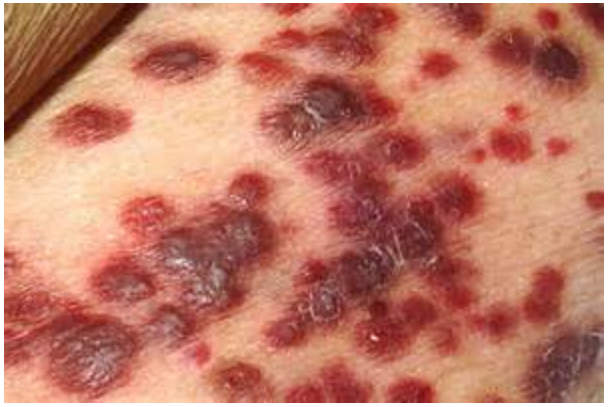
NTP
National Toxicology Program

Draft RoC Monograph
Kaposi Sarcoma Herpesvirus



Stan Atwood, MS, DABT

Integrated Laboratory Systems, Inc.

Contract support to the ORoC

NTP Peer Review Meeting

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Kaposi Sarcoma Herpesvirus (KSHV)

Outline

Properties and detection

Prevalence and transmission

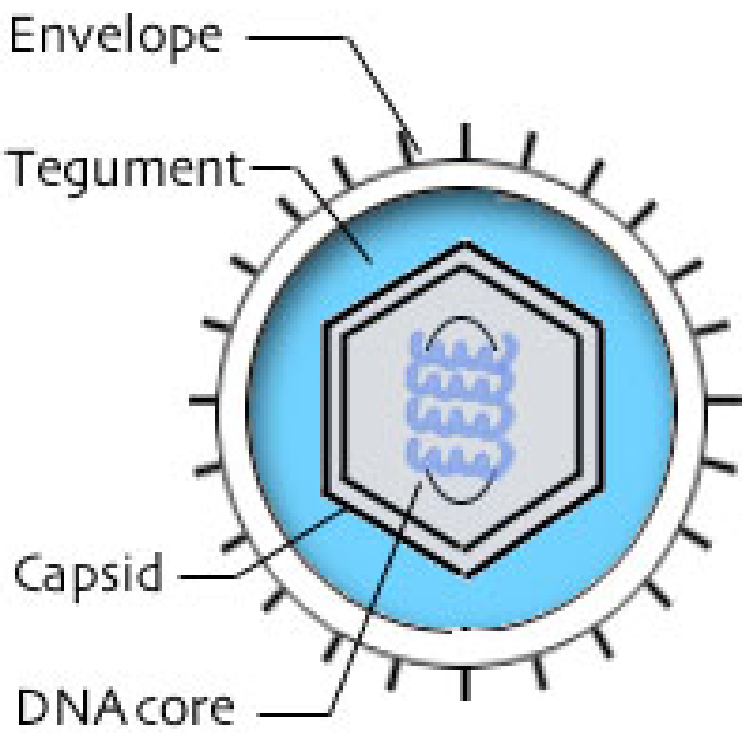
Mechanistic data

Human cancer studies

Preliminary level of evidence summary

KSHV Properties

- Rhadinovirus
  - γ-2 herpesvirus (HHV-8)
  - 1st human member
- 100 to 150 nm
- Linear ds DNA
  - ~165 kbp
  - 87 ORFs
    - >80 viral proteins
    - 17 miRNAs



KSHV Detection in Blood and Tissues

KSHV infects many cell types

- Viral DNA
- Viral proteins
- Anti-KSHV antibodies
  - Latency-associated nuclear antigen (LANA)
  - Lytically expressed capsid antigen K8.1

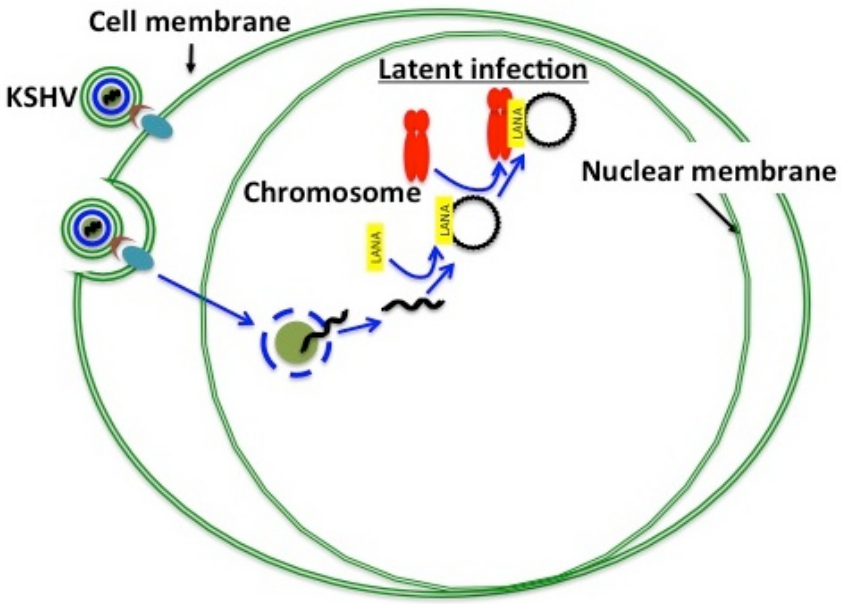
Significant Exposure in the United States

Prevalence and transmission

- Prevalence
  - High endemic; sub-Saharan Africa (30%-70%)
  - Low endemic; Mediterranean (10%-25%)
  - Non-endemic, general population (<10%)
    - United States (0.5%-7%)
    - HIV+ men (30%-60%)
    - HIV negative men that have sex with men (MSM) (20%-30%)
- Transmission
  - Saliva (major)
  - Blood
  - Organ transplant
  - Sexual activity (higher risk in MSM regardless of HIV status)

KSHV Latent Infection and Replication

Maintained as episome



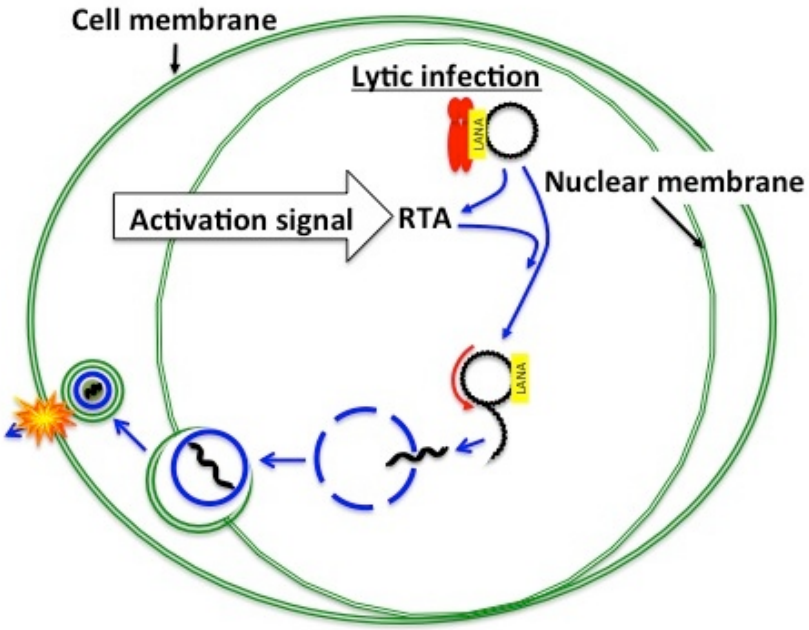
Characteristics

- No new virions produced
- CD19+ B cells long-term reservoir
- Low copy number
- Latent genes
  - LANA
  - vCyclin
  - vFLIP
  - Kaposins A and B
  - miRNAs

LANA = latency associated nuclear antigen, vFLIP = viral FLICE-inhibitory protein, miRNAs = micro RNAs

KSHV Lytic Reactivation and Replication

New virions produced



Characteristics

- Induced by cell stressors
- Produces new virions
- Infects new cells
- All genes expressed
  - RTA
  - K1, K15
  - vIRFs
  - vIL-6
  - vCCLs
  - vGPCR

RTA = replication and transcription activator, vIRFs= viral interferon response factors, vIL-6 = viral interleukin-6, vCCLs = viral-encoded chemokines, vGPCR = viral G-protein coupled receptor

Latent and Lytic Genes Role in Malignancy

Inadvertent consequence of viral survival mechanisms

- Mechanisms
  - Evading immune response (e.g., vFLIP, K3, K5, vIRFs)
  - Dysregulated cell-cycle progression (e.g., vCyclin, LANA)
  - Evading apoptosis (e.g., LANA, K1, K15, vFLIP, vBCL-2)
  - Angiogenesis (e.g., LANA, K1, vCCL, vIL-6, vGPCR)
  - Cell transformation (e.g., LANA, Kaposins, K1, vGPCR, RTA)
- Immune-compromised host
  - Lytic infection escapes immunosurveillance
  - Abortive lytic/paracrine mechanisms promote oncogenesis
    - Early lytic genes expressed without full execution of lytic cycle and are transformed back to less immunogenic latent form
    - Subset of cells express early lytic genes and paracrine-acting growth factors (VEGF, PDGF, IL-6) drive cell proliferation, angiogenesis, inflammation, support immune escape

vBCL2 = viral B-cell lymphoma 2, VEGF = vascular endothelial growth factor, PDGF = platelet-derived growth factor

Endpoints with sufficient or limited evidence

- Kaposi sarcoma
  - Epidemic (HIV/AIDS-related)
  - Iatrogenic (organ transplant recipients)
  - Classic (older males, Mediterranean, E. European Jews)
  - Endemic (adults and children, sub-Saharan Africa)
- Primary effusion lymphoma
- Multicentric Castleman disease

KSHV and Kaposi Sarcoma (KS)

Sufficient level of evidence from human studies

Data source	Results
Epidemiology Studies with positive associations	38/39 case-control[a] and all 16 cohort/nested case-control; ORs/RRs – mostly significant and some very high (10- to >100-fold) Elevated RR in both HIV- and HIV+ populations and in all subtypes of Kaposi sarcoma Dose-response in several studies
Human tissue	
Clonality	Oligoclonal, monoclonal in subset of advanced lesions
% KSHV-infected tumors	>99% (H1 copy/cell)
KSHV protein expression	
Latent	LANA-1, vCyclin, vFLIP, Kaposin A and B
Lytic[b]	RTA, K1, vIRFs, vIL-6, vGPCR, vCCLs, K15
Other	Infection precedes KS onset, antiherpesvirus drug protected AIDs patients from new occurrence, KS incidence mirrors KSHV seroprevalence

[a] studies reporting risk estimate

[b] Expressed by small proportion of cells in KS lesions

OR = odds ratio, RR = relative risk, LANA = latency–associated nuclear antigen, vFLIP = viral FLICE-inhibitory protein, RTA = replication and transcription activator, vIRFs, = viral interferon response factors, vIL-6 = viral interleukin 6, vGPCR = viral G-protein-coupled receptor, VCCLs = viral encoded chemokines

KSHV and Primary Effusion Lymphoma (PEL)

Sufficient level of evidence from human studies

Data source	Results
Epidemiology Studies with positive associations	Found in 109 cases; H76 individual case reports & 31 cases in three case-series & two comparison studies Found mostly in HIV+ cases but also in HIV – cases 50% cases develop Kaposi sarcoma
Human tissue	
Clonality	Monoclonal
% KSHV-infected tumors	100% (50-100 copies/cell)
KSHV protein expression	Similar to Kaposi sarcoma
Other	KSHV is part of diagnostic criteria, previous Kaposi sarcoma diagnosis associated with increased risk of PEL, expression of KSHV viral genes required for survival of PEL cells in culture.

KSHV and Multicentric Castleman Disease

Limited level of evidence from human studies

Data source	Results
Epidemiology Positive associations	4/4 case comparisons studies; very high ORs
Human tissue	
Clonality	Typically polyclonal, monoclonal B-cell expansions
% KSHV-infected tumors	~100% HIV-1+ and <50% HIV-1-
KSHV protein expression	LANA-1, vIL-6, KSHV lytic phase
Other	Frequently found with KS and/or PEL; KSHV inhibitors show some therapeutic success

OR = odds ratio

KSHV and Cancer Risk Cofactors

- Immunosuppression
  - HIV-1 (all clinical subtypes)
  - Organ transplant recipients (liver and kidney)
  - Immunosenescence (elderly, classic subtype)
- Risk factors with limited evidence
  - Co-infection with other viruses (e.g., EBV, HPV)
  - Diabetes, oral corticosteroids (classic subtype, HIV negative)

KSHV Preliminary Level of Evidence Summary

- Cancer sites with sufficient evidence
  - Kaposi sarcoma
  - Primary effusion lymphoma
- Cancer sites with limited evidence
  - Multicentric Castleman disease
- Immunosuppression is an important cofactor
- Role of viral transcripts
  - Latent transcripts promote host cell proliferation, maintain latency and inhibit apoptosis
  - Lytic transcripts dysregulate cell signaling pathways and contribute to the angiogenic and inflammatory oncogenic phenotype via paracrine mechanisms

Clarifications?

Peer Reviewer Comments

All sections: Comment on whether the information is clear and technically accurate and identify any information that should be added or deleted,

Properties, Detection and Human Exposure

- and whether adequate information is presented to document past and/or current human exposure.

Human Cancer Studies

- and provide any scientific criticisms of NTP's cancer assessment of the epidemiologic studies of exposure to the virus.

Mechanistic and Other Relevant Data

- and provide any scientific criticisms of the NTP's synthesis of these data assessing effects of the virus.

Level of Evidence Conclusion (Vote)

KSHV is known to be a human carcinogen based on sufficient evidence in humans.

- Cancer sites with sufficient evidence
  - Kaposi sarcoma
  - primary effusion lymphoma
- Cancer sites with limited evidence
  - multicentric Castleman disease

Preliminary Listing Recommendation (Vote)

KSHV

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 between KSHV infection and multicentric Castleman disease.

Draft Substance Profile

- Contains NTP's preliminary recommendation of the listing status of the substance.
- Summarizes the scientific information that is key to reaching a recommendation.
- Provides information on properties, use, production and exposure.
- Provides information on existing federal regulations and guidelines.

Peer Reviewer Comments

- Provide any new comments (e.g., not previously provided on the same facts or issues in the cancer hazard evaluation section) on whether the informtion on properties and detection, human exposure, cancer studies in humans and mechanistic data is clear and technically accurate.
- Comment on whether the substance profile highlights the information on cancer studies in humans and mechanistic data that are considered key to reaching the listing recommendation.