

# Role of viruses in glioblastoma oncogenesis and progression (Review)

IMANE SABRI<sup>1,2</sup>, PAWEŁ MARCINKIEWICZ<sup>3</sup>, NABIL ISMAILI<sup>1</sup>,  
ROGER ABOUNADER<sup>3</sup>, IMANE CHAOUTI<sup>2</sup> and FADILA GUESSOUS<sup>1</sup>

<sup>1</sup>Laboratory of Onco-Pathology, Biology and Cancer Environment, Faculty of Medicine, Mohammed VI University of Health Sciences, 82403 Casablanca, Morocco; <sup>2</sup>Unit of Biology and Medical Research, National Energy Center of Nuclear Science and Technology, 10080 Rabat, Morocco; <sup>3</sup>Department of Microbiology, Immunology and Cancer Biology, University of Virginia, Charlottesville, VA 22904, USA

Received October 30, 2025; Accepted May 27, 2026

DOI: 10.3892/wasj.2026.502

**Abstract.** Glioblastoma (GBM) is a frequent primary brain tumor well-known for its poor prognosis. The underlying cause of this malignancy has not yet been clarified. To date, the only determined risk factors are ionizing radiation exposure and some inherited genetic syndromes. Since a number of viruses have been implicated in the development of cancer, an investigation into of the viral etiology of GBM was conducted.

Numerous exogenous viruses, from the herpesvirus and polyomavirus families, have been detected in GBM tissue samples and despite their prevalence being inconsistent across studies, human cytomegalovirus (CMV) has previously been associated with GBM progression and has also been suggested to serve a direct role in gliomagenesis, as CMV-elicited GBM cells are generated from normal astrocytes. Furthermore, endogenous retroviruses integrated into the human genome, such as human mouse mammary tumor virus-like-2 and human endogenous retrovirus W, can also contribute to certain oncogenic pathways, leading to poor clinical outcomes. Overall, a deeper understanding of how these viruses interact with their hosts could pave the way for new preventive strategies and the identification of new therapeutic targets.

*Correspondence to:* Miss Imane Sabri or Professor Fadila Guessous, Laboratory of Onco-Pathology, Biology and Cancer Environment, Faculty of Medicine, Mohammed VI University of Health Sciences, Mohammed Taïeb Naciri Boulevard, Hay Hassani, 82403 Casablanca, Morocco  
E-mail: isabri@um6ss.ma  
E-mail: fguessous@um6ss.ma

**Abbreviations:** BKV, BK virus; CMV, cytomegalovirus; CNS, central nervous system; ddPCR, droplet digital PCR; EBV, Epstein-Barr virus; EMT, epithelial-to-mesenchymal transition; env, envelope; FAK, focal adhesion kinase; gag, group specific antigen; GBM, glioblastoma; HIF-1, hypoxia-inducible factor 1; HCMV, human CMV; HERV, human endogenous virus; HHV, human herpesvirus; HML-2, human MMTV-like-2; hnRNP, heterogeneous nuclear ribonucleoprotein; HPV, human papillomavirus; hTERT, human telomerase reverse transcriptase; IARC, International Agency for Research on Cancer; IE, immediate early; IHC, immunohistochemistry; ISH, *in situ* hybridisation; JCV, John Cunningham virus; LTRs, long terminal repeats; MCP, monocyte chemoattractant protein; miRNA, micro RNA; NGS, next generation sequencing; PDGFR- $\alpha$ , platelet-derived growth factor receptor- $\alpha$ ; PD-L1, programmed death-ligand 1; PKM2, pyruvate kinase M2; pol, polymerase; pRb, retinoblastoma protein; Pyk2, proline-rich tyrosine kinase 2; RNA-seq, RNA sequencing; RT-PCR, reverse transcription PCR; SCF, stem cell factor; SV40, Simian virus 40; Tag, large T antigen; VP, viral capsid protein

**Key words:** glioblastoma, human cytomegalovirus, Epstein-Barr virus, human herpesvirus 6, John Cunningham virus, simian virus 40, BK virus, human papillomavirus, human endogenous retroviruses

## Contents

1. Introduction
2. Herpesviruses
3. Polyomaviruses
4. HPV
5. Human endogenous retroviruses (HERVs)
6. Conclusion and future directions

## 1. Introduction

According to the International Agency for Research on Cancer (IARC), > 320,000 individuals were diagnosed with primary central nervous system (CNS) tumors globally in 2022 (1). Furthermore, ~80-85% of CNS tumors are gliomas, with glioblastoma (GBM) being the most common and aggressive type (49%) (2). The latest edition of the World Health Organization Classification of CNS tumors ranked GBM as a grade IV glioma characterized by the isocitrate dehydrogenase wild-type gene (3). The GBM incidence increases after the age of 40, reaching its highest rate in individuals aged 75-84, with a clear predominance in men and Caucasian individuals (4). The current standard therapy for newly diagnosed patients involves a combination of neurosurgery, followed by

concurrent radiotherapy and temozolomide chemotherapy and subsequently, adjuvant temozolomide (5). Despite this multimodal approach, GBM still exhibits the worst prognosis among primary brain tumors, with a median overall survival of ~15 months and a 5-year survival rate of only 3-5% (6). The etiology of GBM is not entirely known. Yet, an increased risk has been reported in a small number of GBM patients due to some inherited genetic syndromes (such as Li-Fraumeni syndrome) and prior exposure to ionizing radiation (7,8). Given that viruses are implicated in ~10-15% of human cancers, a number of studies have explored their association with GBM (9). Therefore, in the present review, the potential involvement of select viruses in the etiology of GBM was examined, with the aim of providing insight into their possible role in GBM progression.

## 2. Herpesviruses

*Human cytomegalovirus.* Human cytomegalovirus (HCMV), also known as human herpesvirus (HHV)-5, is an enveloped, double-stranded DNA virus belonging to the *Betaherpesvirinae* subfamily. It is a ubiquitous virus that the majority of individuals worldwide encounter during their lifetime. A meta-analysis of seroprevalence data indicated that ~60% of individuals in developed countries and up to 90% in developing countries have been exposed to HCMV (10). Despite its widespread prevalence, HCMV infection is typically asymptomatic in immunocompetent individuals due to an effective immune response. Similar to other herpesviruses (such as HHV-6, Herpes Simplex Virus and Epstein-Barr Virus), HCMV establishes latency following primary infection. Although HCMV exhibits tropism for a wide range of cell types, including endothelial, epithelial and fibroblast cells, its latent reservoir is primarily in hematopoietic cells, particularly CD34<sup>+</sup> hematopoietic progenitor cells and myeloid lineage cells. In immunocompromised individuals, reactivation from latency can result in severe, potentially life-threatening disease. During its replicative cycle, HCMV gene expression progresses through three phases: Immediate-early (IE), delayed-early (DE) and late. Proteins expressed during the IE phase regulate cellular and viral gene expression. By contrast, DE proteins are key in viral DNA replication, whereas late proteins are primarily structural and involved in viral capsid assembly. Due to its potential involvement in carcinogenesis, the IARC has classified HCMV in group 2B 'possibly carcinogenic to humans' based on limited evidence for human cancers (11).

*Association between HCMV infection and GBM.* In 2002, Cobbs *et al* (12) reported for the first time, the presence of HCMV in all examined GBM samples, while no viral presence was detected in the adjacent non-neoplastic brain tissue. This finding was subsequently corroborated by further independent studies, a number of which found a higher prevalence of HCMV in GBM compared with other gliomas, non-neoplastic encephalopathies or epileptic brain tissues (13,14). Despite this, additional research has reported conflicting results: Either a very low detection rate or a complete absence of the virus in GBM samples, even when using validated standard detection techniques that can identify the virus in positive control samples (15-17).

Given the low viral load of HCMV in GBM, the discrepancy of these results may be primarily attributed to method sensitivity and experimental conditions. Factors such as section age, thickness and slide pretreatment and deparaffinization can affect the results of both immunohistochemistry (IHC) and *in situ* hybridization (ISH) (18). Cobbs *et al* (12) and additional investigators have emphasized the importance of a protocol that includes optimized antigen retrieval and high antibody concentrations to detect low-level viral proteins (19,20). However, others have argued that this optimized protocol may increase staining artifacts, leading to false positive results (16,21). A number of studies have used other assays, including PCR, ISH, and next-generation sequencing, to further determine IHC results, with findings being frequently consistent with ISH data (12,18,22,23).

Conventional PCR and next-generation sequencing (NGS) often yield negative results, as HCMV copy numbers are below the detection thresholds of these assays. For this reason, more sensitive PCRs, such as nested PCR and droplet digital PCR (ddPCR), have been performed; however, the results either remain negative or show lower prevalence compared with IHC and ISH (18,24-26). The main explanation for this phenomenon is the diversity of HCMV strains and the genetic alterations in the viral genome that may influence primer and probe sensitivity and specificity (14). Despite these divisive findings, a recent meta-analysis including 988 patients from 19 studies reported a pooled prevalence of 68% (95% CI: 0.53-0.80;  $P < 0.0001$ ;  $I^2 = 91.4\%$ ), reinforcing the widespread presence of HCMV in GBM tissue (27). Furthermore, preclinical and clinical studies targeting HCMV have demonstrated improved clinical outcomes in patients with GBM, supporting the hypothesis that HCMV is involved in gliomagenesis. This will be expanded upon in the present review.

Given that HCMV has failed to transform normal human cells *in vitro*, 'oncomodulation' has been proposed as an alternative model to explain how the virus promotes gliomagenesis. This paradigm describes the indirect role of HCMV, where it enhances the oncogenic properties of pre-transformed cells. Despite this, recent findings by El Baba *et al* (28) and Guyon *et al* (29) challenged this paradigm. The authors isolated clinical high-risk strains and successfully generated CMV-elicited GBM cells which exhibited GBM-like features, including plasticity, stemness and invasion, capable of forming tumors in xenografted mice (28,29). This evidence suggests that HCMV may also exert a direct oncogenic effect though the precise conditions governing this capacity remain to be defined, warranting further investigation into its role in GBM pathogenesis.

*Mechanisms of HCMV in gliomagenesis.* All mechanisms as further described are summarized in Fig. 1. i) Effects of HCMV on cell cycle and cellular survival. HCMV contributes to gliomagenesis by promoting cellular proliferation and survival through a number of mechanisms, starting from its attachment to the host cell to its downstream signaling and gene regulation. Platelet-derived growth factor receptor  $\alpha$  (PDGFR $\alpha$ ), a tyrosine kinase receptor frequently overexpressed in GBM cells, serves a pivotal role in this process. PDGFR $\alpha$  is important in HCMV internalization, gene expression and viral particle production (30). Through its interaction with this receptor, HCMV facilitates the activation

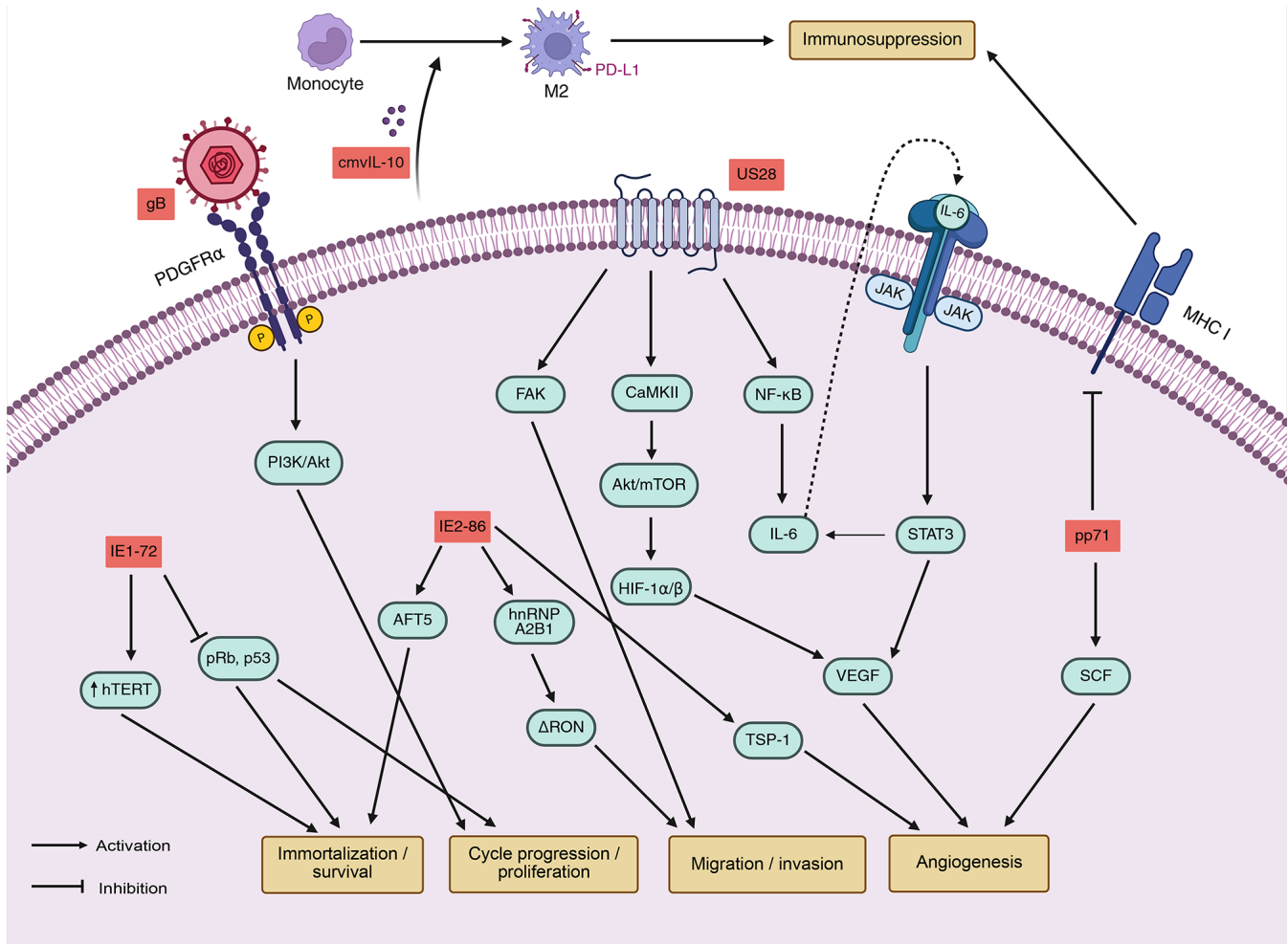


Figure 1. Role of HCMV gene products in GBM. HCMV binds to PDGFR $\alpha$  through gB to enter the cell, leading to PDGFR $\alpha$  phosphorylation and activation of the PI3K/Akt signaling which promotes cell proliferation and survival. IE1-72 enhances cell immortalization and cycle progression by upregulating hTERT expression, inactivating pRb and p53 and stimulating PI3K/Akt. IE2-86 promotes cell survival, migration and angiogenesis by activating AFT5,  $\delta$ -RON and TSP-1, respectively. US28 induces angiogenesis through the activation of VEGF through the Akt/mTOR/HIF-1 signaling and IL-6/STAT3 axis, it also activates FAK which stimulates cellular migration. pp71 upregulates the expression of the proangiogenic factor SCF and inhibits the surface expression of MHC-I resulting in the cancer cell being unrecognizable by the immune system. HCMV-infected cells secrete cmvIL-10, which causes the conversion of monocytes to an immunosuppressive phenotype (M2) and the expression of PD-L1. Created in BioRender (<https://BioRender.com/cqjd1fa>). HCMV, human cytomegalovirus; GBM, glioblastoma; gB, glycoprotein B; PDGFR $\alpha$ , platelet-derived growth factor receptor- $\alpha$ ; cmvIL-10, cytomegalovirus IL-10; PD-L1, programmed death-ligand 1; JAK, janus kinase; FAK, focal adhesion kinase; SCF, stem cell factor; hTERT, human telomerase reverse transcriptase; AFT5, activating transcription factor 5; pRb, retinoblastoma protein; RON, récepteur d'origine nantis; TSP-1, thrombospondin 1; MHC-I, major histocompatibility complex class I; hnRNP, heterogeneous nuclear ribonucleoprotein; CaMKII, calcium/calmodulin-dependent protein kinase II; IE2-86, 86-kDa immediate-early 2 protein; HIF-1, hypoxia-inducible factor 1.

of oncogenic pathways that may support tumor development and progression.

HCMV binds to PDGFR $\alpha$  through its viral envelope glycoprotein B (gB), leading to PDGFR $\alpha$  phosphorylation and subsequent activation of the PI3K/Akt signaling pathway. This activation enhances cellular proliferative activity and inhibits apoptosis, thereby promoting viral persistence and potentially contributing to oncogenic processes in the host cell (31). At the intracellular level, the viral protein IE1-72 has been shown to activate the PI3K/Akt signaling pathway, contributing to cell survival and proliferation (32). The hyperactivation of this axis is a hallmark of aggressive tumor behavior in GBM, often associated with treatment resistance and poor prognosis (33).

Beyond the dysregulation of growth factor signaling, inactivation of tumor suppressor genes signaling and human telomerase reverse transcriptase (hTERT) gene mutation

are frequent alterations that define the genomic landscape of GBM (34). In HCMV infection, IE1-72 was found to promote cell immortalization and genetic instability of GBM cells by dysregulating the oncosuppressor proteins p53 and retinoblastoma protein (pRb) and increasing hTERT expression (32,35,36). In an experimental study using a panel of GBM cell lines, IE1-72 expression exhibited a divergent effect on cell proliferation, triggering either accelerated proliferation with steady-state expression of pRb and the p53 family or cell cycle arrest with high p53 expression. In the context of oncomodulation, this study suggested that the effect of IE1-72 may be conditioned by genetic alterations already present in these cell lines (32). Despite this, a recent study demonstrated that IE1 of a high-risk HCMV strain (HCMV-DB strain) binds to and degrades p53 and pRb through proteasome-mediated pathways, leading to cell cycle entry and inhibition of apoptosis, similar

to other viral oncoproteins [such as human papillomavirus (HPV) E6 and E7 oncoproteins] (37). Another marked viral protein is 86-kDa immediate-early 2 protein (IE2-86), which contains domains that bind both pRb and the TATA-binding protein, allowing it to induce cell cycle progression and hijack the cellular transcription machinery (38-40). By contrast, IE2 may also block the cell cycle in the G<sub>1</sub> phase independently of p53 and pRb and the underlying mechanism remains to be defined (41). In addition, IE2-86 further enhances cellular survival by inducing acetylation of activating transcription factor 5, thereby increasing its transcriptional activity and inhibiting apoptosis (42,43).

An additional viral protein implicated in HCMV-mediated gliomagenesis is US28, a viral G protein-coupled receptor homologous to the human C-C chemokine receptor type 1. US28 serves a key role in evading host immune responses and has been shown to promote tumorigenic processes by creating a positive loop that activates the IL-6/Janus kinase 1/STAT3 axis initiated by NF- $\kappa$ B activation (44). US28 also activates endothelial nitric oxide synthase, which induces the proliferation of glioma-initiating cells, further contributing to GBM progression (45).

ii) Effects of HCMV on cell invasion and migration. In addition to promoting cellular proliferation and survival, HCMV enhances the invasiveness and migration capacity of GBM cells through numerous molecular mechanisms. The HCMV-encoded protein IE2-86 has been shown to promote GBM migration by upregulating heterogeneous nuclear ribonucleoprotein (hnRNP)-A2B1, a factor involved in precursor-mRNA splicing of the Recepteur d'Origine Nantais (RON) protein (46). hnRNP A2B1 induces the skipping of exon 11 in the mature mRNA, producing a constitutively active  $\delta$ -RON isoform that promotes cell motility and metastasis (46). In addition, the overexpression of the viral G protein-coupled receptor US28 is associated with increased invasiveness in GBM (45). It was demonstrated that US28 activates certain non-receptor cellular kinases, including focal adhesion kinase (FAK) and proline-rich tyrosine kinase 2, involved in cell migration and invasion (47,48). Furthermore, HCMV infection can accelerate epithelial-to-mesenchymal transition (EMT), a key process in tumor invasiveness, as well as strengthen cell invasion while collaborating with TGF- $\beta$ 1 through JNK pathway activation (49). The evaluation of EMT-related genes expression has exhibited an upregulated expression of MMP-2 and vimentin, as well as a low expression of E-cadherin in HCMV-positive GBM cell lines after TGF- $\beta$ 1 exposure, compared with HCMV-negative cell lines (49). These findings suggest a synergistic role for HCMV and host signaling in promoting the invasive phenotype of GBM.

iii) Effects of HCMV on angiogenesis. Angiogenesis is a marked hallmark of GBM that maintains cell proliferation and ensures invasion and potential metastasis. The main regulator of this process is VEGF, the expression of which can be induced by HCMV through numerous mechanisms, primarily mediated by the viral chemokine receptor US28. The first mechanism involves US28-mediated activation of certain transcription factors, including STAT3, hypoxia inducible factor 1 (HIF-1), specificity protein 1 and activator protein 2 through p38 and p44/42 MAPK signaling pathways, which finally induces VEGF promoter activation and increased VEGF

expression (50). Secondly, US28 enhances VEGF production through the IL-6/STAT3 signaling axis, resulting in VEGF secretion and supporting an angiogenic tumor microenvironment (44,45). The third mechanism is also led by US28, which constitutively stimulates G $\alpha$ /q protein-mediated signaling pathways resulting in the activation of calcium/calmodulin-dependent protein kinase II and the Akt/mTOR cascade to increase the HIF-1 $\alpha$  activity. HIF-1 $\alpha$  forms a transcription factor with HIF-1 $\beta$  that binds to hypoxia response element regions of different genes, including the VEGF gene (51). In addition, US28 induces the phosphorylation and dimerization of pyruvate kinase M2 (PKM2), a glycolytic enzyme, giving it a transcriptional activity targeting HIF-1 genes. This creates a HIF-1 $\alpha$ /PKM2 feedforward loop that promotes tumor cell proliferation, angiogenesis and a glycolytic phenotype characteristic of GBM (51).

Besides VEGF, HCMV infection can also alter the expression of a number of other angiogenic factors. The HCMV tegument protein pp71 upregulates stem cell factor (SCF) expression, a potent pro-angiogenic factor in GBM, through activation of NF- $\kappa$ B signalling (52). By contrast, IE1-72 downregulates the expression of thrombospondin-1, a well-established anti-angiogenic factor (35). HCMV infection is also associated with the upregulation of endocan, a molecule involved in angiogenesis and vascular remodeling, as well as arginase-2, which enhances vasculogenic mimicry and tumor invasion by modulating MMP-2 and MMP-9 activity (53,54).

iv) Effects of HCMV on stemness. Cancer stem cells are a small subpopulation of quiescent, pluripotent and self-renewing cancer cells identified in numerous malignant tumors, including GBM (55). These cells are known to initiate tumors and exhibit resistance to chemotherapy and radiotherapy, thereby contributing to recurrence and metastasis (56,57). HCMV infection has been shown to promote stemness in GBM cells by upregulating the expression of GBM stem cell markers, namely, CD133, nestin, Notch1, OCT3/4 and Sox2. It also induces sphere-like proliferation, indicative of self-renewal capacity and maintains the cells in an undifferentiated state (58-60). Furthermore, HCMV-infected GBM cells exhibiting a stem cell-like phenotype have been shown to resist the standard-of-care therapeutic agent: Temozolomide. However, that sensitivity can be reversed with anti-viral treatment using ganciclovir (60). Soroceanu *et al* (61) suggested that the viral IE proteins are implicated in promoting stemness in GBM, as they co-localize with CD133, Sox2 and nestin. Notably, attenuation of their expression was found to be sufficient to suppress neurosphere formation and reduce Sox2 expression. In addition, it was also established that HCMV promotes stemness through the upregulation of enhancer of zeste homolog 2 and Myc expression (22).

v) Effects of HCMV on immune evasion. It has been well established that HCMV has evolved numerous mechanisms to evade host immune detection (62-64). However, it is unclear whether HCMV may also mediate the immune escape of infected GBM cells. A study addressing this question has demonstrated that HCMV tegument protein pp71 blocks the transport of major histocompatibility complex class I molecules to the cell surface, thereby hindering the ability of immune

cells to recognize and target GBM cells (65). In addition, HCMV contributes to the establishment of an immunosuppressive microenvironment by modulating the polarization of tumor-associated macrophages from a M1 to M2 phenotype. HCMV-infected GBM cancer stem cells produce a viral homolog of human IL-10 (cmvIL-10), which facilitates the differentiation of recruited monocytes into macrophages or microglia with an immunosuppressive (M2) phenotype and increased expression of programmed death ligand 1 (PD-L1). Consequently, this stimulation activates viral infection in M2 cells with high IE1 and STAT3 expression, which further enhances oncogenesis by supporting VEGF production, TGF- $\beta$ -mediated immunosuppression and invasion (66). In an additional study, HCMV was also shown to induce M1/M2 polarization through activation of NF- $\kappa$ B and PI3K in infected monocytes/macrophages (67). Furthermore, other immunosuppressive factors, such as TGF- $\beta$ , IL-6, arginase II and PD-L1, were also found to be upregulated by HCMV, creating a robust immunosuppressive microenvironment that supports the expansion and activation of regulatory T cells and myeloid-derived suppressor cells (54,68,69). In a recent study, Long *et al* (70) identified FXYD domain-containing ion transport regulator 6<sup>+</sup> T cells, an immunosuppressive T cell subpopulation, as well as macrophages co-expressing both cancer cell and macrophage markers in HCMV-infected GBM. HCMV appears to mediate crosstalk between these cell populations, contributing to the development of an immunosuppressive microenvironment.

*Targeting HCMV in GBM: Current therapeutic landscape.* Elucidating the oncomodulatory effects of HCMV on GBM offers a promising avenue for adopting this virus as a novel therapeutic target. Numerous treatment modalities have been investigated or are currently under clinical assessment for this purpose.

*Antiviral drugs.* Food and Drug Administration-approved HCMV antiviral agents, typically used to treat immunocompromised patients with HCMV infection, have been tested for efficacy against GBM. Valganciclovir is a prodrug of ganciclovir and a nucleoside analog that blocks viral replication. The first randomized trial (Valcyte Treatment of Glioblastoma Patients in Sweden) didn't show any significant survival benefit of valganciclovir; however, the subgroup analysis demonstrated that patients treated with valganciclovir for >6 months exhibited an improved overall survival (OS) compared with those treated for <6 months (24.1 vs. 13.1 months;  $P < 0.0001$ ) (71). During this trial, new patients requested valganciclovir as an add-on to their standard therapy and a retrospective analysis revealed an unexpectedly higher survival rate, reinforcing the efficacy of valganciclovir in improving patient prognosis (72,73). In addition, a recent meta-analysis of five randomized controlled trials has shown that ganciclovir was associated with a 20% increase in 2 and 4-year survival in the GBM cohort (74). At the molecular level, these antiviral drugs are activated by the viral protein pUL97. Therefore, a mutation of the UL97 gene leads to drug resistance. Surpassing this limitation, cidofovir, an additional HCMV antiviral drug, is activated by host-cell kinases, making it less susceptible to viral resistance. Preclinical evidence has indicated that

cidofovir not only downregulates HCMV gene expression and promotes apoptosis but also acts as a radiosensitizer, enhancing the cytotoxicity of radiotherapy by inhibiting DNA repair mechanisms (75).

*Viral targeted immunotherapy.* Dendritic cells (DC) are antigen-presenting cells known for their important role in controlling the immune response (76,77). Leveraging this, a new treatment modality was developed using these cells to boost immunity within the tumor. The HCMV-targeted DC vaccine is fundamentally patient-derived DCs electroporated with viral pp65 mRNA, enabling them to present pp65 antigen to T cells and induce expansion of antigen-specific immunity after being injected back into the patient. Antitumor Immunotherapy Targeted Against Cytomegalovirus was the first randomized trial to introduce the pp65 DC vaccine in patients with GBM after preconditioning the vaccine site with tetanus-diphtheria toxoid (78). Subsequently, the same group conducted an additional clinical study in a separate cohort to evaluate the combination of the pp65 DC vaccine with a granulocyte-macrophage colony-stimulating factor adjuvant and dose-intensified temozolomide (79). According to the latest follow-up of these trials, both studies showed favorable outcomes: ~33% of cases remained recurrence-free 5 years after diagnosis in the first trial and 36% survived 5 years after diagnosis in the second trial (80).

An additional immunological approach proposed to treat GBM is HCMV-directed adoptive T-cell (ATC) therapy. This approach uses autologous T cells that are expanded *in vitro* after stimulation with HCMV epitopes. Early feasibility studies have demonstrated that infusing these cells to consolidate recurrent GBM treatment was safe and improved patient survival (81,82). In adjuvant settings, a subsequent study extended this strategy to newly diagnosed GBM, determining safety and durable maintenance of HCMV-specific T cells after infusion. This study also reported improved OS among patients treated before recurrence compared with among those treated after progression (23 vs. 14 months;  $P = 0.018$ ) (83). More recently, the Evaluation of Recovery From Drug-Induced Lymphopenia Using Cytomegalovirus-Specific T Cell Adoptive Transfer randomized trial has shown that combining ATC with pp65-loaded DC vaccine increases the polyfunctionality of HCMV-specific CD8<sup>+</sup> T cells and this effect was associated with improved OS (84).

*Epstein-barr virus.* Epstein-Barr virus (EBV), also known as HHV-4, is a ubiquitous  $\gamma$ -herpesvirus first discovered in 1964 by Epstein *et al* (85) in a biopsy from a patient with Burkitt lymphoma. EBV is an enveloped virus with a linear double-stranded DNA genome of ~175 kb encoding >80 open-reading frames (86). Epidemiological studies have indicated that >90% of the adult population worldwide is chronically infected with EBV (87-92). Transmission occurs mainly through saliva, although it can also be spread through organ transplantation or exposure to other bodily fluids (93). The virus infects a number cell types but typically exhibits tropism for B lymphocytes and epithelial cells (94). Furthermore, EBV was the first identified oncovirus associated with numerous malignancies in humans, primarily B cell lymphomas, nasopharyngeal carcinoma and gastric carcinoma (86). It encodes for certain gene products, including

the latent membrane protein 1 oncoprotein and the family of non-coding RNAs microRNA (miR)-BART, responsible for the dysregulation of key signaling pathways involved in oncogenic events (95).

Although not primarily classified as a neurotropic virus, EBV can infect the CNS either directly or indirectly through the infected B cells, leading to numerous CNS disorders, such as Parkinson's disease, multiple sclerosis, meningitis, encephalitis and Alzheimer's disease (96). EBV enters astrocytes primarily through the complement receptor type 2 (or CD21) (97). It has also been implicated in the oncogenesis of primary CNS lymphoma, particularly in immunocompromised individuals, where it is associated with poorer prognosis (96,98,99). Based on these observations, a number of studies have investigated whether EBV may act as an etiological agent in GBM and other gliomas; however, results have been inconsistent. In 2019, Limam *et al* (100) reported the highest detection rate of EBV in gliomas compared with previous studies (Table I) (24,101-105). Using conventional PCR, this study found EBV DNA in 21.4% of the tested gliomas, all of which were GBM. Similarly, two independent studies investigating glioma subtypes also detected the presence of EBV exclusively in GBM samples, but not in lower-grade gliomas (24,101). Furthermore, two research groups focused solely on GBM specimens and reported comparable EBV detection rates of 21.4 and 20.7%, respectively (26,106). By contrast, other studies employing PCR and reverse transcription (RT)-PCR failed to detect EBV in GBM or other gliomas (17,102), highlighting inconsistencies in the literature. Notably, in research investigating novel miRNA biomarkers for GBM, Herman *et al* (107) identified differential expression levels of six EBV-derived miRNAs in the blood of patients with GBM compared with healthy controls. These miRNAs are known to serve roles in the switch from latent to lytic infection and in immune evasion.

Numerous studies have investigated the presence of EBV in glioma specimens using NGS techniques. Cimino *et al* (108) performed targeted NGS on 21 high-grade gliomas using a panel of 115 selected genes and detected EBV DNA in 5 cases. Similarly, Strong *et al* (103) analyzed publicly available whole-genome sequencing datasets from patients with low-grade glioma and GBM to perform a virome assessment. This study identified EBV DNA sequences in 13 GBM datasets. However, due to the low number of viral reads and lack of validation with additional methods, these findings remain inconclusive. By contrast, Khoury *et al* (104), through a bioinformatic analysis of available RNA sequencing (RNA-seq) datasets from low-grade glioma and GBM patients, found no evidence of EBV RNA expression. Notably, to understand GBM pathogenesis, a recent study comparing gene expression between patients with GBM and healthy individuals reported marked enrichment of EBV-associated signaling pathways, such as NF- $\kappa$ B, MAPK, JAK-STAT and Akt, suggesting a potential etiological role for EBV in GBM tumorigenesis (109).

**Human herpesvirus 6 (HHV-6).** HHV-6 is a lymphotropic  $\beta$ -herpesvirus initially identified by Salahuddin *et al* (110) in 1986 in patients with lymphoproliferative disorders. Similarly to all other herpesviruses, HHV-6 is highly prevalent, with >90% of adults harboring the virus asymptomatically (111). Initially believed to have two variants: HHV-6A and HHV-6,

these two were reclassified as distinct species in 2012 by the International Committee on Taxonomy of Viruses based on their biological, immunological and epidemiological characteristics, as separate (112). HHV-6B is more commonly detected in the peripheral blood leucocytes of healthy individuals, whereas the pathogenic role of HHV-6A remains poorly understood (113,114). Transmission of HHV-6 occurs primarily through saliva and the infection usually occurs through the nasopharynx and olfactory entry routes between 6 months and 2 years of age (115,116). HHV-6 exhibits a broad cellular tropism infecting a wide range of cell types both *in vivo* and *in vitro*, including fibroblasts, epithelial cells, neurons, oligodendrocytes and astrocytes (117-121). Despite HHV-6 infection being generally asymptomatic, it can cause roseola infant (*Exanthema subitum*) and a variety of complications in immunocompromised individuals, such as hepatitis, pneumonitis, encephalitis, multiple sclerosis and epilepsy (122-126). Although HHV-6 is not an established oncovirus, it has been detected in numerous malignancies, including hematological cancers, as well as gastrointestinal, gynecological, neurological and head and neck cancers (127). In 1995, Luppi *et al* (128) used PCR to assess HHV-6 prevalence in 37 biopsies of neuroglial tumors and 9 necropsies of normal brain tissue. The frequency of HHV-6 DNA was found to be 66% (6/9) in normal tissue but only 16% (6/37) in tumor samples, five of which were GBM. A subsequent study, with a larger cohort, investigated the presence of HHV-6 in 118 brain tumors and 31 normal brain tissues using nested PCR. The results revealed 14 HHV-6 positive cases out of 31 patients with GBM, a frequency of 45% (129). More recently, Crawford *et al* (130) analyzed the presence of HHV-6 in adult CNS tumors using ISH, nested PCR and IHC. In the large cohort of this study, consisting of >200 adult CNS tumors, 47% of patients were positive for U57 major capsid protein, 24% exhibited the presence of HHV-6A/B early antigen (p41) and 35% had a detectable level of late antigens (glycoprotein 116/64/54), with none of these markers present in any of the 25 control samples. Among grade IV astrocytomas, the respective frequencies were 47, 28, 32%. The detection of both early and late antigens indicated an active HHV-6 infection, as opposed to a latent viral state.

To understand the potential role of HHV-6 in glioma development, a research group evaluated inflammatory cytokines levels in cyst fluid samples from patients with glioma infected with the virus. After determination of the results on astrocytes infected with the isolated HHV-6, this study demonstrated that HHV-6 infection leads to elevated levels of IL-6, IL-8, TNF- $\alpha$  and TGF- $\beta$  in glioma, suggesting that it may provide a pro-inflammatory environment conducive to gliomagenesis (131). Similarly, the HHV-6 encoded direct repeat 7 protein has been shown to upregulate the expression of pro-inflammatory cytokines such as IL-1 $\beta$ , IL-6 and IL-8 in glioma cells. It can also accelerate cell proliferation and promote cell migration and invasion by facilitating extracellular matrix degradation (132). In summary, molecular and epidemiological studies have suggested a potential role for HHV-6 in gliomagenesis; however, further investigations are needed to determine whether this virus is an oncogenic driver or simply an opportunistic pathogen that reactivates in response to microenvironmental immunodeficiency.

Table I. Studies investigating the presence of EBV in GBM and other types of glioma.

## A, Studies including only GBM

| First author, year              | Glioma type | Sample size | Detection method                         | Positive results, n (%) | Notes | (Refs.) |
|---------------------------------|-------------|-------------|------------------------------------------|-------------------------|-------|---------|
| Hashida <i>et al</i> , 2015     | GBM         | 39          | Reverse transcription PCR (LMP-1)        | 0 (0.0)                 |       | (17)    |
| Ghaffari <i>et al</i> , 2021    | GBM         | 42          | Conventional PCR (EBNA3C)                | 9 (21.4)                |       | (26)    |
| Zavala-Vega <i>et al</i> , 2017 | GBM         | 29          | IHC (LMP-1) and ISH (EBER)               | 6 (20.7)                |       | (106)   |
| Cimino <i>et al</i> , 2014      | GBM         | 21          | Next-generation sequencing<br>ISH (EBER) | 5 (24.0)<br>0 (0.0)     |       | (108)   |

## B, Studies including multiple types of glioma

| First author, year            | Glioma type                               | Sample size   | Detection method                                            | Positive results, n (%)         | Notes                                                                                                                                                                      | (Refs.) |
|-------------------------------|-------------------------------------------|---------------|-------------------------------------------------------------|---------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|
| Limam <i>et al</i> , 2019     | Grade I-IV                                | 112 (82 GBM)  | Conventional PCR (BamM region)<br>IHC (LMP-1)<br>ISH (EBER) | 24 (21.4)<br>4 (3.6)<br>4 (3.6) | Detected only in GBM cases                                                                                                                                                 | (100)   |
| Strojnink <i>et al</i> , 2017 | Grade III-IV                              | 45            | RT-PCR (ebna)                                               | 3 (6.7)                         | Detected only in grade IV                                                                                                                                                  | (101)   |
| Lin <i>et al</i> , 2016       | GBM, astrocytoma (grade I-III)            | 63 (45 GBM)   | Multiplex droplet digital PCR (LMP-1)                       | 4 (6.3)                         | Detected only in FFPE GBM samples                                                                                                                                          | (24)    |
| Cosset <i>et al</i> , 2014    | GBM, low-grade astrocytoma and others     | 28 (20 GBM)   | RT-PCR (BALF5)                                              | 0 (0)                           |                                                                                                                                                                            | (102)   |
| Strong <i>et al</i> , 2016    | Low-grade and GBM                         | 701 (170 GBM) | WGS<br>RNA-seq<br>RT-PCR                                    | 0 (0)                           | In this virome assessment, EBV was detected in 12 GBM WGS datasets, but this result was not determined due to low viral reads detected and the potential for contamination | (103)   |
| Khoury <i>et al</i> , 2013    | Low-grade and GBM                         | 215 (168 GBM) | RNA-seq                                                     | 0 (0)                           |                                                                                                                                                                            | (104)   |
| Fonseca <i>et al</i> , 2014   | GBM, astrocytoma (grade I-III) and others | 75 (18 GBM)   | Conventional PCR (BamM region)                              | 11 (14.7)                       | Only one case of GBM was positive for EBV                                                                                                                                  | (105)   |

EBV, Epstein-Barr virus; GBM, glioblastoma; EBNA3C, Epstein-Barr nuclear antigen 3C; IHC, immunohistochemistry; ISH, *in situ* hybridization; LMP-1, latent membrane protein 1; EBER, Epstein-Barr encoded RNA; RT-PCR, reverse transcription PCR; WGS, whole genome sequencing; RNA-seq, RNA sequencing; FFPE, formalin-fixed paraffin-embedded.

### 3. Polyomaviruses

*John Cunningham virus (JCV)*. JCV is an opportunistic neurotropic polyomavirus originally isolated in 1971 from

the brain tissue of a patient with Hodgkin's lymphoma (133). Seroepidemiological studies have indicated that 8-10% of individuals contract the virus before 6 years old, with seroprevalence increasing to ~70% in adulthood (134-138). Fortunately,

Table II. Prevalence of JCV in GBM tissue.

| First author, year                     | Sample size | Detection method (target sequence)                             | Results, n (%) | (Refs.) |
|----------------------------------------|-------------|----------------------------------------------------------------|----------------|---------|
| Del Valle <i>et al</i> , 2001          | 21          | PCR followed by southern blotting hybridization (early region) | 12 (57.1)      | (150)   |
| Afshar <i>et al</i> , 2016             | 199         | RT-PCR (Tag)                                                   | 25 (12.6)      | (151)   |
| Limam <i>et al</i> , 2020              | 82          | Conventional PCR (Tag) and ISH (whole genome)                  | 3 (3.6)        | (152)   |
| Rollison <i>et al</i> , 2005           | 102         | PCR followed by southern blotting hybridization (Tag)          | 2 (2)          | (153)   |
|                                        |             | Real-time PCR (Tag)                                            | 0 (0)          |         |
| Boldorini <i>et al</i> , 2003          | 13          | Nested PCR followed by RFLP (Tag)                              | 7 (53.8)       | (155)   |
| Delbue <i>et al</i> , 2005             | 21          | RT-PCR (Tag)                                                   | 11 (52.4)      | (156)   |
| Eftimov <i>et al</i> , 2016            | 51          | RT-PCR (Tag)                                                   | 20 (39.2)      | (157)   |
|                                        |             | RT-PCR (non-coding control region)                             | 2 (3.9)        |         |
| Caldarelli-Stefano <i>et al</i> , 2000 | 5           | Nested-PCR (Tag)                                               | 0 (0)          | (158)   |

GBM, glioblastoma; RFLP, restriction fragment length polymorphism; RT-PCR, reverse transcription PCR; ISH, *in situ* hybridization; Tag, T antigen; JCV, John Cunningham virus.

the infection is asymptomatic; however, JCV can reactivate from latency under severe immunosuppressive conditions, leading to progressive multifocal leukoencephalopathy (139). JCV has a circular double-stranded DNA genome composed of early and late coding regions separated by a regulatory region (140). Although humans are the primary host of this virus, its tumorigenic potential has been well documented in animal models. Numerous studies have demonstrated that JCV induces a wide variety of brain tumors in non-human primates and rodents (141,142).

Large T antigen (Tag) is a viral protein encoded by the early region of the JCV genome. It is considered an oncoprotein due to its key role in tumorigenesis in experimental animal models (143). Tag drives cell cycle progression into the S-phase by interacting with key regulatory proteins, including pRb and the tumor suppressor p53, thereby disrupting their tumor-suppressive functions (144,145). These interactions impair DNA damage repair mechanisms and enhance uncontrolled cellular proliferation.

Similarly to a number of ubiquitous viruses, the association between JCV and human cancers is a divisive topic. Numerous studies have reported the presence of JCV in human CNS malignancies, such as GBM, medulloblastoma and oligoastrocytoma (146-149). However, the prevalence of JCV in GBM tissues varies markedly across studies (Table II). The highest prevalence has been reported by Del Valle *et al* (150), who examined the presence of JCV DNA sequences and the expression of viral regulatory protein Tag in 85 CNS tumor specimens using PCR and IHC, respectively. JCV DNA was detected in 69% of all samples and 57.1% of GBM cases, whereas the Tag protein expression was observed in only a small subset of cases. In a more recent study, Afshar *et al* (151) evaluated p53 expression levels in JCV-positive and JCV-negative patients with GBM using reverse transcription PCR (RT-PCR). The results revealed a lower expression of p53 in patients infected with JCV. While these findings support a potential etiological role of JCV in gliomagenesis, two independent investigations suggested no association, reporting very low detection rates of the virus

in GBM tissues (3.6 and 2.0%) (152,153). Shifting focus from tumor tissue detection, a serological case-control study assessed the JCV infection history by measuring serum antibodies against the viral capsid protein (VP)-1 in two cohorts. The data revealed no marked association between JCV seropositivity and the risk of all gliomas overall or GBM specifically (154).

*Simian virus 40 (SV40)*. SV40 is a polyomavirus that naturally infects rhesus macaques. Human infection with SV40 is uncommon, with seroprevalence of ~2% among healthy adults, and typically requires close contact with infected monkeys (136,159,160). Between the mid-1950s and early 1960s, millions of individuals were accidentally infected by SV40 worldwide due to contaminated polio vaccines prepared using infected monkey kidney cell cultures (161). Shortly after its discovery, SV40 was found to exhibit strong oncogenic potential. It can transform human cells *in vitro* and induce tumors in animal models, including primary bone and brain cancers, malignant mesotheliomas and lymphomas (162,163). Consequently, a number of investigators have evaluated the presence of SV40 in human tumors to assess its possible role in human carcinogenesis.

Using PCR followed by southern blotting hybridization, Martini *et al* (164) screened for the presence of the SV40 Tag coding sequences in 83 brain tumors, including 30 GBM. SV40 was detected in 33% of GBM samples, while no viral sequences were found in normal brain tissue (164). Using the same methodology, Huang *et al* (165) investigated the presence of SV40 Tag DNA sequence across GBM subtypes, including primary, secondary and giant cell GBM. The results of this study revealed a higher prevalence of SV40 sequence in secondary GBM (59%) compared with other subtypes (Table III), with molecular profiles closely resembling low-grade or anaplastic astrocytomas. Since secondary GBM typically arises from the progression of low-grade astrocytomas, the authors suggest that tumors with slower progression and longer clinical history are more likely to be susceptible to SV40 infection (165). In addition, another study conducted on 32 GBM samples

Table III. Prevalence of SV40 in GBM tissue samples.

| First author, year           | Sample size                                                   | Detection method (targets)                                                                            | Results n (%)                     | (Refs.) |
|------------------------------|---------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|-----------------------------------|---------|
| Limam <i>et al</i> , 2020    | 82                                                            | Conventional PCR (Tag gene, regulatory region and VP1 gene), ISH (whole genome) and IHC (Tag and tAg) | 12 (14.6)                         | (152)   |
| Rollison <i>et al</i> , 2005 | 102                                                           | PCR followed by southern blotting hybridization (Tag) and RT-PCR (Tag)                                | 0 (0)                             | (153)   |
| Delbue <i>et al</i> , 2005   | 21                                                            | Nested-PCR followed by RFLP (Tag)                                                                     | 0 (0)                             | (156)   |
| Martini <i>et al</i> , 1996  | 30                                                            | PCR followed by southern blotting hybridization (Tag)                                                 | 10 (33.0)                         | (164)   |
| Huang <i>et al</i> , 1999    | 28 (Primary GBM)<br>22 (Secondary GBM)<br>18 (Giant cell GBM) | PCR followed by southern blotting hybridization (Tag)                                                 | 7 (25.0)<br>13 (59.0)<br>2 (11.0) | (165)   |
| Kouhata <i>et al</i> , 2001  | 32                                                            | Conventional PCR (regulatory region), DNA sequencing, ISH (mRNA Tag) and IHC (Tag)                    | 3 (9.4)                           | (166)   |
| Sabatier <i>et al</i> , 2005 | 20                                                            | IHC (Tag)                                                                                             | 0 (0.0)                           | (167)   |
| Zhen <i>et al</i> , 2001     | 8                                                             | Immunoprecipitation followed by silver staining and western blotting (Tag)                            | 4 (50.0)                          | (169)   |

GBM, glioblastoma; Tag, large T antigen; tAg, small T antigen; ISH, *in situ* hybridisation; VP1, viral capsid protein 1; IHC, immunohistochemistry; RT-PCR, reverse transcription PCR; RFLP, restriction fragment length polymorphism; SV40, Simian virus 40.

detected the regulatory region of SV40 in three elderly patients, with the findings demonstrated by DNA sequencing and ISH following conventional PCR analysis (166). More recently, Limam *et al* (152) investigated the presence of SV40 DNA in 82 patients with GBM using independent PCR assays targeting the Tag gene, the transcriptional regulatory region and VP1. This approach revealed that 14.5% of GBM samples tested were positive for SV40. Despite these findings suggesting a possible role for SV40 in the initiation or progression of GBM, other studies have reported negative results (153,156,167). In a large series of various brain tumors, Rollison *et al* (153) investigated the presence of SV40 using two independent methods, followed by southern blotting hybridization compared with RT-PCR conducted in two different laboratories. This analysis revealed that SV40 was absent in all GBM samples, providing evidence against a causal association between SV40 and GBM.

An additional study evaluated the association between SV40 infection and GBM by comparing the seroprevalence of the virus among patients with GBM, cancer-free individuals and patients with breast cancer. To minimize potential cross-reactivity due to the high degree of protein homology between SV40, JCV and BK viruses (BKV). Mazzoni *et al* (168) performed indirect ELISA using specific synthetic peptides specifically designed to mimic SV40 VPs. The results revealed a notably higher prevalence of SV40 antibodies in patients with GBM (34%) compared with healthy subjects (15%) and patients with breast cancer (15%).

**BK virus (BKV).** Similar to JCV, the human BKV is a ubiquitous polyomavirus that infects >80% of the immunocompetent population (170). It was first isolated in 1971 from the urine of a renal transplant patient (171). BKV infects the renal epithelium and establishes latency. Upon severe immunosuppressive events, this opportunistic virus can reactivate, leading

to a variety of clinical manifestations, including hemorrhagic cystitis, ureteral stenosis, pneumonia, encephalitis and colitis. Notably, BKV is also a notable cause of acute interstitial nephritis in ~10% of kidney transplant recipients, which leads to graft loss in 15-100% of affected patients (172).

Similarly to polyomaviruses, BKV has demonstrated oncogenic potential by inducing tumors in experimental animal models and transforming normal cells *in vitro* (173). Its genome encodes two key oncoproteins: Small Tag and large Tag, which trigger cell proliferation and immortalization by interacting with tumor suppressor genes, namely p53 and Rb (173-175). Despite this proven oncogenic potential of BKV, its association with human cancer types remains inconclusive. A number of studies have detected BKV in different tumors, including brain, bladder, kidney, prostate and cervix cancers, while others did not (176-181). In GBM, studies have showed contradictory results (Table IV). The highest prevalence has been reported by De Mattei *et al* (182), who found that 88% of GBM specimens were positive for BKV Tag DNA sequences. In addition, a study conducted by Corallini *et al* (183) demonstrated the presence of BKV DNA sequence in 50% of GBM cases using southern blotting hybridization. However, contrasting findings have emerged from other studies, which detected the virus in GBM at low frequencies. For example, Delbue *et al* (156) identified BKV Tag DNA sequence in 9.5% of GBM samples using nested PCR. In a different study including three GBM subtypes, only one primary GBM specimen (4%) was positive for BKV, while all secondary and giant cell GBM samples were negative (165). In line with these low detection rates, Rollison *et al* (153) investigated the presence of BKV in a large series of patients by performing two independent protocols in separate laboratories. Only three GBM samples (2.9%) exhibited positivity for BKV in one laboratory, while no positive cases were detected in the

Table IV. Prevalence of BKV in GBM tissue samples.

| First author, year            | Sample size         | Detection method                                                          | Results n (%) | (Refs.) |
|-------------------------------|---------------------|---------------------------------------------------------------------------|---------------|---------|
| Limam <i>et al</i> , 2020     | 82                  | Conventional PCR (Tag)                                                    | 0 (0)         | (152)   |
| Rollison <i>et al</i> , 2005  | 102                 | PCR followed by southern blotting hybridization (Tag)                     | 3 (2.9)       | (153)   |
|                               |                     | RT-PCR (Tag)                                                              | 0 (0.0)       |         |
| Delbue <i>et al</i> , 2005    | 21                  | Nested PCR (Tag)                                                          | 2 (9.5)       | (156)   |
| Huang <i>et al</i> , 1999     | 28 (Primary GBM)    | PCR followed by southern blotting hybridization (Tag)                     | 1 (4.0)       | (165)   |
|                               | 22 (Secondary GBM)  |                                                                           | 0 (0.0)       |         |
|                               | 18 (Giant cell GBM) |                                                                           | 0 (0.0)       |         |
| De Mattei <i>et al</i> , 1995 | 17                  | PCR followed by southern blotting hybridization (Tag)                     | 15 (88.0)     | (182)   |
| Corallini <i>et al</i> , 1987 | 18                  | Southern blotting hybridization (whole viral genome)                      | 9 (50.0)      | (183)   |
| Dörries <i>et al</i> , 1987   | 5                   | RFLP followed by southern blotting and hybridization (whole viral genome) | 1 (20.0)      | (185)   |

GBM, glioblastoma; RT-PCR, reverse transcription PCR; Tag, large T antigen; BKV, BK virus; RFLP, restriction fragment length polymorphism.

other laboratory. By contrast, Grossi *et al* (180) indicated the absence of BKV DNA sequences in all human tumors tested, including GBM, by applying DNA-DNA reassociation kinetics and blotting hybridization (180). Consistent with this finding, Limam *et al* (152) recently reported the absence of BKV DNA in all GBM tested by PCR assay.

To address the discrepancy in previous findings, two serological studies compared the prevalence of BKV antibodies in patients with glioma and healthy controls to assess the potential association between BKV infection and glioma risk. In 2003, Rollison *et al* (184) conducted a nested case-control study to evaluate the potential association between BKV infection and the risk of primary brain tumors. The study analyzed sera collected years before diagnosis from brain tumor patients and matched controls, assessing the presence of BKV-specific antibodies. The findings revealed a non-marked inverse association between BKV seropositivity and the risk of developing brain tumors, including GBM. In a larger recent case-control study conducted within two prospective cohorts, the research group measured BKV antibody levels in adult patients with glioma and cancer-free controls. The seroprevalence analysis demonstrated no notable association between BKV infection and glioma risk in both cohorts. These results remained consistent even when the analysis was restricted to GBM cases (154).

#### 4. HPV

Papillomaviruses are a large group of DNA viruses that infect the epithelial cells of both animals and humans. To date, >300 genotypes of papillomaviruses have been identified and fully sequenced, including >200 exclusively infecting humans (186). Among them, HPV is responsible for ~4.5% of human malignancies and is the primary cause of 95% of cervical cancer cases (187,188). Notably, not all HPV genotypes are oncogenic. According to the IARC classification, only 12 types are considered carcinogenic: HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 (189). The early region of the HPV genome encodes two major oncoproteins, E6 and E7,

serving a notable role in cellular transformation by binding to tumor suppressor proteins p53 and pRb, respectively, which results in their degradation and promotes DNA damage and uncontrolled cell cycle progression (190).

Over the last decade, emerging evidence has reported the presence of HPV DNA sequence in GBM tissue, suggesting a positive association between GBM and HPV infection (Table V). In 2013, Vidone *et al* (191) analyzed tumor samples from 52 patients with GBM using nested-PCR targeting the major capsid protein L1 gene. Positive genes were subsequently genotyped through PCR product sequencing. HPV DNA was detected in 12 cases (23%), including nine cases positive for the low-risk HPV-6 genome and three cases harboring the high-risk HPV-16 genome. This research group also assessed the association between HPV and the prognosis of GBM, finding that the prognosis was markedly worse in HPV-positive cases aged 50 or older compared with their HPV-negative counterparts (191). Similarly, a study conducted with Japanese patients detected the presence of HPV in 21% of GBM, with HPV-16 being the most prevalent genotype, followed by HPV-18 (17). More recently, Adnan Ali *et al* (192) also detected HPV positivity in 28% of GBM from Pakistani patients, among which 20% had HPV-18, 16% HPV-16 and 11 cases showed coinfection with both genotypes. In addition, this study observed that HPV-positive patients exhibited longer survival compared with non-infected cases, but the difference was not statistically significant. The highest prevalence of HPV in GBM was reported by Limam *et al* (193), who detected HPV DNA in 53.7% (44 out of 82) of Tunisian patients. Genotyping showed that 50% of positive samples carried high-risk genotypes and five samples showed simultaneous infection with HPV18 and HPV16. In a recent study, Arsene *et al* (194) found that HPV was markedly more prevalent in GBM compared with normal brain tissues, determining the results of previous studies (17,191-193), suggesting a potential association between HPV infection and gliomagenesis.

Conversely, a bioinformatics analysis conducted by Strong *et al* (103) utilizing publicly available RNA-seq

Table V. Studies detecting HPV in GBM tissue specimens.

| First author, year            | Detection method<br>(targeted sequence)                                                 | Prevalence, %<br>(positive cases/<br>all patients with GBM) | Genotyping<br>method                                                  | Genotyping<br>results                               | (Refs.) |
|-------------------------------|-----------------------------------------------------------------------------------------|-------------------------------------------------------------|-----------------------------------------------------------------------|-----------------------------------------------------|---------|
| Hashida <i>et al</i> , 2015   | RT-qPCR, nested-PCR<br>(E6 of HPV-16 and L1<br>region of HPV-18) and<br>IHC (L1 region) | 20.5 (8/39)                                                 | RT-qPCR<br>Nested-PCR<br>(E6 of HPV-16<br>and L1 region of<br>HPV-18) | HPV-16 (n=6)<br>HPV-18 (n=2)                        | (17)    |
| Vidone <i>et al</i> , 2014    | Nested-PCR (L1 region)                                                                  | 23.1 (12/52)                                                | PCR and Sanger<br>Sequencing)                                         | HPV-6 (n=9)<br>HPV-16 (n=3)                         | (191)   |
| Adnan Ali <i>et al</i> , 2019 | Conventional PCR<br>(L1 region)                                                         | 28.0 (31/112)                                               | PCR and sanger<br>sequencing                                          | HPV-18 (n=22)<br>HPV-16 (n=18)<br>Other types (n=2) | (192)   |
| Limam <i>et al</i> , 2020     | Conventional PCR<br>(L1 region) and<br>DNA ISH                                          | 53.7 (44/82)                                                | Conventional PCR<br>(E7 of HPV-16 and<br>HPV-18)                      | HPV-16 (n=14)<br>HPV-18 (n=13)                      | (193)   |
| Arsene <i>et al</i> , 2022    | Conventional PCR<br>(L1 region)                                                         | 29.5 (13/44)                                                | N/A                                                                   | N/A                                                 | (194)   |

HPV, human papillomavirus; ISH, *in situ* hybridisation; RT-qPCR, reverse transcription-quantitative PCR; GBM, glioblastoma.

and whole genome sequencing datasets, detected the presence of HPV in four cases of low-grade glioma, but none in GBM (103). Similarly, an additional study analyzed RNA-seq datasets to screen for viral sequences across numerous tumor types and failed to detect any HPV transcripts in GBM (104). These findings suggest that, unlike certain other malignancies, HPV is unlikely to play a role in the pathogenesis of GBM.

## 5. Human endogenous retroviruses (HERVs)

Differing from the aforementioned viruses, HERVs are proviruses that represent ~8% of the human genome (195). They are the remnants of ancient germinal retroviral infections that occurred millions of years ago and have been transmitted vertically through successive generations (196,197). HERVs are considered defective viruses that have lost the ability to replicate and produce infectious particles. A typical proviral sequence comprises viral gag (group-specific antigen), pol (polymerase) and env (envelope) genes framed by long terminal repeats (LTRs). Despite this, some HERVs lack the env gene, while others have lost the entire coding sequence during homologous recombination events (196). The genetic expression of HERVs is suppressed by a number of epigenetic mechanisms in normal adult cells, such as chromatin remodeling and DNA methylation (198,199). Yet, in pathological conditions including cancer, the epigenetic regulators are altered, leading to upregulation of HERVs-encoded transcripts and retroviral proteins. Furthermore, growing evidence has suggested that exogenous viral infections, such as HCMV and EBV, are also implicated in HERVs transactivation (200). Initially dismissed as ‘junk DNA’, HERVs were initially thought to serve no functional role in the human genome. Yet, subsequent discoveries have revealed that certain HERVs have

been co-opted by the host for beneficial purposes. A notable example is the HERV-W env gene, which encodes the protein Syn-1, a key component in placental development (201).

HERV-K is a  $\beta$ -retrovirus-like group that includes the youngest and most active HERV subgroup, human MMTV-like-2 (HML-2) (202). Since HML-2 is the most recent integrated HERV, its genomic sequence remained nearly intact, capable of expressing functional viral proteins and producing retrovirus-like particles (203). These gene products were shown to participate in oncogenesis in different ways. In *in vitro* experiments, env proteins induced cell-cell fusion and interacted with numerous signaling pathways implicated in carcinogenesis, such as c-Myc and Notch pathways. Furthermore, due to non-allelic recombination, HERV-K LTRs can serve as enhancers or alternative promoters for oncogenes (204). Whereas Kessler *et al* (205) suggested that HERV-K does not serve a marked role in gliomagenesis, a recent study discovered that HML-2 transcripts are markedly upregulated in GBM specimens compared with matched normal brain tissue and that high expression of HML-2 full-length retroviral sequence is associated with poor clinical outcome (205,206). Using single-cell RNA-seq, the same research group analyzed cellular subpopulations of GBM and found that HML-2 transcripts were primarily upregulated in neural progenitor cells that trigger cellular plasticity. To further investigate the association between HML-2 expression and stemness in glioma, the depletion of HML-2 utilizing CRISPR interference was conducted in order to assess its functional impact on tumor biology. These results exhibited decreased cellular viability and adhesion, along with reduced neurosphere formation, indicating diminished GBM stemness properties. On the other hand, the transfection of normal glial stem cells with the HML-2 consensus

sequence revealed an upregulation of the stem cell marker OCT4. Since HML-2 LTR5Hs contains a binding motif for the nuclear transcription factor OCT4, it may be suggested that OCT4 sustains HML-2 expression, which creates a positive feedback loop that locks GBM cells in a stem-like state. As stemness in GBM is responsible for tumor invasiveness, dissemination and resistance to therapy, targeting this hallmark can notably improve patient survival. In this manner, an antiretroviral nucleoside retroviral inhibitor (abacavir) that abrogates HML-2 expression was tested to evaluate its impact on GBM outcome. The data indicated a marked decrease in OCT4 and env transcript, cellular viability and improved survival in GBM mouse models. Based on these findings, the researchers suggested that HML-2 can serve as a therapeutic target in GBM (206).

An additional HERV family member has also been implicated in GBM tumorigenesis. Reiche *et al* (207) demonstrated that HERV-W, a member of the  $\gamma$ -retrovirus-like supergroup, expresses its env protein in microglia cells within the microenvironment of the glioma, including GBM tissues. To elucidate the effect of the HERV-W env protein on GBM cell line behavior, this study co-cultured microglial cells with GBM cells and subsequently examined changes in gene expression, morphology, migration and proliferation in GBM cells. In the presence of microglia, the stimulation of GBM cells by HERV-W env protein upregulated the expression of a number of genes, including monocyte chemoattractant protein (MCP)-1, MMP9, colony stimulating factor 1, IL6, IL-1 $\beta$ , TNF $\alpha$ , MCP3, inducible nitric oxide synthase and VEGF, involved in tumor growth, cell survival, angiogenesis, extracellular matrix degradation and invasion. Furthermore, enhanced tumor cell proliferation, migration velocity and tumor cell cluster formation by HERV-W env were indicated by this study. However, no marked effect was observed in apoptosis or necrosis (207).

## 6. Conclusion and future directions

GBM is one of the most aggressive malignancies and its etiology is not fully understood, making it hard to establish predictive and preventive strategies. Over the past decades, numerous studies have suggested that the aforementioned viruses may serve a role in GBM carcinogenesis. Although viral prevalence in GBM varies across studies, HCMV and HERVs were clearly shown to be active participants in the oncogenic process. Current evidence indicates that these viruses act as oncomodulators, driving key GBM hallmarks. Since HCMV may be responsible for HERV reactivation, investigating the crosstalk between these two in GBM could reveal a multi-modal model of gliomagenesis and identify novel therapeutic targets. Furthermore, the viral prevalence discrepancy in GBM needs to be addressed by furthering our understanding of the technical factors involved, in order to establish standardized detection protocols. Finally, current evidence is not sufficient to classify HCMV as an oncovirus; further studies are warranted to conclusively determine its role in GBM initiation.

## Acknowledgements

Not applicable.

## Funding

No funding was received.

## Availability of data and materials

Not applicable.

## Authors' contributions

FG conceptualized the present study and provided overall supervision. IS and NI developed the methodology. IS and PM wrote the original draft. FG, IC and RA reviewed and edited the manuscript. IS created the figure. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## References

1. Ferlay J, Ervik M, Lam F, Laversanne M, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I and Brain F: Global Cancer Observatory: Cancer Today, (GLOBOCAN 2022). International Agency for Research on Cancer, Lyon, 2024.
2. Schaff LR and Mellinghoff IK: Glioblastoma and other primary brain malignancies in adults. A review. *JAMA* 329: 574-587, 2023.
3. Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, Hawkins C, Ng HK, Pfister SM, Reifenberger G, *et al*: The 2021 WHO classification of tumors of the central nervous system: A summary. *Neuro Oncol* 23: 1231-1251, 2021.
4. Grochans S, Cybulska AM, Simińska D, Korbecki J, Kojder K, Chlubek D and Baranowska-Bosiacka I: Epidemiology of glioblastoma multiforme-literature review. *Cancers (Basel)* 14: 2412, 2022.
5. Czarnywojtek A, Borowska M, Dyrka K, Van Gool S, Sawicka-Gutaj N, Moskal J, Kościński J, Graczyk P, Hałas T, Lewandowska AM, *et al*: Glioblastoma Multiforme: The Latest Diagnostics and Treatment Techniques. *Pharmacology* 108: 423-431, 2023.
6. Sabouri M, Dogonchi AF, Shafiei M and Tehrani DS: Survival rate of patient with glioblastoma: A population-based study. *Egypt J Neurosurg* 39: 42, 2024.
7. Smith CJ, Perfetti TA, Chokshi C, Venugopal C, Ashford JW and Singh SK: Risk factors for glioblastoma are shared by other brain tumor types. *Hum Exp Toxicol* 43: 09603271241241796, 2024.
8. Braganza MZ, Kitahara CM, Berrington de González A, Inskip PD, Johnson KJ and Rajaraman P: Ionizing radiation and the risk of brain and central nervous system tumors: A systematic review. *Neuro Oncol* 14: 1316-1324, 2012.
9. Moore PS and Chang Y: Why do viruses cause cancer? Highlights of the first century of human tumour virology. *Nat Rev Cancer* 10: 878-889, 2010.
10. Zuhair M, Smit GSA, Wallis G, Jabbar F, Smith C, Devleeschauwer B and Griffiths P: Estimation of the worldwide seroprevalence of cytomegalovirus: A systematic review and meta-analysis. *Rev Med Virol* 29: e2034, 2019.

11. Karagas MR, Kaldor J, Michaelis M, Muchengeti MM, Alfaite D, Argirion I, Chen X, Cunha C, Hantz S, Koljonen V, *et al*: Carcinogenicity of hepatitis D virus, human cytomegalovirus, and Merkel cell polyomavirus. *Lancet Oncol*: S1470-2045(25)00403-6, 2025 (Epub ahead of print).
12. Cobbs CS, Harkins L, Samanta M, Gillespie GY, Bharara S, King PH, Nabors LB, Cobbs CG and Britt WJ: Human cytomegalovirus infection and expression in human malignant gliomas. *Cancer Res* 62: 3347-3350, 2002.
13. Scheurer ME, Bondy ML, Aldape KD, Albrecht T and El-Zein R: Detection of human cytomegalovirus in different histological types of gliomas. *Acta Neuropathol* 116: 79-86, 2008.
14. Ranganathan P, Clark PA, Kuo JS, Salamat MS and Kalejta RF: Significant association of multiple human cytomegalovirus genomic loci with glioblastoma multiforme samples. *J Virol* 86: 854-864, 2012.
15. Sabatier J, Uro-Coste E, Pommepuy I, Labrousse F, Allart S, Trémoulet M, Delisle MB and Brousset P: Detection of human cytomegalovirus genome and gene products in central nervous system tumours. *Br J Cancer* 92: 747-750, 2005.
16. Baumgarten P, Michaelis M, Rothweiler F, Starzetz T, Rabenau HF, Berger A, Jennwein L, Braczynski AK, Franz K, Seifert V, *et al*: Response to Human cytomegalovirus infection in tumor cells of the nervous system is not detectable with standardized pathologic-virological diagnostics. *Neuro Oncol* 16: 1469-1477, 2014.
17. Hashida Y, Taniguchi A, Yawata T, Hosokawa S, Murakami M, Hiroi M, Ueba T and Daibata M: Prevalence of human cytomegalovirus, polyomaviruses, and oncogenic viruses in glioblastoma among Japanese subjects. *Infect Agent Cancer* 10: 3, 2015.
18. Peredo-Harvey I, Rahbar A and Söderberg-Nauclér C: Presence of the human cytomegalovirus in glioblastomas-a systematic review. *Cancers (Basel)* 13: 5051, 2021.
19. Cobbs C: Response to 'Human cytomegalovirus infection in tumor cells of the nervous system is not detectable with standardized pathologic-virological diagnostics'. *Neuro Oncol* 16: 1435-1436, 2014.
20. Cobbs CS, Matlaf L and Harkins LE: Methods for the detection of cytomegalovirus in glioblastoma cells and tissues. *Methods Mol Biol* 1119: 165-196, 2014.
21. Taha MS, Abdalhamid BA, El-Badawy SA, Sorour YM, Almsned FM and Al-Abadi MA: Expression of cytomegalovirus in glioblastoma multiforme: Myth or reality? *Br J Neurosurg* 30: 307-312, 2016.
22. Mitchell DA, Xie W, Schmittling R, Learn C, Friedman A, McLendon RE and Sampson JH: Sensitive detection of human cytomegalovirus in tumors and peripheral blood of patients diagnosed with glioblastoma. *Neuro Oncol* 10: 10-18, 2008.
23. Rahbar A, Orrego A, Peredo I, Dzabic M, Wolmer-Solberg N, Strååt K, Stragliotto G and Söderberg-Nauclér C: Human cytomegalovirus infection levels in glioblastoma multiforme are of prognostic value for survival. *J Clin Virol* 57: 36-42, 2013.
24. Lin CT, Leibovitch EC, Almira-Suarez MI and Jacobson S: Human herpesvirus multiplex ddPCR detection in brain tissue from low- and high-grade astrocytoma cases and controls. *Infect Agent Cancer* 11: 32, 2016.
25. Bhattacharjee B, Renzette N and Kowalik TF: Genetic analysis of cytomegalovirus in malignant gliomas. *J Virol* 86: 6815-6824, 2012.
26. Ghaffari H, Tavakoli A, Faranoush M, Naderi A, Kiani SJ, Sadeghipour A, Javanmard D, Farahmand M, Ghorbani S, Sedaghati F and Monavari SH: Molecular investigation of human cytomegalovirus and epstein-barr virus in glioblastoma brain tumor: A case-control study in iran. *Iran Biomed J* 25: 426-433, 2021.
27. Moraes FCA, Rodrigues PB, Vital LDS, Talah BAD, Burbano RMR and Hirata MH: Prevalence of human cytomegalovirus in glioblastoma multiforme: A systematic review and meta-analysis. *J Neurooncol* 176: 143, 2025.
28. El Baba R, Pasquereau S, Haidar Ahmad S, Monnien F, Abad M, Bibeau F and Herbein G: EZH2-Myc driven glioblastoma elicited by cytomegalovirus infection of human astrocytes. *Oncogene* 42: 2031-2045, 2023.
29. Guyon J, Haidar Ahmad S, El Baba R, Le Quang M, Bikfalvi A, Daubon T and Herbein G: Generation of glioblastoma in mice engrafted with human cytomegalovirus-infected astrocytes. *Cancer Gene Ther* 31: 1070-1080, 2024.
30. Soroceanu L, Akhavan A and Cobbs CS: Platelet-derived growth factor- $\alpha$  receptor activation is required for human cytomegalovirus infection. *Nature* 455: 391-395, 2008.
31. Cobbs C, Khan S, Matlaf L, McAllister S, Zider A, Yount G, Rahlin K, Harkins L, Bezrookove V, Singer E and Soroceanu L: HCMV glycoprotein B is expressed in primary glioblastomas and enhances growth and invasiveness via PDGFR- $\alpha$  activation. *Oncotarget* 5: 1091-1100, 2014.
32. Cobbs CS, Soroceanu L, Denham S, Zhang W and Kraus MH: Modulation of oncogenic phenotype in human glioma cells by cytomegalovirus IE1-mediated mitogenicity. *Cancer Res* 68: 724-730, 2008.
33. Li X, Wu C, Chen N, Gu H, Yen A, Cao L, Wang E and Wang L: PI3K/Akt/mTOR signaling pathway and targeted therapy for glioblastoma. *Oncotarget* 7: 33440-33450, 2016.
34. Tang J, Karbhari N and Campian JL: Therapeutic targets in glioblastoma: Molecular pathways, emerging strategies, and future directions. *Cells* 14: 494, 2025.
35. Lee K, Jeon K, Kim JM, Kim VN, Choi DH, Kim SU and Kim S: Downregulation of GFAP, TSP-1, and p53 in human glioblastoma cell line, U373MG, by IE1 protein from human cytomegalovirus. *Glia* 51: 1-12, 2005.
36. Strååt K, Liu C, Rahbar A, Zhu Q, Liu L, Wolmer-Solberg N, Lou F, Liu Z, Shen J, Jia J, *et al*: Activation of telomerase by human cytomegalovirus. *J Natl Cancer Inst* 101: 488-497, 2009.
37. El Baba R, Haidar Ahmad S, Vanhulle C, Vreux L, Plant E, Van Lint C and Herbein G: Formation of polyploid giant cancer cells and the transformative role of human cytomegalovirus IE1 protein. *Cancer Lett* 630: 217824, 2025.
38. Sommer MH, Scully AL and Spector DH: Transactivation by the human cytomegalovirus IE2 86-kilodalton protein requires a domain that binds to both the TATA box-binding protein and the retinoblastoma protein. *J Virol* 68: 6223-6231, 1994.
39. Cinatl J Jr, Vogel JU, Kotchetkov R and Wilhelm Doerr H: Oncomodulatory signals by regulatory proteins encoded by human cytomegalovirus: A novel role for viral infection in tumor progression. *FEMS Microbiol Rev* 28: 59-77, 2004.
40. Dziurzynski K, Chang SM, Heimberger AB, Kalejta RF, McGregor Dallas SR, Smit M, Soroceanu L and Cobbs CS: HCMV and Gliomas Symposium: Consensus on the role of human cytomegalovirus in glioblastoma. *Neuro Oncol* 14: 246-255, 2012.
41. Wiebusch L and Hagemeyer C: Human Cytomegalovirus 86-Kilodalton IE2 protein blocks cell cycle progression in G(1). *J Virol* 73: 9274-9283, 1999.
42. Wang T, Qian D, Hu M, Li L, Zhang L, Chen H, Yang R and Wang B: Human cytomegalovirus inhibits apoptosis by regulating the activating transcription factor 5 signaling pathway in human malignant glioma cells. *Oncol Lett* 8: 1051-1057, 2014.
43. Hu M, Wang B, Qian D, Wang M, Huang R, Wei L, Li L, Zhang L and Liu DX: Human cytomegalovirus immediate-early protein promotes survival of glioma cells through interacting and acetylating ATF5. *Oncotarget* 8: 32157-32170, 2017.
44. Slinger E, Maussang D, Schreiber A, Siderius M, Rahbar A, Fraile-Ramos A, Lira SA, Söderberg-Nauclér C and Smit MJ: HCMV-encoded chemokine receptor US28 mediates proliferative signaling through the IL-6-STAT3 axis. *Sci Signal* 3: ra58, 2010.
45. Soroceanu L, Matlaf L, Bezrookove V, Harkins L, Martinez R, Greene M, Soteropoulos P and Cobbs CS: Human cytomegalovirus US28 found in glioblastoma promotes an invasive and angiogenic phenotype. *Cancer Res* 71: 6643-6653, 2011.
46. Liang S, Yu B, Qian D, Zhao R, Wang B and Hu M: Human cytomegalovirus ie2 affects the migration of glioblastoma by mediating the different splicing patterns of RON through hnRNP A2B1. *Neuroreport* 30: 805-811, 2019.
47. Vomaske J, Varnum S, Melnychuk R, Smith P, Pasa-Tolic L, Shuttanandan JI and Streblow DN: HCMV pUS28 initiates pro-migratory signaling via activation of Pyk2 kinase. *Herpesviridae* 1: 2, 2010.
48. Cobbs CS, Soroceanu L, Denham S, Zhang W, Britt WJ, Pieper R and Kraus MH: Human cytomegalovirus induces cellular tyrosine kinase signaling and promotes glioma cell invasiveness. *J Neurooncol* 85: 271-280, 2007.
49. Zhu X, Hu B, Hu M, Qian D and Wang B: Human cytomegalovirus infection enhances invasiveness and migration of glioblastoma cells by epithelial-to-mesenchymal transition. *Int J Clin Exp Pathol* 13: 2637-2647, 2020.
50. Maussang D, Verzijl D, van Walsum M, Leurs R, Holl J, Pleskoff O, Michel D, van Dongen GA and Smit MJ: Human cytomegalovirus-encoded chemokine receptor US28 promotes tumorigenesis. *Proc Natl Acad Sci USA* 103: 13068-13073, 2006.

51. de Wit RH, Mujić-Delić A, ven Senten JR, Fraile-Ramos A, Siderius M and Smit MJ: Human cytomegalovirus encoded chemokine receptor US28 activates the HIF-1 $\alpha$ /PKM2 axis in glioblastoma cells. *Oncotarget* 7: 67966-67985, 2016.
52. Matlaf LA, Harkins LE, Bezrookove V, Cobbs CS and Soroceanu L: Cytomegalovirus pp71 protein is expressed in human glioblastoma and promotes pro-angiogenic signaling by activation of stem cell factor. *PLoS One* 8: e68176, 2013.
53. Xing Y, Wang Y, Wang S, Wang X, Fan D, Zhou D and An J: Human cytomegalovirus infection contributes to glioma disease progression via upregulating endocan expression. *Transl Res* 177: 113-126, 2016.
54. Costa H, Xu X, Overbeek G, Vasaikar S, Patro CP, Kostopoulou ON, Jung M, Shafi G, Ananthasethan S, Tsipras G, *et al*: Human cytomegalovirus may promote tumour progression by upregulating arginase-2. *Oncotarget* 7: 47221-47231, 2016.
55. Biserova K, Jakovlevs A, Uljanovs R and Strumfa I: Cancer stem cells: Significance in origin, pathogenesis and treatment of glioblastoma. *Cells* 10: 621, 2021.
56. Xie XP, Laks DR, Sun D, Ganbold M, Wang Z, Pedraza AM, Bale T, Tabar V, Brennan C, Zhou X and Parada LF: Quiescent human glioblastoma cancer stem cells drive tumor initiation, expansion, and recurrence following chemotherapy. *Dev Cell* 57: 32-46.e8, 2022.
57. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD and Rich JN: Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 444: 756-760, 2006.
58. Fornara O, Bartek J Jr, Rahbar A, Odeberg J, Khan Z, Peredo I, Hamerlik P, Bartek J, Stragliotto G, Landázuri N and Söderberg-Naucler C: Cytomegalovirus infection induces a stem cell phenotype in human primary glioblastoma cells: Prognostic significance and biological impact. *Cell Death Differ* 23: 261-269, 2016.
59. Wang X, Hu M, Xing F, Wang M, Wang B and Qian D: Human cytomegalovirus infection promotes the stemness of U251 glioma cells. *J Med Virol* 89: 878-886, 2017.
60. Liu C, Clark PA, Kuo JS and Kalejta RF: Human cytomegalovirus-infected glioblastoma cells display stem cell-like phenotypes. *mSphere* 2: e00137-17, 2017.
61. Soroceanu L, Matlaf L, Khan S, Akhavan A, Singer E, Bezrookove V, Decker S, Ghanny S, Hadaczek P, Bengtsson H, *et al*: Cytomegalovirus immediate early proteins promote stemness properties in glioblastoma. *Cancer Res* 75: 3065-3076, 2015.
62. Powers C, DeFilippis V, Malouli D and Früh K: Cytomegalovirus immune evasion. *Curr Top Microbiol Immunol* 325: 333-359, 2008.
63. Jackson SE, Mason GM and Willis MR: Human cytomegalovirus immunity and immune evasion. *Virus Res* 157: 151-160, 2011.
64. Shen Y, Zhang L, Xue X and Feng S: Molecular mechanisms and targets underlying immune evasion by human cytomegalovirus. *J Microbiol Immunol Infect* 59: 141-151, 2016.
65. Trgovcich J, Cebulla C, Zimmerman P and Sedmak DD: Human cytomegalovirus protein pp71 disrupts major histocompatibility complex class I cell surface expression. *J Virol* 80: 951-963, 2006.
66. Dziurzynski K, Wei J, Qiao W, Hatiboglu MA, Kong LY, Wu A, Wang Y, Cahill D, Levine N, Prabhu S, *et al*: Glioma-associated cytomegalovirus mediates subversion of the monocyte lineage to a tumor propagating phenotype. *Clin Cancer Res* 17: 4642-4649, 2011.
67. Chan G, Bivins-Smith ER, Smith MS and Yurochko AD: NF-kappaB and phosphatidylinositol 3-kinase activity mediates the HCMV-induced atypical M1/M2 polarization of monocytes. *Virus Res* 144: 329-333, 2009.
68. Qin Z, Zhang L, Xu Y, Zhang X, Fang X, Qian D, Liu X, Liu T, Li L, Yu H and Wang B: TLR3 regulates PD-L1 expression in human cytomegalovirus infected glioblastoma. *Int J Clin Exp Pathol* 11: 5318-5326, 2018.
69. El Baba R and Herbein G: Human cytomegalovirus-induced immune tolerance in glioma. *Curr Clin Micro Rpt* 12: 15, 2025.
70. Long X, Zhang Z, Li Y, Deng K, Gao W, Huang M, Wang X, Lin X, She X, Zhao Y, *et al*: ScRNA-seq reveals novel immune-suppressive T cells and investigates CMV-TCR-T cells cytotoxicity against GBM. *J Immunother Cancer* 12: e008967, 2024.
71. Stragliotto G, Rahbar A, Solberg NW, Lilja A, Taher C, Orrego A, Bjurman B, Tammik C, Skarman P, Peredo I and Söderberg-Naucler C: Effects of valganciclovir as an add-on therapy in patients with cytomegalovirus-positive glioblastoma: A randomized, double-blind, hypothesis-generating study. *Int J Cancer* 133: 1204-1213, 2013.
72. Stragliotto G, Pantalone MR, Rahbar A, Bartek J and Soderberg-Naucler C: Valganciclovir as Add-on to standard therapy in glioblastoma patients. *Clin Cancer Res* 26: 4031-4039, 2020.
73. Söderberg-Naucler C, Pantalone MR, Stragliotto G and Bartek J: Does human cytomegalovirus provide a novel therapeutic target for patients with glioblastoma? *Philos Trans R Soc Lond B Biol Sci* 380: 20240403, 2025.
74. Chang CT, Chen HH, Chuang CC, Chang SH and Hsiao NW: Ganciclovir as a potential treatment for glioma: A systematic review and meta-analysis. *J Neurooncol* 165: 399-411, 2023.
75. Hadaczek P, Ozawa T, Soroceanu L, Yoshida Y, Matlaf L, Singer E, Fiallos E, James CD and Cobbs CS: Cidofovir: A novel antitumor agent for glioblastoma. *Clin Cancer Res* 19: 6473-6483, 2013.
76. Sprent J: Antigen-presenting cells: Professionals and amateurs. *Curr Biol* 5: 1095-1097, 1995.
77. Banchereau J and Steinman RM: Dendritic cells and the control of immunity. *Nature* 392: 245-252, 1998.
78. Mitchell DA, Batich KA, Gunn MD, Huang MN, Sanchez-Perez L, Nair SK, Congdon KL, Reap EA, Archer GE, Desjardins A, *et al*: Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients. *Nature* 519: 366-369, 2015.
79. Batich KA, Reap EA, Archer GE, Sanchez-Perez L, Nair SK, Schmittling RJ, Norberg P, Xie W, Herndon JE II, Healy P, *et al*: Long-term survival in glioblastoma with cytomegalovirus pp65-Targeted vaccination. *Clin Cancer Res* 23: 1898-1909, 2017.
80. Batich KA, Mitchell DA, Healy P, Herndon JE II and Sampson JH: Once, twice, three times a finding: Reproducibility of dendritic cell vaccine trials targeting cytomegalovirus in glioblastoma. *Clin Cancer Res* 26: 5297-5303, 2020.
81. Crough T, Beagley L, Smith C, Jones L, Walker DG and Khanna R: Ex vivo functional analysis, expansion and adoptive transfer of cytomegalovirus-specific T-cells in patients with glioblastoma multiforme. *Immunol Cell Biol* 90: 872-880, 2012.
82. Schuessler A, Smith C, Beagley L, Boyle GM, Rehan S, Matthews K, Jones L, Crough T, Dasari V, Klein K, *et al*: Autologous T-cell therapy for cytomegalovirus as a consolidative treatment for recurrent glioblastoma. *Cancer Res* 74: 3466-3476, 2014.
83. Smith C, Lineburg KE, Martins JP, Ambalathingal GR, Neller MA, Morrison B, Matthews KK, Rehan S, Crooks P, Panikkar A, *et al*: Autologous CMV-specific T cells are a safe adjuvant immunotherapy for primary glioblastoma multiforme. *J Clin Invest* 130: 6041-6053, 2020.
84. Reap EA, Suryadevara CM, Batich KA, Sanchez-Perez L, Archer GE, Schmittling RJ, Norberg PK, Herndon JE II, Healy P, Congdon KL, *et al*: Dendritic cells enhance polyfunctionality of adoptively transferred T cells that target cytomegalovirus in glioblastoma. *Cancer Res* 78: 256-264, 2018.
85. Epstein MA, Achong BG and Barr YM: Virus particles in cultured lymphoblasts from burkitt's lymphoma. *Lancet* 1: 702-703, 1964.
86. Kanda T, Yajima M and Ikuta K: Epstein-Barr virus strain variation and cancer. *Cancer Sci* 110: 1132-1139, 2019.
87. Balfour HH Jr, Sifakis F, Sliman JA, Knight JA, Schmeling DO and Thomas W: Age-specific prevalence of Epstein-Barr virus infection among individuals aged 6-19 years in the United States and factors affecting its acquisition. *J Infect Dis* 208: 1286-1293, 2013.
88. Xiong G, Zhang B, Huang MY, Zhou H, Chen LZ, Feng QS, Luo X, Lin HJ and Zeng YX: Epstein-Barr Virus (EBV) infection in Chinese children: A retrospective study of age-specific prevalence. *PLoS One* 9: e99857, 2014.
89. Puhakka L, Sarvikivi E, Lappalainen M, Surcel HM and Et A: Decrease in seroprevalence for herpesviruses among pregnant women in Finland: cross-sectional study of three time points 1992, 2002 and 2012. *Infect Dis (Lond)* 48: 406-410, 2016.
90. Chen CY, Huang KY, Shen JH, Tsao KC and Huang YC: A large-scale seroprevalence of Epstein-Barr virus in Taiwan. *PLoS One* 10: e0115836, 2015.
91. Winter JR, Jackson C, Lewis JEA, Taylor GS, Thomas OG and Stagg HR: Predictors of Epstein-Barr virus serostatus and implications for vaccine policy: A systematic review of the literature. *J Glob Health* 10: 010404, 2020.
92. Wong Y, Meehan MT, Burrows SR, Doolan DL and Miles JJ: Estimating the global burden of Epstein-Barr virus-related cancers. *J Cancer Res Clin Oncol* 148: 31-46, 2022.
93. Huang W, Bai L and Tang H: Epstein-Barr virus infection: The micro and macro worlds. *Virol J* 20: 220, 2023.

94. Möhl BS, Chen J, Sathiyamoorthy K, Jardetzky TS and Longnecker R: Structural and mechanistic insights into the tropism of Epstein-Barr virus. *Mol Cells* 39: 286-291, 2016.
95. Luo Y, Liu Y, Wang C and Gan R: Signaling pathways of EBV-induced oncogenesis. *Cancer Cell Int* 21: 93, 2021.
96. Zhang N, Zuo Y, Jiang L, Peng Y, Huang X and Zuo L: Epstein-Barr virus and neurological diseases. *Front Mol Biosci* 8: 816098, 2022.
97. Menet A, Speth C, Larcher C, Prodinger WM, Schwendinger MG, Chan P, Jäger M, Schwarzmann F, Recheis H, Fontaine M and Dierich MP: Epstein-Barr virus infection of human astrocyte cell lines. *J Virol* 73: 7722-7733, 1999.
98. Gandhi MK, Hoang T, Law SC, Brosda S, O'Rourke K, Tobin JWD, Vari F, Murigneux V, Fink L, Gunawardana J, *et al*: EBV-associated primary CNS lymphoma occurring after immunosuppression is a distinct immunobiological entity. *Blood* 137: 1468-1477, 2021.
99. Kaulen LD, Denisova E, Hinz F, Hai L, Friedel D, Henegariu O, Hoffmann DC, Ito J, Kourtesakis A, Lehnert P, *et al*: Integrated genetic analyses of immunodeficiency-associated Epstein-Barr virus (EBV) positive primary CNS lymphomas. *Acta Neuropathol (Berl)* 146: 499-514, 2023.
100. Limam S, Missaoui N, Mestiri S, Yacoubi MT, Krifa H, Selmi B and Mokni M: Epstein-Barr virus infection in gliomas. *Curr Res Transl Med* 67: 129-133, 2019.
101. Strojnik T, Duh D and Lah TT: Prevalence of neurotropic viruses in malignant glioma and their onco-modulatory potential. *In Vivo* 31: 221-229, 2017.
102. Cosset É, Petty TJ, Dutoit V, Cordey S, Padialeau I, Otten-Hernandez P, Farinelli L, Kaiser L, Bruyère-Cerdan P, Tirefort D, *et al*: Comprehensive metagenomic analysis of glioblastoma reveals absence of known virus despite antiviral-like type I interferon gene response. *Int J Cancer J Int Cancer* 135: 1381-1389, 2014.
103. Strong MJ, Blanchard E IV, Lin Z, Morris CA, Baddoo M, Taylor CM, Ware ML and Flemington EK: A comprehensive next generation sequencing-based virome assessment in brain tissue suggests no major virus-tumor association. *Acta Neuropathol Commun* 4: 71, 2016.
104. Khoury JD, Tannir NM, Williams MD, Chen Y, Yao H, Zhang J, Thompson EJ: TCGA Network; Meric-Bernstam F, Medeiros LJ, *et al*: Landscape of DNA virus associations across human malignant cancers: Analysis of 3,775 cases using RNA-Seq. *J Virol* 87: 8916-8926, 2013.
105. Fonseca RF, Rosas SL, Oliveira JA, Teixeira A, Alves G and Carvalho Mda G: Frequency of Epstein-Barr virus DNA sequences in human gliomas: São Paulo Med J 133: 51-54, 2014.
106. Zavala-Vega S, Castro-Escarpulli G, Hernández-Santos H, Salinas-Lara C, Palma I, Mejía-Arangur JM, Gelista-Herrera N, Rembao-Bojorquez D, Ochoa SA, Cruz-Córdova A, *et al*: An overview of the infection of CMV, HSV 1/2 and EBV in Mexican patients with glioblastoma multiforme. *Pathol Res Pract* 213: 271-276, 2017.
107. Herman A, Gruden K, Blejec A, Podpečan V, Motaln H, Rožman P, Hren M, Zupančič K, Veber M, Verbovšek U, *et al*: Analysis of glioblastoma patients' plasma revealed the presence of MicroRNAs with a prognostic impact on survival and those of viral origin. *PLoS One* 10: e0125791, 2015.
108. Cimino PJ, Zhao G, Wang D, Sehn JK, Lewis JS Jr and Duncavage EJ: Detection of viral pathogens in high grade gliomas from unmapped next-generation sequencing data. *Exp Mol Pathol* 96: 310-315, 2014.
109. Farsi Z and Allahyari Fard N: The identification of key genes and pathways in glioblastoma by bioinformatics analysis. *Mol Cell Oncol* 10: 2246657, 2023.
110. Salahuddin SZ, Ablashi DV, Markham PD, Josephs SF, Sturzenegger S, Kaplan M, Halligan G, Biberfeld P, Wong-Staal F, Kramarsky B, *et al*: Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science* 234: 596-601, 1986.
111. Agut H, Bonnafous P and Gautheret-Dejean A: Laboratory and clinical aspects of human herpesvirus 6 infections. *Clin Microbiol Rev* 28: 313-335, 2015.
112. Ablashi D, Agut H, Alvarez-Lafuente R, Clark DA, Dewhurst S, DiLuca D, Flamand L, Frenkel N, Gallo R, Gompels UA, *et al*: Classification of HHV-6A and HHV-6B as distinct viruses. *Arch Virol* 159: 863-870, 2014.
113. Di Luca D, Mirandola P, Ravaioli T, Bigoni B and Cassai E: Distribution of HHV-6 variants in human tissues. *Infect Agents Dis* 5: 203-214, 1996.
114. Aimola G, Beythien G, Aswad A and Kaufer BB: Current understanding of human herpesvirus 6 (HHV-6) chromosomal integration. *Antiviral Res* 176: 104720, 2020.
115. Tesini BL, Epstein LG and Caserta MT: Clinical impact of primary infection with roseoloviruses. *Curr Opin Virol* 9: 91-96, 2014.
116. Harberts E, Yao K, Wohler JE, Maric D, Ohayon J, Henkin R and Jacobson S: Human herpesvirus-6 entry into the central nervous system through the olfactory pathway. *Proc Natl Acad Sci USA* 108: 13734-13739, 2011.
117. Luka J, Okano M and Thiele G: Isolation of human herpesvirus-6 from clinical specimens using human fibroblast cultures. *J Clin Lab Anal* 4: 483-486, 1990.
118. Chen M, Popescu N, Woodworth C, Berneman Z, Corbellino M, Lusso P, Ablashi DV and DiPaolo JA: Human herpesvirus 6 infects cervical epithelial cells and transactivates human papillomavirus gene expression. *J Virol* 68: 1173-1178, 1994.
119. Liu D, Wang X, Wang Y, Wang P, Fan D, Chen S, Guan Y, Li T, An J and Luan G: Detection of EBV and HHV6 in the brain tissue of patients with rasmussen's encephalitis. *Virol Sin* 33: 402-409, 2018.
120. Albright AV, Lavi E, Black JB, Goldberg S, O'Connor MJ and Gonzalez-Scarano F: The effect of human herpesvirus-6 (HHV-6) on cultured human neural cells: Oligodendrocytes and microglia. *J Neurovirol* 4: 486-494, 1998.
121. He J, McCarthy M, Zhou Y, Chandran B and Wood C: Infection of primary human fetal astrocytes by human herpesvirus 6. *J Virol* 70: 1296-1300, 1996.
122. Yamanishi K, Okuno T, Shiraki K, Takahashi M, Kondo T, Asano Y and Kurata T: Identification Of human herpesvirus-6 as a causal agent for exanthem subitum. *Lancet* 331: 1065-1067, 1988.
123. Hill JA, Myerson D, Sedlak RH, Jerome KR and Zerr DM: Hepatitis due to human herpesvirus 6B after hematopoietic cell transplantation and a review of the literature. *Transpl Infect Dis* 16: 477-483, 2014.
124. Ishio T, Endo T, Okada K, Shigematsu A, Hashino S and Teshima T: Human herpesvirus-6 pneumonitis around the engraftment of cord blood transplantation following foscarnet prophylaxis in a patient with acute leukemia. *Case Rep Hematol* 2015: 949265, 2015.
125. Wainwright MS, Martin PL, Morse RP, Lacaze M, Provenza JM, Coleman RE, Morgan MA, Huette C, Kurtzberg J, Bushnell C, *et al*: Human herpesvirus 6 limbic encephalitis after stem cell transplantation. *Ann Neurol* 50: 612-619, 2001.
126. Santpere G, Telford M, Andrés-Benito P, Navarro A and Ferrer I: The presence of human herpesvirus 6 in the brain in health and disease. *Biomolecules* 10: 1520, 2020.
127. Eliassen E, Lum E, Pritchett J, Ongradi J, Krueger G, Crawford JR, Phan TL, Ablashi D and Hudnall SD: Human herpesvirus 6 and malignancy: A review. *Front Oncol* 8: 512, 2018.
128. Luppi M, Barozzi P, Maiorana A, Marasca R, Trovato R, Fano R, Ceccherini-Nelli L and Torelli G: Human herpesvirus-6: A survey of presence and distribution of genomic sequences in normal brain and neuroglial tumors. *J Med Virol* 47: 105-111, 1995.
129. Cuomo L, Trivedi P, Cardillo MR, Gagliardi FM, Vecchione A, Caruso R, Calogero A, Frati L, Faggioni A and Ragona G: Human herpesvirus 6 infection in neoplastic and normal brain tissue. *J Med Virol* 63: 45-51, 2001.
130. Crawford JR, Santi MR, Cornelison R, Sallinen SL, Haapasalo H and MacDonald TJ: Detection of human herpesvirus-6 in adult central nervous system tumors: Predominance of early and late viral antigens in glial tumors. *J Neurooncol* 95: 49-60, 2009.
131. Chi J, Gu B, Zhang C, Peng G, Zhou F, Chen Y, Zhang G, Guo Y, Guo D, Qin J, *et al*: Human herpesvirus 6 latent infection in patients with glioma. *J Infect Dis* 206: 1394-1398, 2012.
132. Gu B, Li M, Zhang Y, Li L, Yao K and Wang S: DR7 encoded by human herpesvirus 6 promotes glioma development and progression. *Cancer Manag Res* 11: 2109-2118, 2019.
133. Padgett BL, Walker DL, Zur Rhein GM, Eckroade RJ and Dessel BH: Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. *Lancet* 297: 1257-1260, 1971.
134. Taguchi F, Kajioaka J and Miyamura T: Prevalence Rate and Age of Acquisition of Antibodies against JC Virus and BK Virus in Human Sera. *Microbiol Immunol* 26: 1057-1064, 1982.

135. Padgett BL, Rogers CM and Walker DL: JC virus, a human polyomavirus associated with progressive multifocal leukoencephalopathy: Additional biological characteristics and antigenic relationships. *Infect Immun* 15: 656-662, 1977.
136. Kean JM, Rao S, Wang M and Garcea RL: Seroepidemiology of human polyomaviruses. *PLoS Pathog* 5: e1000363, 2009.
137. Elia F, Villani S, Ambrogi F, Signorini L, Dallari S, Binda S, Primache V, Pellegrinelli L, Ferrante P and Delbue S: JC virus infection is acquired very early in life: Evidence from a longitudinal serological study. *J Neurovirol* 23: 99-105, 2017.
138. Ahye N, Bellizzi A, May D and Wollebo HS: The role of the JC virus in central nervous system tumorigenesis. *Int J Mol Sci* 21: 6236, 2020.
139. Koranik IJ: Progressive multifocal leukoencephalopathy revisited: Has the disease outgrown its name? *Ann Neurol* 60: 162-173, 2006.
140. Pietropaolo V, Prezioso C, Bagnato F and Antonelli G: John Cunningham virus: An overview on biology and disease of the etiological agent of the progressive multifocal leukoencephalopathy. *New Microbiol* 41: 179-186, 2018.
141. London WT, Houff SA, Madden DL, Fuccillo DA, Gravel M, Wallen WC, Palmer AE, Sever JL, Padgett BL, Walker DL, *et al*: Brain tumors in owl monkeys inoculated with a human polyomavirus (JC Virus). *Science* 201: 1246-1249, 1978.
142. Walker DL, Padgett BL, ZuRhein GM, Albert AE and Marsh RF: Human papovavirus (JC): Induction of brain tumors in hamsters. *Science* 181: 674-676, 1973.
143. Krynska B, Otte J, Franks R, Khalili K and Croul S: Human ubiquitous JCV(CY) T-antigen gene induces brain tumors in experimental animals. *Oncogene* 18: 39-46, 1999.
144. Dyson N, Bernards R, Friend SH, Gooding LR, Hassell JA, Major EO, Pipas JM, Vandyke T and Harlow E: Large T antigens of many polyomaviruses are able to form complexes with the retinoblastoma protein. *J Virol* 64: 1353-1356, 1990.
145. Caracciolo V, Macaluso M, D'Agostino L, Montanari M, Scheff J, Reiss K, Khalili K and Giordano A: Cross-Talk Between T-Ag Presence and pRb Family and p53/p73 signaling in mouse and human medulloblastoma. *J Cell Biochem* 110: 182-190, 2010.
146. Del Valle L, Gordon J, Enam S, Delbue S, Croul S, Abraham S, Radhakrishnan S, Assimakopoulou M, Katsetos CD and Khalili K: Expression of human neurotropic polyomavirus JCV late gene product agnoprotein in human medulloblastoma. *J Natl Cancer Inst* 94: 267-273, 2002.
147. Krynska B, Del Valle L, Croul S, Gordon J, Katsetos CD, Carbone M, Giordano A and Khalili K: Detection of human neurotropic JC virus DNA sequence and expression of the viral oncogenic protein in pediatric medulloblastomas. *Proc Natl Acad Sci USA* 96: 11519-11524, 1999.
148. Rencic A, Gordon J, Otte J, Curtis M, Kovatich A, Zoltick P, Khalili K and Andrews D: Detection of JC virus DNA sequence and expression of the viral oncoprotein, tumor antigen, in brain of immunocompetent patient with oligoastrocytoma. *Proc Natl Acad Sci USA* 93: 7352-7357, 1996.
149. Del Valle L, Enam S, Lara C, Ortiz-Hidalgo C, Katsetos CD and Khalili K: Detection of JC polyomavirus DNA sequences and cellular localization of T-antigen and agnoprotein in oligodendrogliomas. *Clin Cancer Res* 8: 3332-3340, 2002.
150. Del Valle L, Gordon J, Assimakopoulou M, Enam S, Geddes JF, Varakis JN, Katsetos CD, Croul S and Khalili K: Detection of JC virus DNA sequences and expression of the viral regulatory protein T-antigen in tumors of the central nervous system. *Cancer Res* 61: 4287-4293, 2001.
151. Afshar RM, Mollaei HR, Zandi B and Iranpour M: Evaluation of JC and Cytomegalo Viruses in Glioblastoma Tissue. *Asian Pac J Cancer Prev APJCP* 17: 4907-4911, 2016.
152. Limam S, Missaoui N, Bdioui A, Yacoubi MT, Krifa H, Mokni M and Selmi B: Investigation of simian virus 40 (SV40) and human JC, BK, MC, KI, and WU polyomaviruses in glioma. *J Neurovirol* 26: 347-357, 2020.
153. Rollison DE, Utaipat U, Ryschkewitsch C, Hou J, Goldthwaite P, Daniel R, Helzlsouer KJ, Burger PC, Shah KV and Major EO: Investigation of human brain tumors for the presence of polyomavirus genome sequences by two independent laboratories. *Int J Cancer* 113: 769-774, 2005.
154. Egan KM, Kim Y, Bender N, Hodge JM, Coghill AE, Smith-Warner SA, Rollison DE, Teras LR, Grimsrud TK and Waterboer T: Prospective investigation of polyomavirus infection and the risk of adult glioma. *Sci Rep* 11: 9642, 2021.
155. Boldorini R, Pagani E, Car PG, Omodeo-Zorini E, Borghi E, Tarantini L, Bellotti C, Ferrante P and Monga G: Molecular characterisation of JC virus strains detected in human brain tumours. *Pathology* 35: 248-253, 2003.
156. Delbue S, Pagani E, Guerini FR, Agliardi C, Mancuso R, Borghi E, Rossi F, Boldorini R, Veggiani C, Car PG and Ferrante P: Distribution, characterization and significance of polyomavirus genomic sequences in tumors of the brain and its covering. *J Med Virol* 77: 447-454, 2005.
157. Eftimov T, Enchev Y, Tsekov I, Simeonov P, Kalvatchev Z and Encheva E: JC polyomavirus in the aetiology and pathophysiology of glial tumours. *Neurosurg Rev* 39: 47-53, 2016.
158. Caldarelli-Stefano R, Boldorini R, Monga G, Meraviglia E, Zorini EO and Ferrante P: JC virus in human glial-derived tumors. *Hum Pathol* 31: 394-395, 2000.
159. Shah KV: Neutralizing antibodies to simian virus 40 (SV40) in human sera from India. *Proc Soc Exp Biol Med* 121: 303-307, 1966.
160. Engels EA, Switzer WM, Heneine W and Viscidi RP: Serologic evidence for exposure to simian virus 40 in North American zoo workers. *J Infect Dis* 190: 2065-2069, 2004.
161. Sweet BH and Hilleman MR: The vacuolating virus, S.V. 40. *Proc Soc Exp Biol Med* 105: 420-427, 1960.
162. Vilchez RA and Butel JS: Emergent human pathogen simian virus 40 and its role in cancer. *Clin Microbiol Rev* 17: 495-508, 2004.
163. Pipas JM: SV40: Cell transformation and tumorigenesis. *Virology* 384: 294-303, 2009.
164. Martini F, Iaccheri L, Lazzarin L, Carinci P, Corallini A, Gerosa M, Iuzzolino P, Barbanti-Brodano G and Tognon M: SV40 early region and large T antigen in human brain tumors, peripheral blood cells, and sperm fluids from healthy individuals. *Cancer Res* 56: 4820-4825, 1996.
165. Huang H, Reis R, Yonekawa Y, Lopes JM, Kleihues P and Ohgaki H: Identification in human brain tumors of DNA sequences specific for SV40 large T antigen. *Brain Pathol* 9: 33-42, 1999.
166. Kouhata T, Fukuyama K, Hagihara N and Tabuchi K: Detection of simian virus 40 DNA sequence in human primary glioblastomas multiforme. *J Neurosurg* 95: 96-101, 2001.
167. Sabatier J, Uro-Coste E, Benouaich A, Boetto S, Gigaud M, Tremoulet M, Delisle MB, Galateau-Sallé F and Brousset P: Immunodetection of SV40 large T antigen in human central nervous system tumours. *J Clin Pathol* 58: 429-431, 2005.
168. Mazzoni E, Gerosa M, Lupidi F, Corallini A, Taronna AP, D'Agostino A, Bovenzi M, Ruggeri G, Casali F, Rotondo JC, *et al*: Significant prevalence of antibodies reacting with simian virus 40 mimotopes in sera from patients affected by glioblastoma multiforme. *Neuro Oncol* 16: 513-519, 2014.
169. Zhen H, Zhang X, Zhang Z, Fei Z, He X, Liang J, Huang W, Liu X and Zhang P: Simian virus 40 large tumor antigen forms specific complexes with p53 and pRb in human brain tumors. *Chin Med J (Engl)* 114: 382-386, 2001.
170. Chong S, Antoni M, Macdonald A, Reeves M, Harber M and Magee CN: BK virus: Current understanding of pathogenicity and clinical disease in transplantation. *Rev Med Virol* 29: e2044, 2019.
171. Gardner SD, Field AM, Coleman DV and Hulme B: New human papovavirus (B.K.) isolated from urine after renal transplantation. *Lancet* 297: 1253-1257, 1971.
172. Siguier M, Sellier P and Bergmann JF: BK-virus infections: A literature review. *Med Mal Infect* 42: 181-187, 2012.
173. Fioriti D, Videtta M, Mischitelli M, Degener AM, Russo G, Giordano A and Pietropaolo V: The human polyomavirus BK: Potential role in cancer. *J Cell Physiol* 204: 402-406, 2005.
174. Harris KF, Christensen JB and Imperiale MJ: BK virus large T antigen: interactions with the retinoblastoma family of tumor suppressor proteins and effects on cellular growth control. *J Virol* 70: 2378-2386, 1996.
175. Harris KF, Christensen JB, Radany EH and Imperiale MJ: Novel mechanisms of E2F induction by BK virus large-T antigen: Requirement of both the pRb-binding and the J domains. *Mol Cell Biol* 18: 1746-1756, 1998.
176. Flaegstad T, Andresen PA, Johnsen JI, Asomani SK, Jørgensen GE, Vignarajan S, Kjuul A, Kogner P and Traavik T: A possible contributory role of BK virus infection in neuroblastoma development. *Cancer Res* 59: 1160-1163, 1999.
177. Monini P, Rotola A, Di Luca D, De Lellis L, Chiari E, Corallini A and Cassai E: DNA rearrangements impairing BK virus productive infection in urinary tract tumors. *Virology* 214: 273-279, 1995.

178. Das D, Shah RB and Imperiale MJ: Detection and expression of human BK virus sequences in neoplastic prostate tissues. *Oncogene* 23: 7031-7046, 2004.
179. Martini F, Iaccheri L, Martinelli M, Martinello R, Grandi E, Mollica G and Tognon M: Papilloma and polyoma DNA tumor virus sequences in female genital tumors. *Cancer Invest* 22: 697-705, 2004.
180. Grossi MP, Meneguzzi G, Chenciner N, Corallini A, Poli F, Altavilla G, Alberti S, Milanesi G and Barbanti-Brodano G: Lack of association between BK virus and ependymomas, malignant tumors of pancreatic islets, osteosarcomas and other human tumors. *Intervirology* 15: 10-17, 1981.
181. Völter C, Hausen H, Alber D and de Villiers EM: Screening human tumor samples with a broad-spectrum polymerase chain reaction method for the detection of polyomaviruses. *Virology* 237: 389-396, 1997.
182. De Mattei M, Martini F, Corallini A, Gerosa M, Scotlandi K, Carinci P, Barbanti-Brodano G and Tognon M: High incidence of BK virus large-T-antigen-coding sequences in normal human tissues and tumors of different histotypes. *Int J Cancer* 61: 756-760, 1995.
183. Corallini A, Pagnani M, Viadana P, Silini E, Mottes M, Milanesi G, Gerna G, Vettor R, Trapella G, Silvani V, *et al*: Association of BK virus with human brain tumors and tumors of pancreatic islets. *Int J Cancer* 39: 60-67, 1987.
184. Rollison DE, Helzlsouer KJ, Alberg AJ, Hoffman S, Hou J, Daniel R, Shah KV and Major EO: Serum antibodies to JC virus, BK virus, simian virus 40, and the risk of incident adult astrocytic brain tumors. *Cancer Epidemiol Biomark Prev* 12: 460-463, 2003.
185. Dörries K, Loeber G and Meixensberger J: Association of polyomaviruses JC, SV40, and BK with human brain tumors. *Virology* 160: 268-270, 1987.
186. Egawa N, Egawa K, Griffin H and Doorbar J: Human papillomaviruses; Epithelial tropisms, and the development of neoplasia. *Viruses* 7: 3863-3890, 2015.
187. de Martel C, Plummer M, Vignat J and Franceschi S: Worldwide burden of cancer attributable to HPV by site, country and HPV type. *Int J Cancer* 141: 664-670, 2017.
188. World Health Organization (WHO): Cervical cancer. WHO, Geneva, 2024.
189. International Agency for Research on Cancer (IARC): IARC Monographs on the Identification of Carcinogenic Hazards to Humans. IARC, Lyon, 2012.
190. Hoppe-Seyler K, Bossler F, Braun JA, Herrmann AL and Hoppe-Seyler F: The HPV E6/E7 oncogenes: Key factors for viral carcinogenesis and therapeutic targets. *Trends Microbiol* 26: 158-168, 2018.
191. Vidone M, Alessandrini F, Marucci G, Farnedi A, de Biase D, Ricceri F, Calabrese C, Kurelac I, Porcelli AM, Cricca M and Gasparre G: Evidence of association of human papillomavirus with prognosis worsening in glioblastoma multiforme. *Neuro Oncol* 16: 298-302, 2014.
192. Adnan Ali SM, Mirza Y, Ahmad Z, Zahid N and Enam SA: Human papillomavirus and human cytomegalovirus infection and association with prognosis in patients with primary glioblastoma in Pakistan. *World Neurosurg* 121: e931-e939, 2019.
193. Limam S, Missaoui N, Hmissa S, Yacoubi MT, Krifa H, Mokni M and Selmi B: Investigation of human cytomegalovirus and human papillomavirus in glioma. *Cancer Invest* 38: 394-405, 2020.
194. Arsene DE, Milanesi E and Dobre M: Viral oncogenesis in tumours of the central nervous system: Reality or random association? A retrospective study on archived material. *J Cell Mol Med* 26: 1413-1420, 2022.
195. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, *et al*: Initial sequencing and analysis of the human genome. *Nature* 409: 860-921, 2001.
196. Bock M and Stoye JP: Endogenous retroviruses and the human germline. *Curr Opin Genet Dev* 10: 651-655, 2000.
197. Jern P and Coffin JM: Effects of retroviruses on host genome function. *Annu Rev Genet* 42: 709-732, 2008.
198. Garcia-Montojo M, Doucet-O'Hare T, Henderson L and Nath A: Human endogenous retrovirus-K (HML-2): A comprehensive review. *Crit Rev Microbiol* 44: 715-738, 2018.
199. Li J, Liao L, Liu X, Zhu Y, Sun D, Zhang C and Jiang Y: Decoding neurological mysteries: The potential impact of endogenous retroviruses on brain health. *Stress Brain* 4: 1-30, 2024.
200. Evans EF, Saraph A and Tokuyama M: Transactivation of human endogenous retroviruses by viruses. *Viruses* 16: 1649, 2024.
201. Li F and Karlsson H: Expression and regulation of human endogenous retrovirus W elements. *APMIS* 124: 52-66, 2016.
202. Williams ZH, Imedio AD, Gaucherand L, Lee DC, Mostafa SM, Phelan JP, Coffin JM and Johnson WE: Recombinant origin and interspecies transmission of a HERV-K(HML-2)-related primate retrovirus with a novel RNA transport element. *Elife* 13: e80216, 2024.
203. Curty G, Marston JL, de Mulder Rougvié M, Leal FE, Nixon DF and Soares MA: Human endogenous retrovirus K in cancer: A potential biomarker and immunotherapeutic target. *Viruses* 12: 726, 2020.
204. Bannert N, Hofmann H, Block A and Hohn O: HERVs new role in cancer: From accused perpetrators to cheerful protectors. *Front Microbiol* 9: 178, 2018.
205. Kessler AF, Wiesner M, Denner J, Kämmerer U, Vince GH, Linsenmann T, Löhr M, Ernestus RI and Hagemann C: Expression-analysis of the human endogenous retrovirus HERV-K in human astrocytic tumors. *BMC Res Notes* 7: 159, 2014.
206. Shah AH, Rivas SR, Doucet-O'Hare TT, Govindarajan V, DeMarino C, Wang T, Ampie L, Zhang Y, Banasavadi-Siddegowda YK, Walbridge S, *et al*: Human endogenous retrovirus K contributes to a stem cell niche in glioblastoma. *J Clin Invest* 133: e167929, 2023.
207. Reiche L, Plaack B, Lehmkuhl M, Weyers V, Gruchot J, Picard D, Perron H, Remke M, Knobbe-Thomsen C, Reifemberger G, *et al*: HERV-W envelope protein is present in microglial cells of the human glioma tumor microenvironment and differentially modulates neoplastic cell behavior. *Microbes Infect* 27: 105460, 2025.



Copyright © 2026 Sabri et al. This work is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0) License.