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EDITORIAL
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Targeting hypoxia-inducible factors in malignancies caused by Kaposi's sarcoma associated herpesvirus

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Abstract: In this editorial, we highlight the potential use of inhibitors of hypoxia-inducible factors (HIFs) for the use in Kaposi's sarcoma associated herpesvirus (KSHV) (also known as human herpesvirus-8) related malignancies. The past 20 years has accumulated detailed knowledge of the role of these factors in ensuring the maintenance of the KSHV in infected cells, in aiding the growth of the virus infected cells and aiding in the spread of virus from infected cells by inducing lytic reactivation. Today, a wide range of inhibitors for HIFs are currently being clinically evaluated for use in treating a variety of cancers. We discuss the current state of this research area as it relates to KSHV malignancies and describe pre-clinical and clinical evidence of drugs that target HIF to back up the idea that these inhibitors could be a novel way to treat KSHV related diseases.

***Keywords*:** hypoxia inducible factor, Kaposi sarcoma associated herpesvirus, human herpesvirus 8, Kaposi's sarcoma

The discovery of hypoxia-inducible factors (HIFs) and their role in orchestrating the response to hypoxia in 1995 was a major advance for which the discoverers were awarded the Nobel Prize (*1*). After their discovery, it soon became apparent that many cancers, especially solid tumors, usurp HIF-induced pathways to promote tumor cell survival, tumor growth, angiogenesis, and even tumor metastasis (*2,3*). Healthy cells generally do not activate the hypoxic pathway except when exposed to low oxygen tension (as in wound healing) or other stresses, and for this reason, HIFs are being studied as potential targets for anti-cancer therapy. There are two principal HIFs, HIF-1α and HIF-2α (also known as endothelial PAS domain-containing protein 1 [EPAS1]). These factors rapidly accumulate in cells exposed to hypoxia, bind to hypoxia response elements (HREs) in the genome, and upregulate specific genes that promote angiogenesis, maintain cell survival and growth etc. The degree of expression of these two forms of HIF varies among different cell types.

It has become apparent that HIFs play a central role in the pathogenesis of Kaposi sarcoma (KS) and other tumors or hyperproliferative diseases caused by Kaposi's sarcoma associated herpesvirus (KSHV) (*4*). KSHV-induced diseases include primary effusion lymphoma (PEL), multicentric Castleman disease (MCD), and KSHV inflammatory cytokine syndrome (KICS). KSHV is a gammaherpesvirus and like other herpesviruses, has both latent and lytic gene expression programs. The promoters of several key KSHV genes were found to have HREs and to be upregulated by HIFs; these include latency associated nuclear antigen (LANA), the lytic switch gene (RTA), and the ORF34-37 lytic gene cluster (*4,5*). Exposure of PEL cells to hypoxia induces lytic replication through the upregulation of RTA by HIF working in concert with LANA. Interestingly, KSHV infection itself leads to an increase in HIFs, so there is a positive feedback loop (*6*). One way HIF inhibitors could help benefit patients with PEL or other KSHV-mediated tumors is to block lytic activation of the infected cells and thereby prevent production of KSHV-encoded cytokines and virus spread. Also, Shrestha *et al*. have shown that if the HIF-1α gene is knocked out in PEL lines, cell growth is severely impaired (*7*). They also showed that the HIF-1 inhibitor, PX-478, which downregulates the mRNA for HIF-1α, severely impairs the growth of PEL cells in normoxia but has no effect on uninfected lymphoma cells (*7*). These studies provide a rationale for exploring the use of HIF inhibitors in the treatment of PEL. In addition, our group and others have demonstrated that KSHV-infected cells harness a significant portion of the hypoxic gene expression signature even under normoxic conditions, suggesting that these specific hypoxic pathways (such as increased glycolysis) could also be targeted in KSHV malignancies (*8,9*). While PEL involves KSHV-infected B-cells, KS primarily involves KSHV-infected endothelial cells, which generally express HIF-2α rather than HIF-1α (*10*). It has been demonstrated that HIF-2α but not HIF-1α is responsible for activating lytic replication in certain KSHV-infected cells by localizing HIF-2α to the endoplasmic reticulum, thus allowing for translation

Table 1. Selected list of drugs shown to inhibit HIF and to have pre-clinical or clinical activity against KSHV-induced tumors

Drugs	Clinical status	Mechanism of HIF inhibition	Effect on KSHV malignancy	*Ref.*
Echinomycin	In clinical use*	Inhibits HIF-1α activity and cMyc	Inhibits cell growth in PEL and KS xenograft mice models	(*14*)
Pomalidomide	Approved for KS	HIF-1α inhibition in endothelial cells	Inhibits PEL cell growth *in vitro*; effective against KS patients	(*7,12*)
Lenalidomide	In clinical use*	HIF-1α inhibition in endothelial cells	Inhibits PEL cell growth *in vitro*	(*13,15*)
Rapamycin	Clinically used in transplant-associated KS	Indirectly inhibits HIF-1α and HIF-2α *via* mTOR pathway inhibition	Inhibits PEL cell growth *in vitro*; effective against KS in renal transplant recipients	(*16,17*)
Rapamycin plus digoxin	Both drugs in clinical use**	Both drugs inhibit HIF-1α and HIF-2α levels and activity	Prevents the growth of KS-like tumors in nude mice	(*18*)
Everolimus	In clinical use*	Indirectly inhibits HIFs *via* mTOR pathway inhibition	Induces apoptosis and inhibits KSHV gene expression and virus production in PEL cell lines	(*19*)
PX-478	Phase I	Inhibits HIF-1α mRNA and protein levels	Selectively inhibits the growth of PEL cell lines *in vitro*	(*7*)

* In clinical use, but not for KS or other KSHV-induced diseases. ** Both drugs in clinical use, but not together against KS or other KSHV-induced diseases. HIF, hypoxia inducible factor; KS, Kaposi sarcoma; PEL, primary effusion lymphoma.

of viral mRNAs (*4*). Therefore, in the case of KS, treating patients with a HIF-2α inhibitor rather than a HIF-1α inhibitor, might be beneficial. Interestingly, a specific HIF-2α inhibitor, belzutifan, which prevents the dimerization of HIF-2α with its partner HIF-1β (needed for its activity) was recently approved by the FDA for the treatment of patients with von Hippel-Lindau disease which leads to overexpression of HIF-2α (*11*).

Several pre-clinical studies have described strategies for targeting HIFs, alone or with other targets, that may be worth exploring in KSHV diseases. In this regard, certain therapies now used to treat KS and other KSHV diseases are known to have indirect or direct effects on HIFs, and this may contribute to their activity. For example, the mTOR pathway is upregulated in KSHV-infected cells by a variety of mechanisms (*4*) and this can increase and stabilize the levels of HIF protein. This may explain in part why rapamycin, which targets mTOR, is useful in the treatment of KS in renal transplant patients. Another FDA-approved treatment for KS is the immunomodulator pomalidomide, an analog of thalidomide (*12*). While it remains unclear why exactly pomalidomide works in KS patients, it has been shown that lenalidomide and pomalidomide decrease angiogenesis and HIF expression in endothelial cells (*13*). Several drugs now in clinical use for other conditions have been shown to inhibit HIF and to have activity in certain models of KS. One is echinomycin, which targets both HIF-1α and cMyc; this has been shown to have activity in pre-clinical models of both PEL and KS (*14*). Table 1 shows selected compounds known to have inhibitory effects on HIF and how they affect KSHV infected cells and/or patients.

In summary, for reasons that are still unclear, KSHV has evolved so that HIFs play an important role in the viral life cycle and in the pathogenesis of KSHV-induced diseases. As such, HIFs may represent a potential target for the treatment of these diseases. Several therapies that modulate HIFs as well as other targets have been shown to be effective in KS, and the effect on HIF may contribute to their activity. It is possible that other HIF-specific approaches may be found to be active in these diseases, and this is an area for future study.

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Conflict of Interest: R. Yarchoan reports receiving research support from Celgene (now Bristol Myers Squibb), CTI BioPharma (a Sobi A.B. Company), PDS Biotech, and Janssen Pharmaceuticals through CRADAs with the NCI. Dr. Yarchoan also reports receiving drugs for clinical trials from Merck, EMD-Serano, and Eli Lilly and preclinical material from Lentigen Technology through CRADAs or MTAs with the NCI. R. Yarchoan and DA. Davis are co-inventors on U.S. Patent 10,001,483 entitled "Methods for the treatment of Kaposi's sarcoma or KSHV-induced lymphoma using immunomodulatory compounds and uses of biomarkers". An immediate family member of R. Yarchoan is a co-inventor on patents or patent applications related to internalization of target receptors, epigenetic analysis, and ephrin tyrosine kinase inhibitors. All rights, title, and interest to these patents have been assigned to the U.S. Department of Health and Human Services; the government conveys a portion of the royalties it receives to its employee inventors under the Federal Technology Transfer Act of 1986 (P.L. 99-502).

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