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University of Nevada, Reno

**Kaposi's Sarcoma: Literature Review on the mechanism of Kaposi's Sarcoma
encoded Latency-Associated Nuclear Antigen and A Clinical Case Study**

A thesis submitted in partial fulfillment
of the requirements for the degree of

BACHELOR OF SCIENCE IN BIOCHEMISTRY AND MOLECULAR BIOLOGY

By

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We recommend that the thesis
prepared under our supervision by

Jessica Chen

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Dr. Josh Baker, Ph.D., Thesis Advisor

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Abstract

Whether genetic, geographic, or acquired; a multitude of factors can bring about Herpesvirus 8 induced Kaposi's Sarcoma, of which four subtypes have been identified: Classic, African endemic, AIDS-related, and iatrogenic Kaposi's Sarcoma. A Case Study has been incorporated to illustrate the clinical manifestations of the latter type of Kaposi's Sarcoma; a rather atypical occurrence in which the patient does not present with the characteristic purple, cutaneous lesions. A mechanistic approach examines just how this tumor establishes latency in infection and how the Latency-Associated Nuclear Antigen (LANA) that Herpesvirus 8 encodes for, downregulates various regulatory proteins, such as tumor suppressor cells: p53, VHL, and the Retinoblastoma protein and therefore promote oncogenesis.

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Introduction: An overview of the Disease

Overview:

Historically having been erroneously referred to as the “AIDS rash,” Classic Kaposi’s Sarcoma which is characterized by purple or red lesions was first discovered in 1872 by Moritz Kaposi, in older males of Jewish or Mediterranean descent. However, AIDS related or epidemic Kaposi’s Sarcoma is one of the four types of this cancer, later described in 1981 when an epidemic of Kaposi’s Sarcoma was observed in 95% of young heterosexual or bisexual males. Before then, around the 1950’s, an endemic took hold in Africa, particularly near the equatorial region affecting about 1 female in every 10-15 males. Lastly, patients who were iatrogenically immunosuppressed as seen in transplant patients, for instance, were found to be at a significantly increased risk of developing Kaposi’s Sarcoma due to weakened immune responses from the drugs they are required to take to prevent graft or donor tissue rejection. Obviously, Kaposi’s Sarcoma can be transmitted sexually, vertically from mother to child, via blood transfusions, but the horizontal route by saliva is also a common mode of transmission (Pica F, Volpi A).

Nevertheless, these four types of Kaposi’s Sarcoma are still distinguished by the dermal lesions that may first appear as a flat patch or plaque and progress into a raised nodule or tumor. Although the lesions are generally found on the skin, they can also develop in the gastrointestinal tract or respiratory tract and cause complications such as abdominal pain, bloody stool, and shortness of breath. According to clinical observations, Kaposi’s Sarcoma has been classified as a multifocal rather than metastatic neoplasm. Furthermore, blood vessels become leaky and high amounts of the iron-storage complex,

haemosiderin deposits into red blood cells. It wasn't until 1994 when biopsies indicated that Herpesvirus 8 was the pathogen responsible for Kaposi's Sarcoma and the term Kaposi's Sarcoma-associated Herpesvirus was coined. What was discovered in these lesions and tumors was that KSHV attacked endothelial cells, particularly spindle cells; however more than just the integumentary system, KSHV targeted B lymphocytes as well (Mesri, E.A., Cesarman, E., and Boshoff, C).

Disease progression:

There is no universal system for staging Kaposi's Sarcoma; however a proposed system is as follows: Macronodular is the first stage, characterized by small lesions usually confined to the lower extremities. Larger lesions such as plaques constitute the Stage II: Infiltrative Stage, while larger plaques and nodules define Stage III: Florid Stage. Stage IV: Disseminated Stage extends beyond the lower extremities and is characterized by multiple large lesions (Roy, Sampurna, M.D.).

Virology/Pathology:

Kaposi's Sarcoma-associated Herpesvirus (KSHV) or also known as Herpesvirus 8 belongs to the gammaherpesvirus family and targets B lymphocytes as well as endothelial cells for infection. KSHV is approximately 160 – 170 kilobase and has double stranded, linear DNA; however like all herpesviruses which can express latent or lytic infection, upon latent infection by KSHV, the viral genome exists as an episome (double stranded circular DNA). Typically the latent phase predominates but in immunocompromised patients in particular, latent cells can be reactivated into lytic replication which releases virions. The viral genome in lytic infection exists instead as a linear, double stranded DNA molecule (Ye, F., Lei, X., Gao, S.J.). A part of the coding region of Herpesvirus 8's open reading frame (ORF) consists of at least 87 genes flanked by G-C rich terminal repeats that act as the origin of replication, thus the virus couples to the host's cellular replication machinery. On each terminal repeat unit are two LANA (Latency-Associated Nuclear Antigen) binding sites: LBS1 and LBS2, the former has a greater affinity than the latter. (Verma, S.C., et al). Herpesvirus 8 tethers its genome to

the histones of the host chromosome and recruits the host cell's replication machinery. The virus does so by detecting cell signals and having regulatory sites on its promoter that bind host cell factors and thus triggers viral replication (Alka, P., Lu, M., Lukac, D., Zeichner, S).

A number of genes that are viral homologues with oncogenic properties have been discovered to be associated with Herpesvirus 8 such as type D-cyclin, kaposin, vFLIP, and LANA. Type-D Cyclin which is also called HHV-8-v-cyclin and encoded by ORF72 (West, J.T., Wood, C.), is associated specifically with cyclin-dependent kinase 6 (Cdk6) which plays a role in control of the cell cycle checkpoints as well as in regulating tumor suppressor protein Retinoblastoma (Rb). Three types of kaposin, encoded by ORF12 (Wen, K.W., Damania, B.) exist: A, B, and C and it has been shown in transfected Rat cells that Kaposin A has oncogenic potential in that it induces aggregation of lymphocytes (Zuckerman, A.J.) which explains why Kaposi's Sarcoma is classified as a lymphoma. Cell proliferation is further progressed by vFLIP (viral FLICE-like inhibitory proteins) which inhibits apoptosis and as a result contributes to tumor survival (Zuckerman, A.J.). The key gene product of interest is LANA-1: latency-associated nuclear antigen, encoded by ORF73, which plays multifunctional roles to maintain the episome and bind the viral genome to the host chromosome as mentioned earlier (Verma, S.C, et al.). In order to maintain latency, LANA represses the transcriptional activity of a gene which activates the lytic phase involving viral replication and release of virions: RTA which stands for Replication and Transcription Activator, encoded by ORF50 (Mesri, E.A., Cesarman, E., Boshoff, C). Despite its crucial role during latency, LANA

interferes with many regulatory proteins; in particular, tumor suppressors, of which will be the main focus of this paper.

LANA targets proteins for degradation via ubiquitylation, such as the well known tumor suppressor proteins: p53, von- Hippel Lindau, and Retinoblastoma and thus in turn promotes tumorigenesis. p53 responds to cellular stresses by regulating apoptosis, controlling the cell cycle, sensing DNA damage, amongst other critical functions. Similarly, von Hippel-Lindau (VHL) regulates cell division and other genes but more importantly, the protein plays a role in sensing of oxygen levels. Its main function is to target the hypoxia-inducible transcription factor (HIF-1 α) for degradation as HIF-1 α promotes angiogenesis. Via direct and indirect mechanisms, which will be addressed in the second chapter, LANA also affects tumor suppressor protein Rb. Therefore, when LANA interferes with the functions of these tumor suppressors, viral proliferation persists.

As for lytic infection by KSHV, which is reactivated by activation of Replication and Transcription Activator (RTA), gene expression in the host is repressed as a result of accelerated mRNA turnover mediated by viral SOX protein expression, which is involved in processing and packaging the genome of the herpesvirus. The SOX (shut off and exonuclease) protein degrades mRNA by reducing the half life of the mRNAs targeted and thereby activates host transcription of angiogenic or oncogenic factors. In summary, infection by KSHV of human endothelial cells leads to genetic instability, which is a hallmark of cancer in humans, causing aberrant formation of the spindle cell, chromosomal misalignment, duplication of centrosomes, and multinucleation (Si, H., Robertson, E.S.).

Diagnosis:

Often times Kaposi's Sarcoma patients can be asymptomatic; however as mentioned earlier, one of the most apparent manifestations of Kaposi's Sarcoma is the distinct red or purple lesions. Consequently, diagnosis of the cancer is confirmed via a biopsy of dermal lesions and to check for the more life-threatening lesions that may be in the lungs or gastrointestinal tract, a chest x-ray or bronchoscopy and a gastrointestinal endoscopy is performed. A CT (computerized tomography) scan can also be used to detect Kaposi Sarcoma in the lymphatic system. Since it is unlike other cancers and the type of Kaposi's Sarcoma is a factor for consideration, staging the disease is taken by a case by case approach and stratification follows the system developed by the AIDS Clinical Trial Group which classifies risk by tumor, immune status, and systemic illness as summarized in *table 1* below. The reason CD4 cell count is considered, is because LANA downregulates the Major Histocompatibility Complex II (MHC II), which associates with the CD4 marker on Helper T cells.

Table 1: Diagnostic Scheme for Evaluating Kaposi's Sarcoma

Shows the general diagnostic guide by which Kaposi's Sarcoma is evaluated according to whether the risk is high or low depending on three main factors (Oncolink.org).

	Good Risk	Low Risk
Tumor	Confined to skin and/or lymph nodes - Minimal oral disease (non-nodular KS confined to palate)	-Tumor-associated edema or ulceration -Extensive oral KS -Gastrointestinal KS -Respiratory KS
Immune Status	CD4 cell count >200/ μ L	CD4 cell count <200/ μ L
Systemic Illness	-No history of opportunistic infections or thrush -No "B" symptoms- unexplained fever, night sweats, <10 percent involuntary weight loss, or diarrhea persisting more than two weeks. -Karnofsky performance status >70 (independent and functional without assistance)	- History of OI or thrush - "B" symptoms present - Karnofsky performance status <70 Other HIV-related illness (e.g., neurologic disease, lymphoma)

Treatment:

Currently, there is no cure for Herpesvirus 8; thus the objective for treating Kaposi's Sarcoma is to build immunity and induce regression. For patients diagnosed with AIDS-related Kaposi's Sarcoma, HAART, which stands for highly active antiretroviral therapy has been implemented as a means of improving immunity in immunosuppressed patients via a mix of drugs, popularly referred to as the "AIDS cocktail." The cocktail includes three major of classes of drugs aimed at reducing the virus: nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors, and integrase inhibitors (Stuart, A). These inhibitors suppress the enzymes which are necessary for the

herpesvirus to produce more progeny. Inhibiting HIV-1 cofactors that promote tumor growth and reducing cytokine and pro-angiogenic production are some possible mechanisms of HAART that have been proposed to lead to regression of Kaposi's Sarcoma. Radiation therapy, Intralesional Chemotherapy are other rational treatments, as well as the use of topical retinoids for the lesions. Potential drugs that target vascular endothelial growth factor (VEGF), vFLIP, and other factors that cause Kaposi's Sarcoma are also being investigated, in which the biological effects include inhibiting angiogenesis, inflammation, and proliferation of spindle cells. Another potential therapy for treating Kaposi's Sarcoma, particularly tumors is using Nutlin-3 which is a small imidazoline analog that disrupts the interaction between murine double minute (mdm2) and p53, thus activating the tumor suppressor (Sarek, G. et al).

Literature Review

Latency-Associated Nuclear Antigen (LANA) of Kaposi's Sarcoma Herpesvirus (KSHV) inhibits tumor suppressor proteins: p53, VHL, and Retinoblastoma and thus promotes oncogenesis.

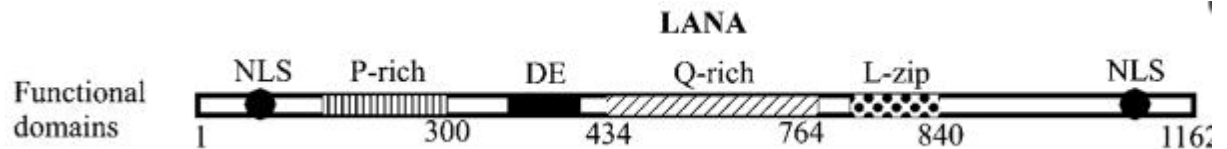
Human Herpesvirus 8 or Kaposi's Sarcoma-Associated Herpesvirus (KSHV) is unlike other viruses in that it not only pirates host genes but encodes many cellular homologues, one of which has been expressed in a significant majority of Kaposi's Sarcoma malignancies: Latency-associated nuclear antigen (LANA). LANA is a protein encoded by open reading frame 73 on the viral genome and serves a crucial role in maintaining the episome during latency in infected host cells. In a lytic infection, the viral genome persists in a linear form. In order to maintain latency, LANA is capable of affecting many regulatory pathways such as apoptosis, cell cycle control, and transcription.

The Structure of LANA

Three major domains (as shown in *Figure 1* below) make up LANA's amino acid sequence: a 337-amino acid N-terminal domain, 240-amino acid C terminal domain, and a 585 amino acid central domain comprised primarily of repeats of the amino acids: glutamic acid, aspartic acid, and glutamine (Verma, S.C., Lan, K., Robertson, E.). Both the N-terminal and the C-terminal contain a nuclear localization signal (NLS). The N-terminal domain is rich in proline residues and is where LANA tethers to the histones of the host chromosome during mitosis, particularly to H2A and H2B while the C-terminal domain consists of a leucine zipper motif which is important in interactions with cellular proteins such as p53 (Hu, J., Garber, A.C., Renne, R.). It is these functionalities of the

LANA domains that help recruit the host replication machinery so that viral DNA can be replicated.

Figure 1: Domains of LANA



In addition to contributing to the proliferation of Herpesvirus 8, LANA supports viral persistence and tumorigenesis by interfering with tumor suppressor proteins. In inhibiting the p53 pathway which serves an anticancer function in regulating cell proliferation, LANA binds p53 to its C terminus, which plays a role in maintaining genetic stability, regulating programmed cell death and arresting the cell cycle at the G1 checkpoint to prevent excessive cell growth in response to stressors such as hypoxia, oncogenes, and DNA damage. According to an experiment conducted by Si Huaxin and Erie Robertson, the LANA transfected cell lines that they generated not only demonstrated an increase in cells entering S phase, thereby amplifying cell proliferation, the human B cells (from the BJAB cell line) expressing LANA indicated chromosomal instability which was marked by multinucleation and abnormal centrosomes, as well as micronuclei. More than 15% of the cells expressing LANA had three or more centrosomes per cell compared to the less than 2% in the controls without LANA expression (Si, H., Robertson, E.S.).

Inhibition of the p53 pathway by LANA

The mechanism by which LANA inhibits p53 function involves recruiting the EC₅S Ubiquitin Complex, (illustrated in *Figure 2*) which tags the tumor suppressor protein for degradation via the ubiquitylation process. The Ubiquitin Complex accomplishes the tagging process with the help of an E3 ligase encoded by the SOCS-box-motif (Suppressors of Cytokine Signaling box) of LANA and its interaction with E1 and E2 to transfer the ubiquitin to the protein targeted for degradation. E1 is an ubiquitin-activating enzyme which transfers ubiquitin to E2, a conjugating enzyme. In other words, LANA interferes by mimicking a molecule that adapts specifically to the E3 complex. The E2-SOCS-box protein associates with an Elongin BC Complex through binding of a Cul5/Rbx1 hemagglutinin module which stabilizes LANA. It has been shown that Elongin C of the Elongin BC Complex interacts with the amino-terminal of LANA (Cai, Q., et al.). Together, these components comprise of the EC₅S Ubiquitin Complex that marks p53 and VHL for degradation. Phosphorylation of p53 by various kinases can lead to interaction of the tumor suppressor to either activators of transcription or negative regulators such as the human double minute 2 protein (hdm2) through its E3 ubiquitination ligase activity that targets p53 for degradation (Chen, W. et al.). In mice, the formation of tumors were observed in the presence of mdm2 (murine double minute 2) expression which is homologous to hdm2 therefore indicating that hdm2/mdm2 is an oncogene. Furthermore when Chen et al., immunoprecipitated hdm2, LANA was detected, thus suggesting that LANA also interferes with this p53 negative regulator. It has been suggested that hdm2 either interacts with LANA directly or indirectly by changing the conformation of p53 so that it has affinity in binding LANA. It is through

the release of hdm2, along with other posttranslational modifications, that activate p53 when DNA damage is detected. As mentioned in the treatment section of Chapter 1, the Nutlin-3 molecule has cytotoxic effects which interferes with the interaction between p53 and mdm2, leading to apoptosis in KSHV-infected cells. Nutlin disrupts this interaction by acting as a competitive inhibitor (Sarek, G., et al.).

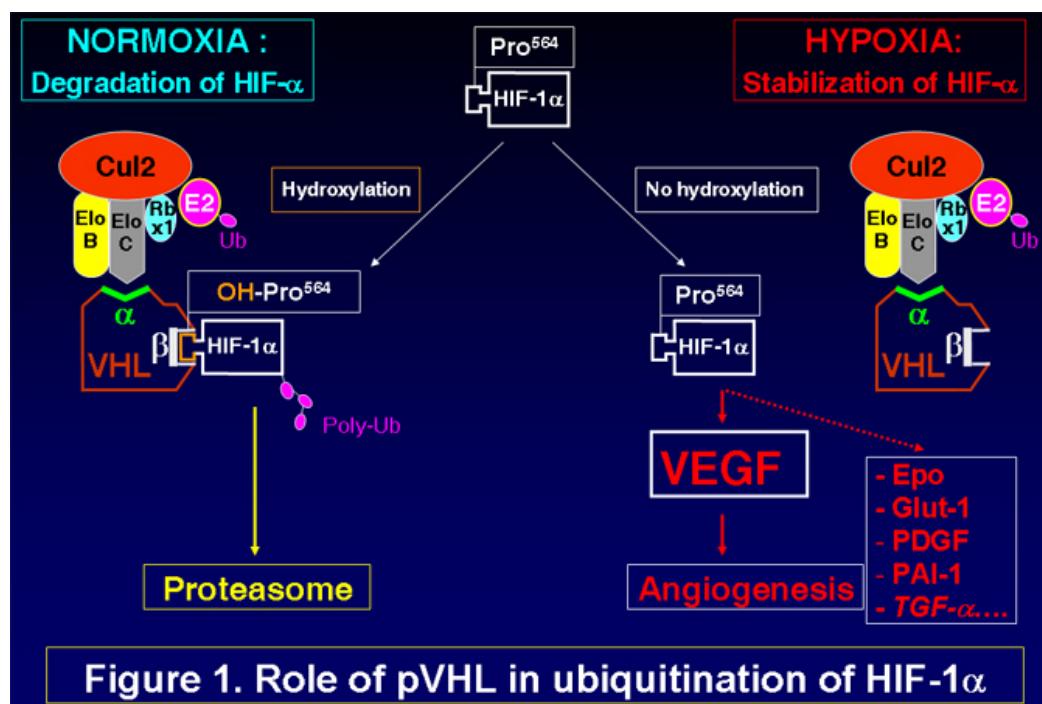
Another mechanism by which LANA downregulates p53 is in upregulating the abnormal expression of Aurora A kinase which has been known to regulate tumorigenesis and induce chromosomal instability. In fact, overexpression of Aurora A is actually quite common in many tumor type cancers in humans, so unsurprisingly increased mRNA transcript of Aurora A was found in KSHV positive tissue but not in KSHV negative tissue in the experiment done by Cai, et al. In other words, this kinase which is a checkpoint protein in mitosis associated with the centrosome, enhances the affinity of p53 for binding to LANA and destabilizes p53 by upregulating an oncogene (Cai, Q., et al). To confirm that LANA does affect Aurora A and therefore p53, Cai, et al. knockdowned LANA and as a result they observed a reduction in Aurora A transcripts. The mechanism behind this reveals that LANA targets one of the three cis elements comprising the promoter of Aurora A: transcription factor Sp1. Furthermore, Aurora A enhances the first mechanism discussed: ubiquitylation of p53 by augmenting the tagging function of LANA's SOC-motif. Phosphorylation at Serine residues 215 and 315 also facilitate LANA-induced degradation of the tumor suppressor.

Downregulation of VHL by LANA

As with p53, the von-Hippel-Lindau (VHL) tumor suppressor which regulates oxygen concentration and gene expression (refer to *Figure 2*), is degraded by the ubiquitin protein in the same manner described above for the deregulation of p53. Regulation by VHL is necessary because under conditions of hypoxia (low oxygen), hypoxia-inducible factors (HIFs), in particular HIF-1 α activates a number of genes that promote angiogenesis, which feed tumors the necessary nutrients it needs to flourish. As a result of deregulation of von-Hippel-Lindau by LANA, HIF-1 α is stabilized and the hypoxia pathway therefore prevails, contributing to the development of tumors as paracrine angiogenic and inflammatory factors such as VEGF (Vascular Endothelial Growth Factor) are secreted (Mesri, E.A., Cesarman, E., Boshoff, C.).

Figure 2: VHL Regulatory Activity

Shows VHL regulation of oxygen levels and its activity. Under conditions of Normoxia, VHL tags HIF-1 α for degradation via ubiquitylation; however in conditions of hypoxia, HIF-1 α activates factors that increase the levels of oxygen. *It should be noted, that Cul2 should actually be Cul 5 as confirmed in a study done by Qi-Liang Cai, et al.

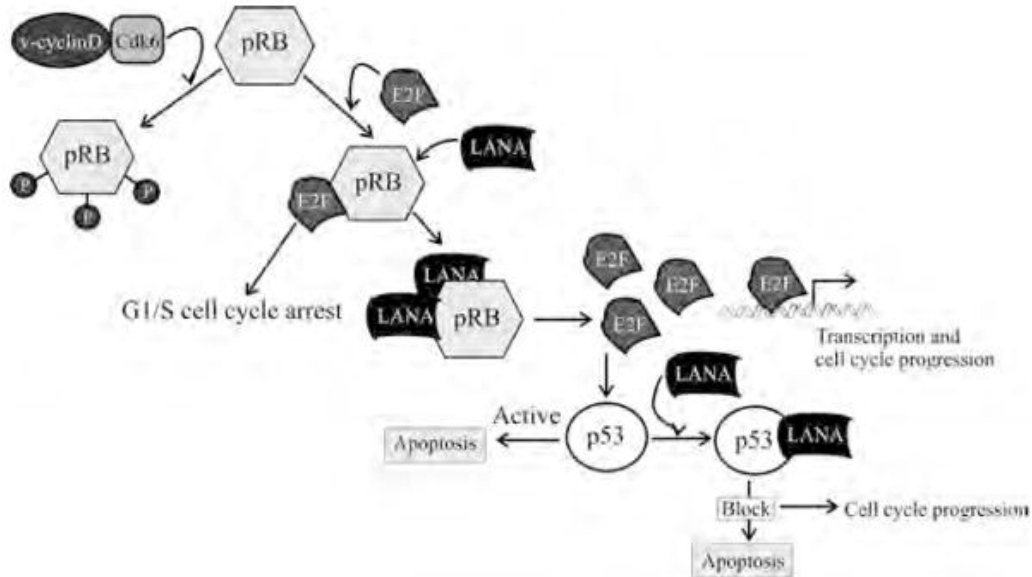


LANA deregulates the Retinoblastoma protein

Retinoblastoma (pRb) is another tumor suppressor protein that belongs to the “pocket binding protein” family and is responsible for regulation of the cell cycle at the G₁ checkpoint to assure that damaged DNA does not proceed into the S phase for replication. pRb interacts with the E2F family of transcription factors, triggering suppression of cell growth (Verma, S.C., Lan, K., Robertson, E.). When pRb is in its inactive state which is induced by Cyclin Dependent Kinases (Cdks) phosphorylation (hyperphosphorylation), cell cycle progression occurs while hypophosphorylation turns the protein on by causing the E2F factors to dissociate so that progression into the S phase can be inhibited. Typically D type cyclins are associated with Cdk 6 which initiates a cascade that phosphorylates protein Retinoblastoma. However as mentioned in Chapter 1, the KSHV encoded homolog v-cyclin, which acts like D-type cyclins, have been known to hyperphosphorylate pRb, thus deregulating pRb and mediating cell proliferation, providing an explanation for the progression of Kaposi’s Sarcoma into the nodular phase. Biopsies of Kaposi’s Sarcoma lesions expressing v-cyclin transcripts further confirm the role of v-cyclin in promoting oncogenesis (Kennedy, M.M, et al.). Just as with p53, VHL, and many other proteins, LANA also binds to pRb, competing with the E2F factors as shown in *Figure 3* (Verma, S.C., Lan, K., Robertson, E.). Via an indirect mechanism, LANA also stimulates cells in the resting G₀ phase to enter the cell cycle by stabilizing β -catenin through binding to the Glycogen Synthase Kinase-3-beta (GSK-3-beta). The gene that codes for the protein, β -catenin can serve as an oncogene and overexpression of this protein has been found in Kaposi’s Sarcoma tissue (Fujimuro M., et al).

Figure 3: LANA interference with Retinoblastoma protein

LANA competes with the E2F transcription factors in binding to the Retinoblastoma tumor suppressor and thus disrupts pRb regulation of the cell cycle.



Conclusion:

As the mechanisms addressed have shown, the Latency-Associated Nuclear Antigen of Herpesvirus 8 has a broad binding affinity for many regulatory proteins in our bodies. Of particular interest, is the fact, that deregulation of such proteins as the tumor suppressors: p53, VHL, and Retinoblastoma promote their proliferation and persistence in latently-infected hosts by upregulating factors favorable for oncogenesis. Much research is still being conducted on this newest member of the Herpesvirus Family, with particular emphasis on its encoded LANA and its interaction. Future directions for research hold potential for possibly finding an effective treatment for Kaposi's Sarcoma extending beyond palliation.

Clinical Case Study

ID and Chief Complaint:

Elijah is a 30 year old African American male who came in to the office, complaining of chest pain and shortness of breath (dyspnea).

History of Present Illness:

Elijah came in for a doctor's visit, presenting with chest pain which he has been experiencing for about a month now and which has progressively gotten worse over this time. The chest pain started off as intermittent, but has now become more frequent and the pain much more intolerable. On a scale of 10, Elijah describes the chest pain as a 7, stating that it feels as if bricks have been placed on his chest, making it difficult for him to expand his cavity when he breathes. Elijah reports that the chest pain usually lasts for a few minutes and does not radiate to any other part of the body, such as the neck or arms. He has not taken any medication to alleviate his chest pain.

With his chest pain, he has also been experiencing shortness of breath over the past 3 weeks, where his inhalations are very shallow and as mentioned earlier, there is a feeling of tightness in his chest. For Elijah, who is a very active individual, shortness of breath is very uncommon; however recently it has become a recurring problem for him. He has also noted that the shortness of breath not only occurs during light exercise but is more prominent following the onset of his chest pain and he mentions that his symptoms have prohibited him from doing the activities he enjoys on a daily basis. Although Elijah experiences chest pain, on average, once or twice a day, his shortness of breath occurs

more often throughout the day. There have also been a two or three episodes where he has woken up from his sleep due to shortness of breath.

Elijah is otherwise a healthy being and has not been in contact with any sick individuals. His immunizations are up to date and he recently received his influenza vaccination which he developed a sore throat and runny nose in response to the shot, but these symptoms lasted only a few days.

Past Medical History:

- End stage renal disease due to rhabdomyolysis
- Surgeries
 - Kidney Transplant in January 2010
 - ACL reconstruction 2009
- Medications
 - Cyclosporine (by mouth) for renal transplant
 - Melatonin
- Allergies
 - No known drug allergies

Family History:

- Sister is lactose intolerant
- Grandfather is dyslipidemic (high blood cholesterol)
- No FHx of diabetes, obesity, cancer, or cardiac disease

Social/Sexual History:

- -Marital status: Engaged and lives with fiancé
- -Sexually active; use of condoms
- -No children, but plan on having one in the near future
- -Occupation: business executive and fiancé is an elementary school teacher
- -Tobacco: denies use
- -Illicit drugs: denies use
- Alcohol: occasional beer or two a month
- Caffeine: a cup a day in the morning
- Exercise: active-enjoys working out, rock climbing, racquetball, and snowboarding
- Diet: balanced-meat, vegetables, fruits, grains
- Has not traveled anywhere in the last few years

Review of Systems:

- Constitutional: fevers at night starting two days ago, no chills, nor any weight loss or gain or loss of appetite, some fatigue.
- HEENT:
 - ◇ *head*: no dizziness; however occasional headaches (side effect of Cyclosporine), no neck pain
 - ◇ *ears*: no loss of hearing or earache/ear infection
 - ◇ *eyes*: no changes in visual acuity
 - ◇ *nose*: no runny nose, sinuses, or congestion, no tinnitus (ringing)

◇ *throat*: no dysphagia (difficulty swallowing), no toothache or mouth sores, tonsils present

- Cardiovascular: chest pain, no tachycardia or brachycardia, intolerance of exercise,
- Respiratory: shortness of breath; Paroxysmal Nocturnal Dyspnea (PND-sudden shortness of breath when sleeping- 2 to 3 occurrences), occasional cough (side effect of Cyclosporine), hemoptysis (began coughing up blood the last two days), no wheezing
- GI: no abdominal pain, no nausea, vomiting, or indigestion, no abdominal pain, stool is normal; no constipation or diarrhea, no heartburn or acid reflux. No eating disorder
- GU (genitor-urinary): kidney transplant, no rejection of transplant detected, no nocturia or polyuria, no incontinence (unintentional urine loss), no difficulties urinating, no erectile dysfunction or testicular pain, no history of STDs
- Integumentary: no bruises, rashes, irregular growths, dermal lesions, or skin irritation, no jaundice, redness, or any skin discoloration, no hair loss, nails appear healthy
- MS (musculoskeletal): no hip, joint, shoulder, or back pain, some swelling in legs, and feeling of muscle weakness, ACL injury, no stiffness or restrained movements
- Neurological: no seizures, numbness, or dizziness, coordination and balance is fine

- Mental: no depression, confusion, anxiety, or any other mental health disorder.
No alcohol or substance abuse. No memory loss

Physical Exam

- Vitals:
 - ◇ Temperature: 99°F (rechecked: 99.3°F)
 - ◇ Blood pressure: 84/50 mmHg (rechecked: 82/50)
 - ◇ Pulse: 88 beats per minute
 - ◇ Respirations: 20 breaths per minute
 - ◇ Pulse Oximetry: 86% Saturation
 - ◇ Height: 5' 8"
 - ◇ Weight: 148 lbs
 - ◇ BMI: 22.5
- HEENT:
 - ◇ NCAT (Normocephalic atraumatic): size and shape of head appears normal, no tenderness upon palpitation
 - ◇ PERRL: (pupils equally round and reactive to light), red eye reflex intact, no conjunctivitis
 - ◇ EOMI: (extraocular muscles intact), tympanic membrane clear, no fluid or significant earwax, no inflammation
 - ◇ clear discharge from nose, moist mucous membranes, no sinuses
 - ◇ no JVD (jugular venous distention) noted, slight tenderness and swelling of lymph nodes in neck region

- Cardiovascular: heart rate within normal range, no tachycardia or bradycardia, murmurs/bruits, gallops, or irregular palpitations
- Respiratory: dyspnea, hypoxemia
 - ◇ Observation: use of some accessory muscles, no difficulty speaking, difficulty breathing especially during inhalation, slight inclination/slouching forward upon inhalation, no barrel chest, no cyanosis (turning blue) or pursed lips
 - ◇ Palpation: trachea is medial, Fremitus (vocal vibratory sensation) is asymmetric-decreased, chest pain
 - ◇ Percussion: dull resonance
 - ◇ Auscultation: exhalations are louder than inhalations, no crackles, rales (scratchy sounds), ronchi (gurgling), moderate wheezing
- Abdomen:
 - ◇ Observation: not obese, symmetrical, no rashes or visible abnormalities , evidence of renal transplant surgery
 - ◇ Palpation: no hernia or protuberances, some tenderness in both upper left and right quadrants with deep palpitations
 - ◇ Percussion: liver span approximates 10cm with dull resonance, tympanitic stomach resonance, detection of fluid was negative
 - ◇ Auscultation: no irregular bowel movement sounds, no bruits (sound of turbulent arterial flow)

- Extremities: some edema in lower extremities, particularly near groin region, ACL reconstruction scar, no clubbing either, 2 second capillary refill in toes.
- GI: no enlarged organs or distentions detected
- GU: no tenderness, rashes, or growths
- Integumentary: normal skin coloration, health nailbeds, some swelling noted in leg; otherwise no lesions or rashes
- MS: normal flexion/extension and abduction/adduction movements, no muscular atrophy; however 3/5 muscle strength, all ranges of motion (active and passive) are normal and painless, no effusion of joints noted, McMurray's Test for torn meniscus-negative, shoulder rotations are fine
- Neurological: intact sensations to pain and touch, patellar and all other reflexes are normal, normal proprioception, gait, and responses to cranial nerve assessments, no numbness, dizziness, or loss of consciousness
- Mental: no mood swings, anxiety, or depression

Labs and Radiology:

- | | |
|--|----------------------------------|
| • <u>CBC</u> : results suggest anemia | <i>Reference Ranges</i> |
| -WBC count: 3,500/ μ L | (4,500-11,000 cells/ μ l) |
| -RBC count: 3.9million/ μ L | (3.8-5.8million cells/ μ l) |
| -CD4+ T-cell count: 300 cells/ mm^3 | (440-1600 cells/ mm^3) |
| -Hemoglobin: 12.8 g/dl | (13.2-17.3g/dl) |
| -Hematocrit: 38% | (39-49%) |
| -MCH: 32.8pg | (27-34pg) |

-MCV: 97 fL	(80-99fL)
- MCHC: 33.7 g/dl	(32-37g/dl)
-Platelet count: 100,000	(150,000-400,000 cells/ μ l)
-Total lymphocytes: 30%	(normal: 33%)
-Neutrophils: 51%	(54-80%)
-Eosinophils: 3%	(0-5%)
-Basophils: 1%	(0-2%)
-Monocytes: 15%	(0-10%)
• <u>Iron panel</u>	
-Total iron: 350 ng/ml	(60-170 ng/ml)
-Ferritin: 310 ng/mL	(18-250 ng/ml)
• <u>Lipid panel</u>	
-LDL cholesterol: 80 mg/dl	(62-130 mg/dl)
-HDL cholesterol: 70 mg/dl	(35-135 mg/dl)
-triglycerides: 130 mg/dl	(0-200 mg/dl)
• B.U.N: 24 mg/dL	(8-20 mg/dl)
• Creatine: 1.6 mg/dL	(0.7-1.4 mg/dl)
• Bilirubin (direct): 0.4 mg/dL	(0-0.3 mg/dl)
• Alkaline phosphatase: 148 U/L	(20-125 U/L)
• Serum potassium: 5.8 mEq/L	(3.5-5.5 mEq/L)
• <u>Serological exam</u>	
-HIV: (-)	
-TB antibody: (-)	

- Cardiac markers/enzymes

-troponin: 0 ng/mL (<0.1 ng/ml)

-CPK: 85 Units/L (38-174 units/L)

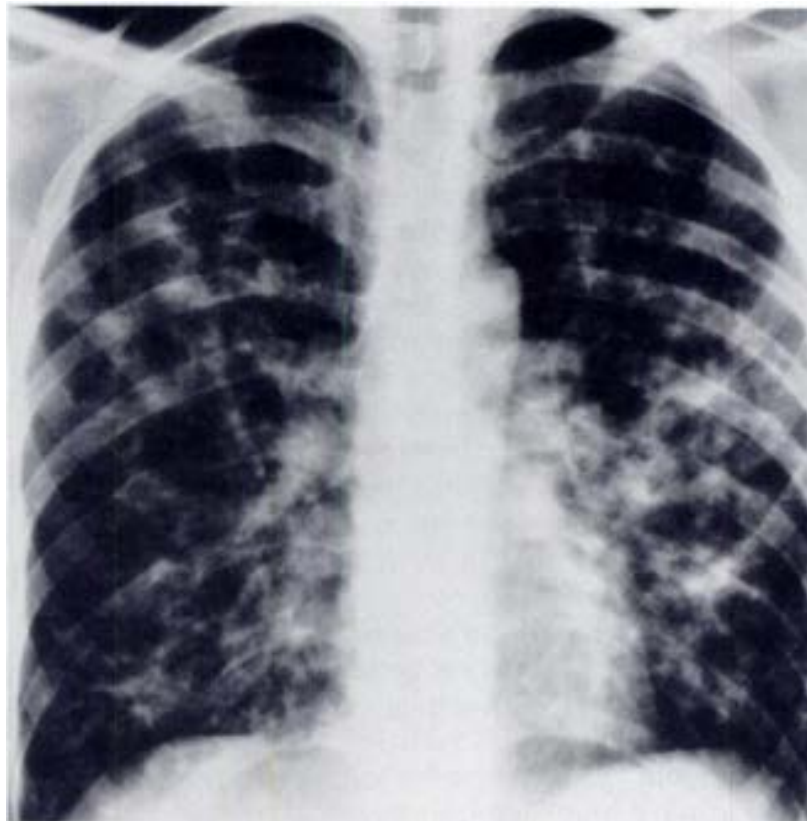
-myoglobin: 75 ng/mL (10-95 ng/ml)

- EKG

- tall tented T-waves were noted which is a possible indication of moderate hyperkalemia. The ventricular extrasystoles observed likely correspond to the shortness of breath and chest pain. P waves were absent and the QRS complex was rather broad.

- Figure 4: Chest X-ray

An X-ray of the patient's chest showed a cloudy appearance, indicating mild pleural effusion. Ill-defined nodules and opacities ranging from 4-12mm in diameter were also noted, particularly on the upper quadrants of both lungs.



- Figure 5: CT scan

Peribronchovascular septal thickening was noted in the CT scan of the patient's kidneys.



- Bronchoscopy

-Bright red lesions noted in lower portion of bronchi

- Lung Biopsy

-spindle cell proliferation confirmed

Problem List

- Dyspnea
- Chest pain

- Anemia
- Hyperkalemia
- Hemoptysis
- Thrombocytopenia

Differential Diagnosis

- Cardiac complications
 - Coronary heart disease
 - Congestive heart failure
 - myocardial infarction
- Pulmonary
 - COPD
 - bronchitis
 - tuberculosis
 - Pneumonia
 - acute pulmonary edema
 - sarcoidosis of lungs
- Other
 - Kaposi's Sarcoma

Assessment

Elijah's primary symptoms of chest pain and shortness of breath suggested that whatever was ailing him must be pulmonary or associated with the cardiovascular system; therefore an EKG and x-ray were done. The wave pattern of his EKG with

extrasystoles reflected his chief complaint; however tented T waves were also observed, indicating that the patient had hyperkalemia. Lab results confirmed elevated levels of potassium. Despite an irregular EKG, angina was deemed an unlikely differential and therefore atherosclerosis/coronary heart disease were ruled out upon normal results obtained from a lipid panel. Because Elijah had some swelling in his lower extremities, anemia, and his chest X-ray showed pleural effusion, myocardial infarction and congestive heart failure had initially been considered a possible differential; however a test with cardiac markers: troponin and CPK indicated otherwise.

The pleural effusion noted in Elijah's chest x-ray and septal thickening shown on a CT scan of his lungs pointed to many possible pulmonary related differentials but what led to the ultimate diagnosis was the decision to proceed with a biopsy of the ill-defined nodules found on the upper quadrants of both lungs from the bronchoscopy. Remarks from pathology of the samples included spindle cell proliferation and along with all the lab results thus far, the diagnosis was ascertained to be Kaposi's Sarcoma.

Although typical cases of Kaposi's Sarcoma present as defined bluish-purple skin lesions, Elijah was diagnosed with iatrogenic Kaposi's Sarcoma. Looking back at his medical history, the fact that Elijah has been taking cyclosporine, as prescribed for his renal transplant, explains the elevated bilirubin and hyperkalemia as the immunosuppressant drug warns of these side effects. Such immunosuppressant drugs, and particularly, cyclosporine, increase the risk of transplant patients being diagnosed with Kaposi's Sarcoma as it promotes excessive production of Vascular Endothelial Growth Factor (VEGF). The low numbers from the CBC count verified

immunosuppression in the patient where the characteristic Kaposi's Sarcoma nodules manifested on Elijah's lungs which led to the chest pain and shortness of breath he was experiencing and development of these lesions eventually caused hemoptysis.

Treatment Plan and Follow up

The immunosuppressant drug, Cyclosporine has been shown to increase the risk of transplant patients becoming susceptible to being diagnosed with Kaposi's Sarcoma as it promotes excessive VEGF (vascular endothelial growth factor) production and cancer progression, rather than targeting Kaposi's Sarcoma Associated Herpesvirus (KSHV or Herpesvirus 8) for apoptosis or the lytic cycle. Instead, an alternative immunosuppressant, Sirolimus not only provides the protection from graft rejection, but has been shown to be effective in the regression of Kaposi's Sarcoma. Therefore, in treating Elijah, Cyclosporine was discontinued and Sirolimus was prescribed and a follow up appointment in three months has been set . By then, the lesions should disappear. Typically, Kaposi's Sarcoma patients can undergo HAART (Highly Active Antiretroviral Therapy); however this treatment option better suits patients who have are HIV positive. The patient was advised against strenuous exercise and Ibuprofen, taken as needed, was suggested for temporary relief due to chest pain. In a 3 month follow up appointment, Kaposi's Sarcoma should have regressed with reduction or disappearance of the lesions as well as the swelling.

Oftentimes, secondary cancers can develop succeeding the onset of Kaposi's Sarcoma, one common disease is non-Hodgkin lymphoma and the prognosis assessed for

Elijah is that this is a concern as Kaposi's Sarcoma had progressed fairly well in its stage. Although lesions did not manifest on his skin, their presence in the lungs particularly pose greater urgency as the other systems of the body can be severely affected by the respiratory complications associated. Furthermore, some swelling of lymph nodes was noted in Elijah, near the groin region; therefore future office visits will follow up with this prognosis.

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