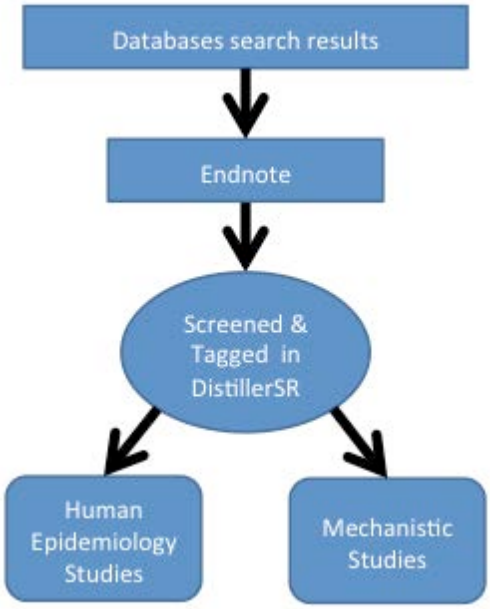

Figure 1: Literature processing flow

Search strings for KSHV Searches

Pubmed: 2008-2015

"Herpesvirus 8, human"[mh] OR "Kaposi's sarcoma-associated herpes virus"[tiab] OR "Kaposi's sarcoma-associated herpesvirus"[tiab] OR "Kaposi sarcoma-associated herpes virus"[tiab] OR "Kaposi sarcoma-associated herpesvirus"[tiab] OR KSHV[tiab] OR "human herpesvirus 8"[tiab] OR "human herpes virus 8"[tiab] OR HHV8[tiab] OR "HHV-8"[tiab]

WOS and Scopus: 2008-2015

"Kaposi's sarcoma-associated herpes virus" OR "Kaposi's sarcoma-associated herpesvirus" OR KSHV OR "human herpesvirus 8" OR "human herpes virus 8" OR HHV8

Part 2

Draft Profile

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Kaposi Sarcoma Herpesvirus

CAS No.: none assigned

Known to be a human carcinogen[1]

Also known as KSHV or human herpesvirus 8 (HHV-8)

Carcinogenicity

Kaposi sarcoma herpesvirus (KSHV) is *known to be a human carcinogen* based on sufficient evidence from studies in humans. This conclusion is based on evidence from epidemiological and molecular studies, which show that KSHV causes Kaposi sarcoma and primary effusion lymphoma, and on supporting mechanistic data. There is also limited evidence for a causal association of between KSHV infection and multicentric Castleman disease.

KSHV causes cancer, primarily in immunosuppressed people, by expression of latent proteins, lytic proteins, and microRNAs that work together to transform cells and promote cancer progression. These viral transcripts dysregulate several cellular signaling pathways, including those involved in cell-cycle progression, angiogenesis, apoptosis, immune evasion and modulation, signal transduction, and antiviral responses (Mesri *et al.* 2010, Wen and Damania 2010, IARC 2012). Although latent infection is predominant in KSHV-infected cells *in vivo* and *in vitro*, both latent and lytic viral genes contribute to the malignant phenotype (Mesri *et al.* 2010, Fukumoto *et al.* 2011).

Cancer Studies in Humans

The majority of human cancer studies of KSHV have focused on Kaposi sarcoma; however, other cancer end points, including certain B-cell non-Hodgkin lymphomas (primary effusion lymphoma and multicentric Castleman disease), also have been linked to KSHV. Molecular data show that tumor tissue from essentially all cases of Kaposi sarcoma, primary effusion lymphoma, and human immunodeficiency virus type 1 (HIV-1) -positive multicentric Castleman disease contains KSHV DNA. The data were inadequate to evaluate the association of KSHV with other cancer end points (such as multiple myeloma, other lymphomas, or prostate cancer).

Kaposi Sarcoma

There is credible evidence for an association between KSHV infection and Kaposi sarcoma based on consistent findings of increased risk in epidemiological studies with different designs and in different populations and on evidence of a dose-response relationship. The body of epidemiological studies evaluating Kaposi sarcoma risk and KSHV infection consists of approximately 25 cohort or nested case-control studies and 80 case-control studies conducted in patients with all four main epidemiological types of the disease (epidemic or HIV-1-related, iatrogenic, classic, and endemic) (see Section 3, Human Cancer Studies, Cancer Hazard Evaluation Component). Over 50 of the studies reported effect estimates or information from which to calculate estimates. In the 39 case-control studies with risk estimates, risk was often increased over 10-fold and sometimes over 100-fold. Most cohort and nested case-control studies reported risk to be increased 2- to 16-fold. Increased risk of Kaposi sarcoma was found in both HIV-positive and HIV-negative KSHV-infected individuals, the latter consisting of organ

[1]NTP preliminary listing recommendation proposed for the RoC.

transplant recipients (iatrogenic type), patients with classic Kaposi sarcoma (mostly older males in specific populations, such as in Mediterranean countries or among East Europeans of Jewish descent), or patients with endemic Kaposi sarcoma (found in sub-Saharan Africa, mostly among men but also among children). Positive associations with Kaposi sarcoma were found in studies measuring antibodies to lytic and latent antigens and KSHV DNA, and risk increased with increasing titer of antibodies to KSHV antigens (Sitas *et al.* 1999, Newton *et al.* 2003a,b, Albrecht *et al.* 2004, 2006).

KSHV has been identified in over 80% of Kaposi sarcoma cases by serology and in virtually all Kaposi sarcoma tumors and is considered to be a prerequisite for diagnosis of this cancer (Chang *et al.* 1994, Mesri *et al.* 2010, Cavallin *et al.* 2014). Furthermore, KSHV seroprevalence is highest in areas of high Kaposi sarcoma incidence, and KSHV infection precedes onset of the disease. Most Kaposi sarcoma lesions of all four epidemiologic forms exhibit an oligoclonal pattern (with independent viral clones in different lesions); however, monoclonal expansion does occur, supporting an etiologic role for the virus (Judde *et al.* 2000, Duprez *et al.* 2007). Furthermore, multifocal Kaposi sarcoma lesions generally arise from independent clones rather than via metastatic dissemination. The principal cofactor is immune suppression (e.g., resulting from HIV-1 infection or in organ transplant patients). Acquiring HIV-1 infection prior to KSHV infection may increase the risk of epidemic Kaposi sarcoma by 50% to 100% compared with HIV-1 infection acquired at the same time as or after KSHV infection.

Primary Effusion Lymphoma

There is evidence for an association between KSHV infection and primary effusion lymphoma, based on case reports, case series, and two case-comparison studies involving approximately 109 KSHV-infected patients (IARC 2012, see Section 3, Human Cancer Studies, Cancer Hazard Evaluation Component), together with histological confirmation of the tumors' specific morphological and immunophenotypical features. These studies led to the adoption of the presence of KSHV as a diagnostic criterion for the specific pathological entity that constitutes KSHV primary effusion lymphoma. Primary effusion lymphoma (also called body-cavity-based B-cell lymphoma) is a rare type of B-cell non-Hodgkin lymphoma that presents primarily as pleural, peritoneal, or pericardial lymphomatous effusions and comprises approximately 2% to 4% of HIV-1-related non-Hodgkin lymphomas (Simonelli *et al.* 2003, Sullivan *et al.* 2008). Although the majority of cases occur in HIV-1-positive patients, cases also occur in HIV-1-negative patients (IARC 2012). Approximately half of HIV-1-positive patients with primary effusion lymphoma also have Kaposi sarcoma.

Molecular evidence for an etiologic role of KSHV in primary effusion lymphoma in humans includes the following findings: (1) primary effusion lymphoma cells contain 50 to 100 copies of KSHV DNA, and the expression pattern of KSHV transcripts is almost identical to that observed in Kaposi sarcoma, (2) several KSHV-infected cell lines established from human primary effusion lymphoma cells retain a stable latent viral genome, and (3) primary effusion lymphoma lesions are monoclonal, indicating that KSHV infection precedes tumor growth (Katano *et al.* 1999, Judde *et al.* 2000, Fukumoto *et al.* 2011, IARC 2012, Giffin and Damania 2014). Immune suppression resulting from HIV-1 infection is a cofactor for development of primary effusion lymphoma among KSHV-positive individuals. Primary effusion lymphoma in HIV-1-positive patients exhibits a rapid progression with short survival times, whereas

progression in HIV-1-negative and immunocompetent patients appears to be much slower (IARC 1997).

Multicentric Castleman Disease

There is limited evidence for an association between KSHV infection and multicentric Castleman disease, based on consistent evidence from three case-series studies, four case-comparison studies, and molecular studies in humans that KSHV is associated with some forms of multicentric Castleman disease (see Section 3, Human Cancer Studies, of the Cancer Hazard Evaluation Component). Similar to primary effusion lymphoma, multicentric Castleman disease is a rare polyclonal lymphoproliferative B-cell lymphoma that can develop into plasmablastic lymphoma. Among multicentric Castleman disease patients, KSHV occurs in almost all HIV-1-positive individuals and in less than half of HIV-1-negative individuals (Parravicini *et al.* 1997, Oksenhendler *et al.* 2002, 2013). Kaposi sarcoma and multicentric Castleman disease frequently occur together in the HIV-positive population (Cronin and Warnke 2009, Reddy and Mitsuyasu 2011). The role of KSHV in multicentric Castleman disease among HIV-1-negative individuals is not clear (van Rhee *et al.* 2010). It is unclear whether other viruses, such as Epstein-Barr virus, or other cofactors play a role in the development of these conditions; their rarity has precluded large-scale studies of these factors.

In contrast to primary effusion lymphoma, in which cells predominantly express latent KSHV antigens and are monoclonal, KSHV-associated multicentric Castleman disease cells appear to express lytic KSHV antigens, including a virally encoded interleukin 6 (IL-6), and are polyclonal (Aoki *et al.* 2001, Burbelo *et al.* 2010, Fukumoto *et al.* 2011, Giffin and Damania 2014). The level of KSHV DNA in peripheral blood monocytes or plasma corresponds with the occurrence of symptoms during multicentric Castleman disease flares in HIV-positive individuals (Reddy and Mitsuyasu 2011). Multicentric Castleman disease has an aggressive course and a poor prognosis (IARC 1997).

Studies on Mechanisms of Carcinogenesis

KSHV-associated cancer develops through a complex process that involves interactions among many viral, host, and environmental factors (Mesri *et al.* 2014, Fukumoto *et al.* 2011). Although many of the mechanisms by which KSHV establishes and maintains infection are known, and many KSHV-encoded proteins and their functions have been characterized *in vitro,* some aspects of the infection and transformation still are not well understood (Fukumoto *et al.* 2011, Giffin and Damania 2014). In a healthy host, KSHV infection is not oncogenic, because it leads to latent infection that cannot transform cells (Cavallin *et al.* 2014). Although lytic infection leads to expression of oncogenic and angiogenic genes, it is cytopathic and immunogenic in immunocompetent hosts and does not transform cells. However, in an immune-compromised host, lytic-infected cells escape immunosurveillance and are able to express the full repertoire of oncogenic genes, including genes that induce angiogenesis and inflammation (Cavallin *et al.* 2014, Mesri *et al.* 2014). In addition, some latently infected cells express some of the early lytic genes but do not complete the lytic cycle (abortive lytic replication). These cells may become progressively transformed and acquire oncogenic alterations that allow them to switch back to a less immunogenic latent form.

Latent proteins and microRNAs play an important role in KSHV-associated neoplasia through inhibition of apoptosis and maintenance of latency, while KSHV lytic proteins mimic or disrupt host cytokine signals, resulting in microenvironments that promote tumor growth through

paracrine mechanisms (paracrine neoplasia or oncogenesis) (Mesri *et al.* 2010, Fukumoto *et al.* 2011, Cavallin *et al.* 2014). Paracrine-acting factors important for tumor growth include vascular endothelial growth factor, platelet-derived growth factor, and IL-6, and can drive latently infected cell proliferation, angiogenesis, inflammation, and immune evasion. One or more KSHV transcripts are expressed in all KSHV-associated tumors and are required for survival of tumor cells in culture (IARC 2012). *In vitro* and animal models have been developed that accurately reproduce many features observed in KSHV-associated cancer (Mutlu *et al.* 2007, Mesri and Cesarman 2011, Ashlock *et al.* 2014).

Biological Properties

KSHV is an enveloped double-stranded DNA gamma-2 herpesvirus (rhadinovirus) that was first identified in humans in 1994 in association with acquired immunodeficiency syndrome (AIDS) (Chang *et al.* 1994, IARC 1997, Fukumoto *et al.* 2011). A lipid membrane envelope surrounds a layer made up of six types of viral proteins, which encloses a viral capsid with five different proteins and a linear 165-kb genome (IARC 1997, 2012, Fukumoto *et al.* 2011, Giffin and Damania 2014). KSHV infects endothelial cells, epithelial cells, B lymphocytes, dendritic cells, monocytes, keratinocytes, fibroblasts, and prostate cells (IARC 1997, 2012, Fukumoto *et al.* 2011, Campbell *et al.* 2014, Giffin and Damania 2014). $CD19^+$ B lymphocytes are a long-term latency reservoir for the virus. KSHV glycoproteins bind to several host-cell receptors and initiate viral entry through inward budding of the host plasma membrane encapsulating the virus-receptor complex (endocytosis or macropinocytosis) (Griffin and Damania 2014). The virus can reproduce by cell lysis or can remain latent as a viral episome and replicate along with the host genome, using host-cell machinery.

Detection

KSHV is detected most commonly by measurement of anti-KSHV antibodies, but also by detection of DNA and viral antigens in tissues (Parravicini *et al.* 1997, Fukumoto *et al.* 2011, Bhutani *et al.* 2015, Xu *et al.* 2015). Serological tests for detecting the latent and lytic antigens encoded by KSHV have varied in sensitivity and specificity. Different antigens are expressed during different phases of the viral life cycle, and an individual's antibody response to these antigens varies, resulting in uncertainty about the comparative prevalence of KSHV in different populations (IARC 2012). The level of anti-KSHV antibodies depends on the phase of the infection, with low levels in latently infected individuals and higher levels during lytic infections or in patients with Kaposi sarcoma (IARC 2012). Antibodies against lytic or latent antigens can also help differentiate the predominant phase (IARC 1997, Fukumoto *et al.* 2011). Viral DNA can be detected in Kaposi sarcoma, primary effusion lymphoma, or multicentric Castleman disease tissues by polymerase chain reaction (IARC 1997, Fukumoto *et al.* 2011, Campbell *et al.* 2014). Patients with Kaposi sarcoma or multicentric Castleman disease also tend to have higher viral loads in peripheral blood mononuclear cells than do other KSHV-infected individuals. The viral homolog of IL-6 encoded by KSHV can be detected in multicentric Castleman disease and primary effusion lymphoma patients (in the tumor cells or in the blood), but not in Kaposi sarcoma patients (Parravicini *et al.* 1997, Fukumoto *et al.* 2011, Bhutani 2015).

Exposure

Prevalence studies measuring antibodies to KSHV have shown that a significant number of people in the United States are exposed to KSHV.

Transmission

Transmission of KSHV appears to be primarily via saliva (IARC 2012). The presence of KSHV in peripheral blood suggests that transmission via blood also is possible, and transmission has been reported in injection drug users, transfusion recipients, and organ transplant recipients. In populations with high endemic KSHV prevalence, both horizontal and vertical (mother to child) transmission appear to occur, primarily in children between the ages of 6 and 10 years (particularly if the mother is infected), and infection rates increase with age. Risk factors for infection may include contact with infected family members, contaminated water, possibly insect bites (Coluzzi *et al.* 2003, Amodio *et al.* 2011) and, in particular, HIV infection (IARC 2012); factors that increase the risk of HIV infection (e.g., number of sexual partners) also increase the risk of infection with KSHV. (Smith *et al.* 1999, Engels *et al.* 2007, IARC 2012); however, uninfected heterosexual individuals have been reported to have a higher risk of infection if a sexual partner is KSHV-positive than if both partners are KSHV-negative, despite apparent similarities in factors related to frequency and type of sexual activity (Dupuy *et al.* 2009).

Seroprevalence Studies

In the first systematic evaluation of KSHV epidemiology in the general public in the United States (based on enzyme immunoassays of serum samples from the Third National Health and Nutrition Examination Survey, 1988–1994), overall KSHV seroprevalence was approximately 7% and was similar in men and women (Engels *et al.* 2007). A previous study of 1,000 U.S. blood donors (sampled in 1994 and 1995) reported KSHV seropositivity estimates ranging from 0.5% to 5% (Pellett *et al.* 2003, IARC 2012). KSHV prevalence rates appear to vary widely in different populations, from 2% to 3% in northern Europe to over 50% in some sub-Saharan African populations (IARC 2012).

Diseases (Non-Cancer), Prevention, and Treatment

Most otherwise healthy individuals who are infected with KSHV are asymptomatic (DHHS 2013a, ACS 2014, NCI 2014). There are very few reports of primary infection with KSHV; symptoms associated with initial KSHV infection include a febrile maculopapular skin rash (Andreoni *et al.* 2002), diarrhea, fatigue, localized rash, lymphadenopathy (Wang *et al.* 2001), fever, splenomegaly, cytopenia, and bone-marrow failure with plasmacytosis (Luppi *et al.* 2000a). Active KSHV infection may be associated with fever, cutaneous rash, and hepatitis (Luppi *et al.* 2000b). There is conflicting evidence regarding suggested associations between KSHV infection and pemphigus vulgaris, pemphigus foliaceus, and actinic keratosis (Ablashi *et al.* 2002). KSHV has been found in inflammatory cells in isolated cases of interstitial pneumonitis, in sarcoid tissue, and in histocytic necrotic lymphadenitis, but an etiologic role of KSHV in these diseases has not been established.

Because KSHV transmission is associated with KSHV shedding in saliva and occasional shedding in genital secretions, avoiding salivary exposure (e.g., via kissing or sharing food, drink, or toothbrushes) and following safe sexual practices may theoretically prevent transmission (Chang-Moore Laboratory 2009, DHHS 2013a,b). Some drugs have been reported

to reduce KSHV shedding and others have been reported to inhibit KSHV shedding; however, currently there are no FDA-approved drugs for treatment of KSHV infection. Highly active antiretroviral therapy was associated with an 89% decrease in KSHV shedding frequency (Cattamanchi *et al.* 2011). There is no vaccine against KSHV although limited vaccine development efforts are ongoing (Wu *et al.* 2012, ACS 2014).

Regulations

Department of Transportation (DOT)

Infectious substances are considered hazardous materials, and special requirements have been set for marking, labeling, and transporting these materials.

Occupational Safety and Health Administration (OSHA)

Comprehensive regulations have been developed for employers to develop and adhere to exposure control plans for bloodborne pathogens.

All work-related needlestick injuries and cuts from sharp objects that are contaminated with another person's blood or other potentially infectious material must be recorded.

First-aid training program trainees must have adequate instruction in the value of universal precautions for preventing infectious diseases.

Guidelines

Food and Drug Administration (FDA)

The FDA has issued numerous guidance documents prescribing procedures (e.g., use of standardized labels, abbreviated donor screening questionnaires) for reducing the risk of virus transmission by blood and blood products (FDA 2015).

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