New Approaches to the Treatment of Human Herpesvirus 8-Associated Disease

Running Head: New Therapies for HHV-8

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gB, glycoprotein B

HAART, highly active antiretroviral therapy

KSHV, Kaposi’s sarcoma-associated herpes virus

KS, Kaposi’s sarcoma

MCD, multicentric Castleman disease

MMP, matrix metalloproteinases

MSM, men who have sex with men

PEL, primary effusion lymphoma

PT, phosophotransferase

TK, thymidine kinase

VEGF, vascular endothelial growth factor

Summary

Human herpesvirus 8 (HHV-8, also known as Kaposi sarcoma-associated herpesvirus or KSHV) is the etiologic agent of Kaposi sarcoma (KS) and primary effusion lymphoma (PEL), as well as many cases of Castleman disease. Despite significant advances in understanding the biology and natural history of these diseases, current treatment options have important limitations, and strategies to prevent their development in high-risk individuals are lacking. This article reviews the scope of HHV-8-associated disease, as well as the efficacy of current treatment options. Finally, novel approaches to treatment and prevention are described, including antiviral agents, targeted molecular therapy, and a combination of these modalities.

Summary Word Count: 100

Introduction

The human herpesviruses are among the world's most ubiquitous infections, with almost all eight viruses infecting the majority of the global population. While there is some heterogeneity in the prevalence of these infections in different populations, only one shows a curious pattern of both geographic and demographic restriction. Human herpes virus 8 (HHV-8, also known as KSHV), was first identified as the etiologic agent of KS in 1994[1], and is now known to be prevalent in populations of men who have sex with men (MSM), as well as persons from the Southern Mediterranean, Middle East, Africa, and parts of South America and Asia[2-5].

The reason for these distinct "pockets" of infection has not been well-characterized. Epidemiologic and virologic data suggest that HHV-8 is transmitted predominantly through saliva[6-9], similar to other human herpesviruses such as HSV-1, EBV, CMV, and HHV-6. It has been hypothesized that the relative restriction of HHV-8 infection may be attributable to differences across populations in susceptibility to infection, control of viral replication at mucosal sites, and/or behavioral, biologic or infectious co-factors for acquisition.

Prevalence of HHV-8-associated Disease

Infection with HHV-8 is most frequently asymptomatic[10], but may progress to one of three neoplastic or lymphoproliferative disorders: KS, multicentric Castleman disease (MCD), or PEL[11]. The burden of HHV-8-associated disease is greatest in areas where HHV-8 infection is endemic[12]. However, while HHV-8 infection is required for development of KS, PEL or some forms of MCD, it is not likely to be sufficient. The presence of co-factors such as immunosuppression may enhance progression to disease[13], and therefore there are clear disparities between the prevalence of HHV-8 infection and

the incidence of KS. For example, countries in Africa have among the highest incidence of KS in the world, but the incidence varies widely even in countries with a similar seroprevalence of HHV-8 and HIV infection (Figure 1).

KS is the commonest HHV-8-associated disease. In the height of the HIV epidemic, 1 in 5 HIV-infected persons in the USA developed KS[13], though the incidence has dropped dramatically with the introduction of highly active antiretroviral therapy (HAART)[14]. Today, KS remains the most frequent malignancy in persons with HIV in the USA[14]. In many parts of Africa, KS is the most common cancer in the entire population[12], and KS likely accounts for 1% of all cancers in resource-poor areas[15]. MCD and PEL remain relatively rare complications of HHV-8 infection. No comprehensive registry tracks the incidence of these diseases, and thus their frequency in the population is difficult to ascertain. It is estimated that less than 10,000 people are afflicted with MCD in the USA[16], with even fewer cases of PEL under medical care.

Efficacy and Limitations of Current Treatment Strategies

Despite significant progress over the past decade in understanding the pathophysiology of HHV-8-assocaited disease, treatment of these diseases today remains both toxic and incompletely efficacious. Response to HAART alone in persons with KS depends on the burden of preexisting disease; one metaanalysis found that 81% of persons without advanced KS experienced a complete response to HAART, but only 5 *cases* of response to HAART alone could be documented in the medical literature among persons with more advanced KS[17]. A recent examination of persons who developed KS in the era of HAART in Seattle, Washington, found that half remain with persistent disease in the 3 years after diagnosis, despite treatment with HAART and chemotherapy[18]. The

vast majority of patients with KS, MCD or PEL will be co-infected with HIV, and control of HIV infection is an essential component of their care. No studies have documented responses in MCD or PEL with HAART alone. These data suggest that in MCD and PEL, as well as cases of advanced KS, treatment with HAART alone is unlikely to be sufficient.

For patients requiring adjunctive therapy to HAART, the mainstay of current treatment is conventional chemotherapy. The use anthracyclines, antimitotic agents, microtubule stabilizers, or other chemotherapeutic agents alone or in combination for the treatment of KS have been shown in small clinical trials to result in response rates ranging from 25 to 88%[19]. Response varies with burden of disease, associated co-morbidities, and control of underlying immunodeficiency when applicable. Also, it is important to note that many who "respond" to conventional chemotherapy will still have residual disease. Considerably less information is available regarding the treatment of MCD and PEL. In small case series, treatment of these diseases with conventional chemotherapy has been associated mostly with short-lived responses and high mortality[16,20].

Therapies Under Investigation

The limited response rates and significant toxicity seen with conventional adjunctive chemotherapy have spurred investigators to search for novel therapeutics for the treatment and prevention of HHV-8-associated disease.

Antiviral Therapy

In each of the HHV-8-associated diseases, ongoing viral replication plays a key role in the development or sustenance of disease. The presence of replicating HHV-8 in

the peripheral blood has been shown to be one of the strongest predictors for the development of KS[21-24], and *in vitro* work has revealed that a small amount of lytic HHV-8 infection is required for the initiation and maintenance of KS tumors[25]. MCD is characterized by episodic reactivation of HHV-8 replication, accompanied by high levels of HHV-8 in the peripheral blood[26] and an almost exclusively lytic viral gene program[27]. PEL falls somewhere between KS and MCD in the spectrum of lytic replication[27]. These observations imply that antiviral therapy aimed at abrogating HHV-8 replication may have a role in the prevention or treatment of HHV-8-associated disease.

In 1978, a safe and effective compound was developed with considerable activity against the human herpesviruses[28]. In the subsequent three decades, a series of DNA synthesis inhibitors were developed with variable activity against each of the 8 human herpesviruses[29]. Data supporting the efficacy of antiviral medications in suppressing HHV-8 replication come from both basic science and observational studies.

The herpesvirus DNA synthesis inhibitors rely on the ability of a nucleoside analogue to be incorporated into a growing viral DNA chain. The different antiherpetic antivirals differ in their mechanism of action. Aciclovir, penciclovir, famciclovir, and ganciclovir all are phosphorylated by the herpesvirus thymidine kinase (TK) and/or UL97 phosphotransferase[30], though each herpesvirus enzyme may have a different affinity for each nucleoside analogue. Foscarnet and cidofovir both work independently of the herpesvirus TK /UL97; the former acts directly on the pyrophosphate binding site of the DNA polymerase while the latter is diphosphorylated by cellular enzymes. In the first set of analyses to determine whether any of the current antiviral agents would be active against HHV-8, it was found that the HHV-8 TK and PT share homology with those in

other human herpesviruses, and that they are capable of phosphorylating ganciclovir[31]. Subsequently, a set of novel experiments were designed to test the antiviral susceptibility of HHV-8 *in vitro.* These new methods were required because the virus does not produce a cytopathic effect in culture, obviating the traditional "gold standard' for susceptibility testing, the plaque reduction assay. Though these experiments differed in their methodology, most measured the degree to which a given antiviral would reduce the production of HHV-8 lytic gene products (either viral DNA or lytically-expressed proteins) when added to a cell culture containing an immortalized, latently-infected HHV-8 cell line induced to lytic replication. The results were uniformly consistent in that ganciclovir, foscarnet and cidofovir were the only agents with activity against HHV-8[32-36]. HHV-8 research is also hampered by the lack of a facile animal model, but productive infection may be established in immunocompromised mice, and this infection is blocked by administration of ganciclovir immediately after inoculation with HHV-8[37].

Evidence supporting the efficacy of antiviral medication in the prevention or treatment of HHV-8-associated disease in humans is found either in retrospective studies or case series. Two separate retrospective studies found that the incidence of KS was significantly reduced among persons who had received foscarnet or ganciclovir for CMV retinitis, but no reduction in KS was seen among users of aciclovir[38,39]. Similarly, a study investigating the efficacy of systemic ganciclovir for the treatment of CMV retinitis in AIDS patients found substantial reductions in the incidence of KS among persons treated with the drug, though this outcome was a secondary endpoint and no measurements of HHV-8 replication were obtained[40].

In the first and only clinical trial to date to evaluate the effect of antiviral medication on HHV-8 replication, 26 HHV-8 infected men (including 16 with HIV and 10 without) were randomized to either oral valganciclovir, 900 mg daily, or placebo once daily for 8 weeks[41]. After a 2-week washout period, participants were randomized to the opposite treatment arm for an additional 8 weeks. Oral swabs were collected for the quantification of HHV-8 DNA by PCR daily throughout the 18-week study. The use of valganciclovir was associated with a 46% reduction in oral HHV-8 replication (Figure 2), an effect that was found to be independent of the reduction in CMV replication.

Only one trial has evaluated the use of antiviral medication for the treatment of KS, finding that cidofovir was ineffective by itself for the treatment of both epidemic and classic KS[42]. Interferon-alpha, which has both antiviral and immunomodulatory properties, however, may find a role as adjunctive therapy in persons with advanced KS. Five (38%) of 13 patients with epidemic KS responded to interferon-alpha 2b in conjunction with HAART[43].

Although KS is more common, it might be expected that the HHV-8-associated diseases which are characterized by more extensive viral replication would be the best candidates for treatment with antiviral therapy. Successful treatment of PEL, either alone or with adjunctive chemotherapy, immunotherapy, or HAART, has been described to date with both ganciclovir[44,45] and cidofovir[44,46,47]. Of note in some of these case series is a documented decline in HHV-8 viral load coinciding with administration of antiviral therapy[44,46], and in one case, rebound with gaps in therapy[44]. Similar results have been observed in MCD patients treated with ganciclovir[48,49], but failures have been reported with cidofovir[50]. Recent basic science data also find that it may be feasible to induce cells

latently-infected with HHV-8 to lytic replication with valproic[51] or glycyrrhizic[52] acid or bortezomib[53], thereby "sensitizing" the tumor to antiviral therapy. Several clinical trials are currently enrolling patients to assess the efficacy of antiviral or inductive therapy in the treatment of HHV-8-associated disease

Novel Chemotherapy

In the effort to make chemotherapy more effective and less toxic, great progress has been made in the past decade to target specific molecules involved in oncogenesis. Many HHV-8-associated conditions exhibit features making them ideal targets for molecularly-directed therapy[54].

Vascular Endothelial Growth Factor Inhibitors. KS is among the most vascular tumors, and the interactions between HHV-8 and the human vascular endothelial growth factor (VEGF) pathway are essential for tumorigenesis. VEGF receptors 2 and 3 are expressed in KS lesions[55], and HHV-8 glycoproteins K1 and glycoprotein B (gB) increase the expression of VEGF and activate VEGF-R3 (respectively)[56,57]. Clinical trials of early VEGF inhibitors, pentosan and tecogalan, were characterized by moderate toxicity and minimal efficacy[58-60]. A phase II study of IM862, an antiangiogenic compound which both inhibits VEGF formation and activates natural killer cells, showed that 36% of 44 patients with epidemic KS had at least a partial response[61]. Of note, no relationship between plasma VEGF levels and either administration of IM862 or tumor response could be identified, suggesting that either the effects were local or the drug worked in a manner independent of VEGF. A subsequent Phase III placebo controlled study, however, found the drug to be no more efficacious than HAART alone, suggesting

that the efficacy seen in the Phase II study may have been attributable to HAART use[62]. A Phase I study of a novel antisense oligonucleotide to VEGF (VEGF-AS) led to more dramatic decreases in VEGF plasma levels and a complete remission of KS in a patient who was refractory to chemotherapy and HAART, but dose-limiting toxicities were common[63].

Tyrosine Kinase Inhibitors. The observation that KS tumors express *c-kit*, the tyrosine kinase receptor for stem cell factor, suggested to some that HHV-8-associated malignancies may be treatable with *c-kit* inhibitors[64]. Limited experience with imatinib showed that 5 of 10 patients with epidemic KS displayed a partial response to the drug, though diarrhea limited the dose of imatinib which could be administered in 6 of 10 participants[65]. Clinical trials are currently ongoing to examine the role of other *c-kit* inhibitors, such as sorafenib, in the treatment of KS.

Monoclonal Antibody Therapy. Deciphering the unique pathobiology of MCD has revealed two targets against which monoclonal antibody therapy could be effectively directed. Many of the signs and symptoms of MCD may be mediated by the production of viral IL-6, an analogue of the human cytokine which has been pirated by HHV-8. In an animal model, it has been shown that the expression of IL-6 in mice produces a disease which histologically and clinically resembles MCD[66]. Furthermore, high levels of IL-6 are universally detected in patients with HHV-8-assocaited MCD[26]. Administration of a murine monoclonal antibody against IL-6 to a patient with MCD resulted in a dramatic resolution of symptoms and signs, but the illness rapidly returned after the infusion was

discontinued[67]. Subsequently, a humanized antibody to the IL-6 receptor was developed and was administered to 28 patients with the plasma cell variant of MCD in Japan[68]. Two of these participants were infected with HHV-8, and none had HIV. 12 (52%) of 23 participants who had lymphadenopathy saw a significant reduction in the size of their lymph nodes at one year, and C-reactive protein levels normalized in 18 (64%) of 28. This study represents the largest clinical trial of person with MCD to date; the reasonable efficacy, and lack of serious adverse reactions, makes IL-6 receptor antagonists a promising choice for the treatment of MCD. The role of IL-6 in other HHV-8-associated diseases is less clear, but it is unlikely that these drugs will find indications in either PEL or KS. However, both MCD and PEL are primarily diseases of B lymphocytes. Consequently, monoclonal antibody therapy (rituximab) directed at CD20, expressed on all mature B-cells (Figure 3), has been used successfully in the treatment of both MCD and PEL[69-77].

Matrix Metalloproteinases. Matrix metalloproteinases (MMP) are overexpressed in KS tumors, and may play a role in oncogenesis by helping the tumor to invade the basement membrane. COL-3 is a topical MMP inhibitor which is similar in structure to tetracycline. In a Phase II clinical trial comparing 2 doses of COL-3, 41% of 75 patients responded to the lower (50 mg) dose[78]. Participants frequently discontinued using the drug, and photosensitivity was common.

Summary

In the quarter of a century since epidemic KS heralded the beginning of the HIV pandemic, much progress has been made in characterizing the frequency, spectrum,

pathophysiology and treatment of HHV-8-associated disease. The persistent global burden of KS, coupled with the few effective treatment options for MCD and PEL, argue for continued research on prevention and therapy for HHV-8-associated disease. Recent translational studies have identified several new targets for potential future therapies, including inhibitors of viral replication, cell signaling, inflammation, and angiogenesis, but the efficacy of these strategies can only be established through careful controlled clinical trials.

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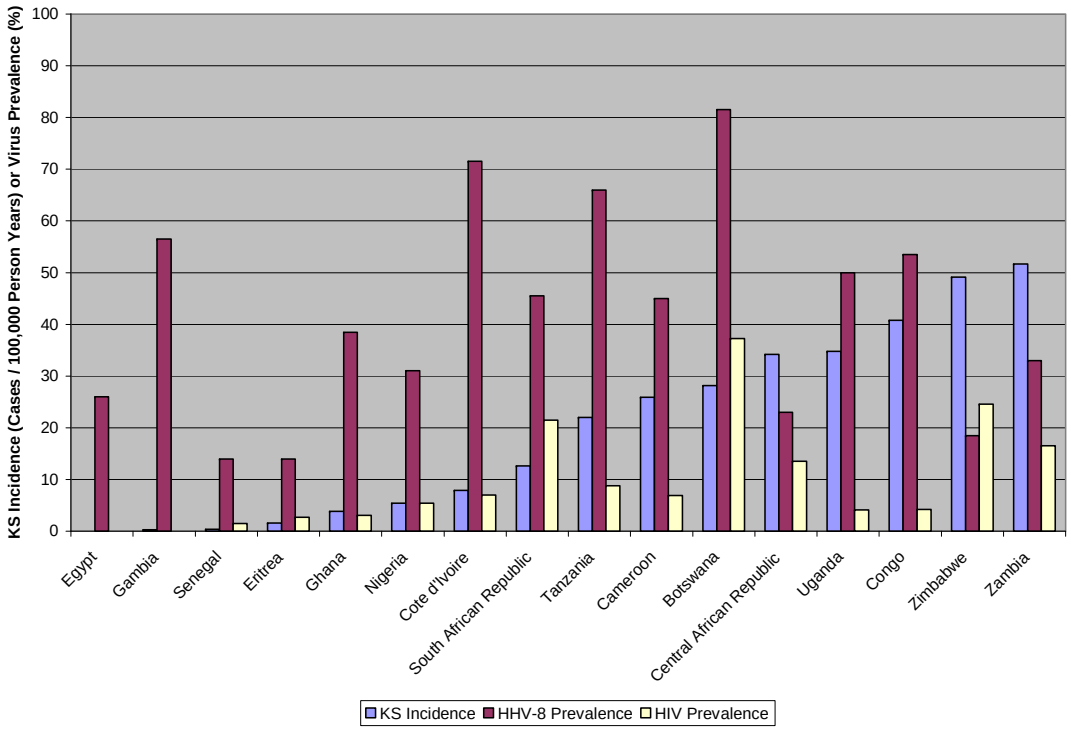
Figures

Figure 1. Prevalence of HHV-8 and HIV infection compared with the incidence of Kaposi sarcoma in countries of Africa, ranked according to their incidence of Kaposi's sarcoma



Footnotes:

HHV-8 Prevalence is estimated based on aggregates of studies since 1996 showing seroprevalence of HHV-8 in general population. HIV prevalence statistics are as of 2005 and taken from UNAIDS/WHO Global HIV/AIDS Online Database (available: http://www.who.int/GlobalAtlas/predefinedReports/EFS2006/index.asp). KS Age-Standardized Incidence Rates obtained from *Cancer Incidence in Five Continents*).

Figure 2. Effect of Valganciclovir on Human Herpesvirus 8 Oral Shedding, By HIV Status

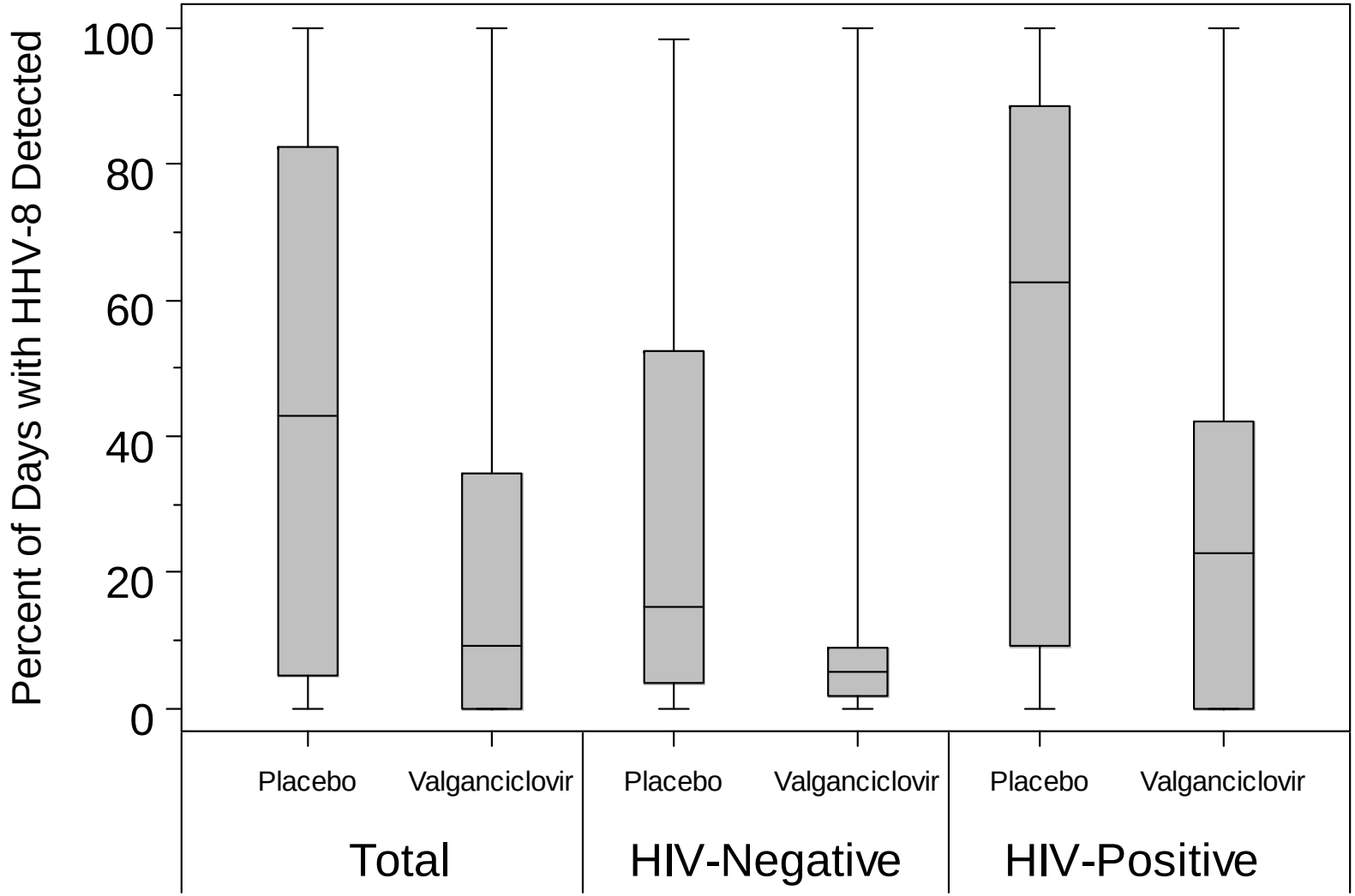


Figure 3. Histopathology of Germinal Center in Hyaline Vascular Variant Multicentric Castleman Disease Stained with Antibodies to CD20