Clinical implications of cytomegalovirus in glioblastoma progression and therapy

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Glioblastoma (GBM) is one of the deadliest brain cancers with a median survival of only 15 months. This poor prognosis has prompted exploration of novel therapeutic targets for GBM patients. Human cytomegalovirus (HCMV) has been implicated in GBM; however, its impact remains poorly defined, and there is conflicting data over the presence of HCMV in tumors. Nonetheless, clinical trials targeting HCMV have shown promising initial data, and evidence suggests that HCMV may negatively impact GBM patient survival by multiple mechanisms including changes in GBM cell behavior and the tumor microenvironment (TME) that potentiate tumor progression as well as therapy-induced virus reactivation. Moreover, HCMV has many effects on host immunity that could impact tumor behavior by altering the TME, which are largely unexplored. The goal of this review is to describe these potential interactions between HCMV and GBM. Better understanding of these processes may allow the development of new therapeutic modalities to improve GBM patient outcomes.

Glioblastoma (GBM) is the most common primary malignant brain tumor and one of the deadliest types of cancer[1]. GBM comprises 50% of all malignant brain tumors with over 14,000 new cases expected in 2023 in the US[1]. Current treatment includes surgical resection followed by radiotherapy and concomitant chemotherapy using temozolomide (TMZ)[2]. Since 2005 the only new therapies that have been FDA approved for GBM patients are TMZ, Bevacizumab and Optune, a wearable tumor treating fields (TTF) electrical device[3,4]. Patients are also administered corticosteroids, such as dexamethasone, to help mitigate peritumoral edema.

Modern diagnosis of GBM is based on pathological features and molecular markers. These features include high mitotic index, presence of necrosis, microvascular proliferation and hyperplasia. *MGMT* (O-6-methylguanine-DNA methyltransferase) promoter methylation is assessed as an indicator of TMZ sensitivity. However, regardless of *MGMT* methylation status at diagnosis, resistance to treatment inevitably occurs leading to recurrent disease. Molecularly, GBM is characterized by *TERT* promoter mutation, gain of chromosome 7 and loss of chromosome 10[5,6]. GBM is further distinguished by the expression of wild-type isocitrate dehydrogenase (IDH1/2), in contrast to astrocytoma where somatic point mutations in IDH1/2 are a defining feature. Overall, genetic mutations in GBM are heterogeneous and variable between patients. These mutations generally result in activation of tyrosine kinase receptor/PI3K signaling, as well as altered p53 and retinoblastoma (Rb) tumor suppressor pathways[7].

GBM can broadly be transcriptionally defined as proneural (PN), mesenchymal (MES) or classical, which reflects its biological heterogeneity[8]. Single-cell studies further highlight the heterogeneity and plasticity of these tumors at the transcriptomic level[9]. GBM is resistant to immune checkpoint blockade (ICB), which is in part because GBM is considered a 'cold' tumor characterized by an immunosuppressive tumor microenvironment (TME)[10] and low mutational burden compared with ICB-responsive tumor types. The GBM TME is dominated by brain-resident microglial cells and bone marrow-derived macrophages, with relatively low levels of T cells[10,11]. Despite great strides in understanding GBM biology there is still a complete lack of effective GBM targeted therapeutics.

Recent studies suggest that targeting human CMV (HCMV) in GBM patients may be a promising therapeutic avenue[12–14]. HCMV is a β-herpesvirus that establishes lifelong latency with a seroprevalence of 90% in developing countries and 60–80% in developed countries[15]. Latent HCMV is thought to reside in bone marrow hematopoietic progenitor cells and circulating monocytes[16]. Glycoproteins on the viral envelope engage with cell surface receptors to allow virus entry including platelet-derived growth factor receptor alpha (PDGFRα), neuropilin 2, epidermal growth factor receptor (EGFR) and integrin-mediated src-family kinase signaling[17]. HCMV transmission typically occurs via prolonged contact with infected bodily fluids and rarely through blood transfusions[18]. Infection is usually mildly symptomatic and well-controlled in immunocompetent hosts, but

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infection and reactivation can cause serious pathology in immunocompromised individuals. Examples include end organ disease, HCMV-induced retinitis and congenital hearing loss in neonates, which poses a major public health concern[17].

Over the last 20 years, numerous studies have investigated the link between HCMV and GBM. Initial studies described the presence of HCMV proteins and nucleic acids in patient specimens, and identified effects on GBM cell growth, invasion and stemness. However, there is a lack of clarity over the presence of HCMV within tumors, and recent discoveries suggest that the interactions between HCMV and GBM are complex and nuanced, involving potential effects on immune cells and the TME. The goal of this review is to comprehensively discuss the complex and multifactorial nature of the relationship between HCMV and GBM with an in-depth examination of the current landscape of HCMV as a potential therapeutic target in GBM therapy.

Human Cytomegalovirus in Glioblastoma

Viruses influence cancer development and progression in multiple and complex ways[19]. Several oncoviruses have been widely described in human cancer, including human papillomavirus (HPV), Epstein-Barr virus (EBV), hepatitis B virus (HBV), hepatitis C virus (HCV), Kaposi sarcoma-associated herpesvirus (KSHV), human T-lymphotropic virus-1 (HTLV-1) and Merkel cell polyomavirus (MCPyV)[20]. Oncogenic mechanisms induced by these viruses include direct inactivation of tumor suppressors such as Rb and p53 in addition to inhibition of apoptosis and immune surveillance[20]. Oncogenic viruses can also act indirectly through increased genetic mutation frequency, chronic inflammation and chromosomal mutagenesis[19] To date no direct viral causation has been established in GBM. However, the studies outlined in the sections below suggest multiple potential oncomodulatory mechanisms mediated by HCMV. It is important to note that unlike other herpesviruses, there is no clear oncogenic mechanism established for HCMV[21].

Detection of HCMV in GBM

HCMV was first reported to be present in human GBM specimens in 2002[22] and several subsequent studies reported oncomodulatory effects of HCMV associated with increased tumorigenesis[23]. These specimens were measured by immunohistochemistry (IHC) and polymerase chain reaction (PCR)[22]. Notably, HCMV was detected in all tumors tested but was not seen in normal brain and tissues from other brain pathologies. These findings have been confirmed by subsequent work employing various techniques such as in situ hybridization (ISH), immunofluorescence (IF), PCR and IHC[24–30]. Recent investigations have detected the 65 kDa phosphoprotein (pp65/UL83) HCMV protein in both intra- and extra-axial brain tumors and HCMV microRNA (miR-UL112-3p) in blood samples of GBM patients[31,32]. Additionally, IF staining demonstrated the presence of HCMV in human brain sections, where virus co-localized with perivascular NG2+ cells, suggesting a potential residency of HCMV in pericytes[33] and implying that HCMV might be sheltered by various cell types within the TME. The same study also showed detection of the 72 kDa immediate early protein 1 (IE1) gene UL123 through PCR with absolute quantification, but at very low levels. Furthermore, an independent study detected HCMV in 17 out of 18 tumor specimens through the detection of IE1 (UL123)[33].

Conflicting reports challenging this link have also been published. An analysis of 41 GBM samples in The Cancer Genome Atlas (TCGA) RNAseq dataset did not detect HCMV transcripts[34]. A study involving a cohort of 39 Japanese subjects and another from an Israeli cohort failed to detect HCMV DNA using quantitative and nested PCR[35,36]. Another study using deep-coverage whole genome sequencing analyzed 52.6 billion DNA reads from 34 GBM samples found no traces of the virus. Other recent studies have taken multi-pronged approaches, and failed to detect HCMV in central nervous system tumors[37–39].

These discrepancies in detecting HCMV in GBM clinical specimens could either reflect a true absence of virus or be attributed to the varying sensitivities required by different assays to detect specific HCMV viral proteins like IE1 (UL123), glycoprotein B (gB/UL55), or pp65 (UL83). Variability in HCMV detection might also be due to very low viral loads in tissue samples which we have previously reported[40] and may be below the threshold of some bulk RNAseq approaches. This would mean that levels could be below the detection thresholds of many standard detection approaches. These conflicting data underscore the need for further investigation and more refined approaches to detect HCMV in tumors and definitively answer this question. It is also important to note that while there has been difficulty in identifying HCMV in GBM specimens, this does not rule out the possibility of virus reactivation within the tumor and its surrounding environment during treatment, which is more difficult to assess due to the lack of sample availability. Finally, lack of detection could be explained by a "hit and run" mechanism involving a transient initial infection that, though resolved, has long-lasting effects on the cellular environment that may contribute to tumor progression[41].

Oncomodulation by HCMV

The concept of "oncomodulation," which refers to the virus-associated enhancement of malignancy[42], has been observed in various types of cancers. Several studies have documented indications of oncomodulation by HCMV in diverse contexts including controlled laboratory environments (in vitro), clinical case reports, and further substantiated through in vivo GBM mouse models[40,43]. In these models, infection with murine CMV (MCMV), the HCMV equivalent used to model disease, led to more aggressive tumor growth. Clinical evidence further supports the pro-oncogenic function of HCMV. It has been observed that HCMV seropositive GBM patients have significantly shorter overall survival than those who test seronegative[44]. Specifically, HCMV seropositive GBM patients exhibited an average survival of approximately 404 days, while seronegative patients had a longer average survival of around 530 days. These correlations between HCMV and more aggressive GBM disease support an oncomodulatory function of CMV.

In another retrospective study, no connection between HCMV serostatus and patient outcomes was observed. However, this study used samples collected from patients in the early 1990s, prior to the use of the current standard of care (SOC), and also prior to the discovery of the IDH mutation or *MGMT* methylation. Furthermore, this study does show a trend to poorer survival associated with HCMV which did not reach statistical significance. Interestingly, this study also showed an inverse correlation between the α-herpesvirus varicella zoster virus (VZV) and GBM although the mechanism is not defined[45,46].

Oncomodulation could occur via multiple mechanisms including direct effects of HCMV infection on tumor cells, its impact on the TME, or a combination of both, and there is evidence supporting all of these possibilities, with HCMV being implicated in multiple cancer hallmarks as shown in Fig. 1[42,47–59]. HCMV triggers key pro-oncogenic signaling pathways including PDGFRα, which has been shown to be phosphorylated as a consequence of HCMV infection in various cell types leading to the activation of PI3K signaling and increased GBM cell proliferation[60]. Aberrant activation of the PI3K-Akt pathway is a well-known driver of tumorigenesis[61]. Moreover, expression of HCMV IE1 (UL123) dysregulated tumor suppressor proteins including p53 family proteins (p53, p63, p73) and Rb in GBM cell lines results in differential growth in vitro[7]. Another significantly studied HCMV protein, the G protein-coupled receptor (GPCR) US28, has been reported to activate NF-κB and the IL6-JAK1-STAT3 signaling axis leading to interleukin 6 (IL6) production[62]. This induces the expression of vascular endothelial growth factor (VEGF), crucial for supporting tumor angiogenesis[63]. Furthermore, HCMV-induced NF-κB activation may prompt a shift in GBM cells from a proneural (PN) to a mesenchymal (MES) state through upregulation of c-MET[64].

The signal transducer and activator of transcription 3 (STAT3) protein plays a critical role in GBM pathology where its activation and signaling significantly impact disease progression. In particular, aberrant activation of STAT3 has been identified to promote cell proliferation and resistance to apoptosis in GBM[65]. In studies conducted in vitro, GBM tumor spheres
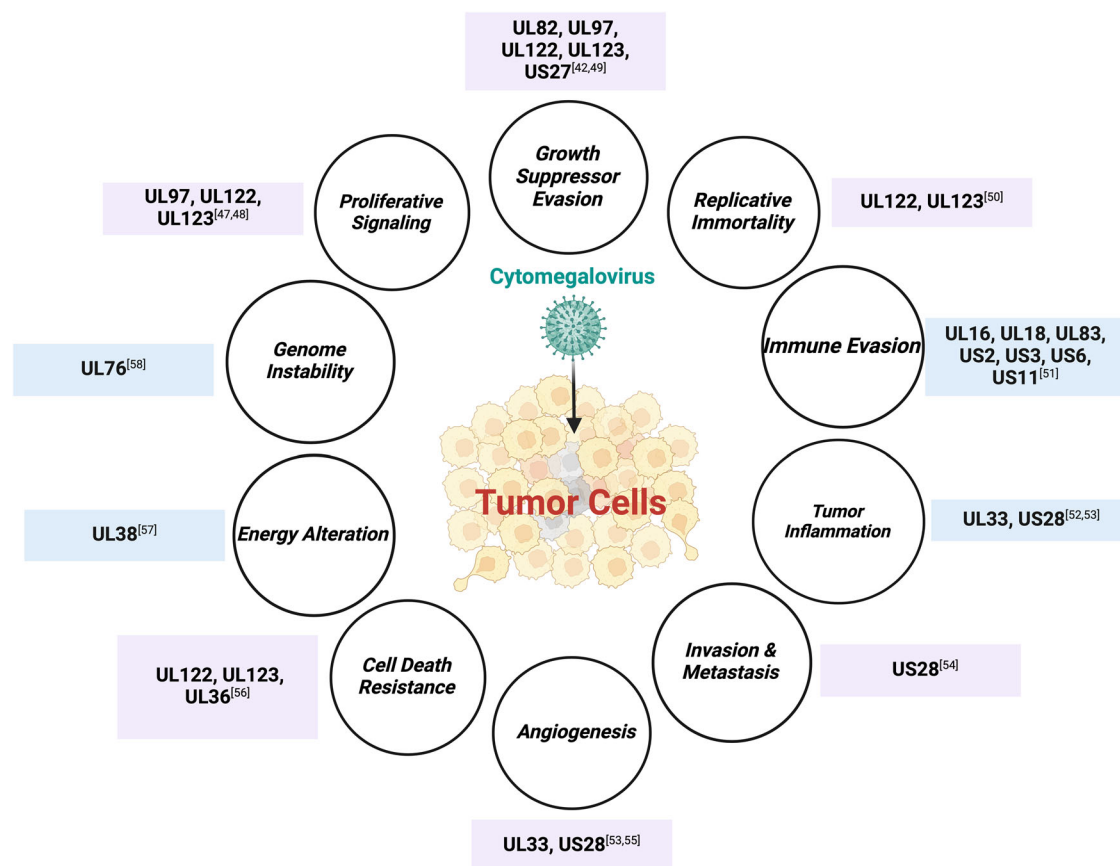
Fig. 1 | Association of HCMV with cancer hallmarks. HCMV genes have been reported to induce multiple cancer hallmarks as defined by Hanahan and Weinberg[120,121], highlighted in blue are four emerging hallmarks.

displayed elevated phosphorylation of STAT3 following infection with HCMV[40,66]. In addition, co-localization of phosphorylated STAT3 and HCMV US28 in vascular regions has been reported in GBM patient samples[67]. Furthermore, HCMV US28 has demonstrated its capacity to activate STAT3 in a sphingosine-1-phosphate-dependent fashion, thereby increasing the survival and proliferation of GBM cells[59]. This further emphasizes the potential role of HCMV-encoded proteins, especially HCMV US28, in modulating STAT3 activity, contributing to the aggressive behavior of GBM cells.

Notable support for the oncomodulatory role of HCMV in GBM comes from studies conducted in mouse models designed to study the impact of MCMV in GBM progression[40]. In this study, two well-characterized murine GBM cell lines, GL261 and CT-2A, were intracranially implanted into C57BL/6 mice. In these models, MCMV infection was found to accelerate GBM growth, resulting in significantly shorter survival times for MCMV-infected mice compared to those without MCMV infection. The MCMV-infected mice displayed earlier onset of clinical signs, such as faster deterioration, neurologic symptoms, and weight loss. Histological examination using Ki67 and CD31 markers revealed an increase in proliferation and angiogenesis in MCMV-infected mice as well as detectable virus within tumors[42].

Together, evidence from cell-based models, mouse models, and human studies support an oncomodulatory role of CMV. HCMV might influence GBM via direct effects on GBM (stem) cells, the TME, or both. Moreover, these effects could extend beyond the infected cells and potentially impact neighboring cells through paracrine signaling. These data suggest that GBM could display more aggressive characteristics in patients harboring HCMV and there are multiple potential mechanisms through which the virus might modulate tumor progression, highlighting potential avenues for exploring new therapeutic strategies.

HCMV Latency and Reactivation in GBM

In healthy individuals, the immune system prevents HCMV reactivation from latent pools. However, under immunosuppressive conditions, the host will no longer be able to prevent the virus from reactivating and re-establishing infection along with viral spreading. The process by which HCMV reactivates involves several modulatory pathways including cellular differentiation, inflammatory-associated signaling and epigenetic regulation of the immediate early locus[68–70]. Of importance, differentiation of myeloid progenitors into circulating monocytes and dendritic cells has been reported to play an important role in latency and subsequent viral spread[71,72].

In addition to the detection of HCMV in GBM tumor specimens and its potential oncomodulatory effects, various lines of evidence suggest that viral reactivation may play a role in the pathogenesis of GBM. Firstly, prospective cohort studies demonstrate a correlation between HCMV reactivation and neurological decline in GBM patients, leading to HCMV-induced encephalopathy that can be reversed with antiviral treatment[73,74]. Interestingly, dexamethasone administered during treatment is associated with increased risk of both reactivation and encephalopathy[75]. Secondly, data from human and murine studies show that antiviral treatment delays tumor progression and prolongs survival[33,76]. Altogether, these data suggest that immunosuppression from GBM and its attendant therapies (e.g., steroids, radiation, chemotherapy) may allow HCMV reactivation propelling tumor progression and patient decline. Therefore, antiviral therapies have the potential to slow tumor growth by impeding viral activity.

Potential Influence of HCMV on The GBM Immune Landscape

HCMV is a highly immunomodulatory virus, but its effects on the immune landscape in tumors have not been studied in any depth. GBM is considered phenotypically 'cold' due to its highly immunosuppressive TME and low

infiltration by cytotoxic T cells[11,77]. TME immunosuppressive factors including TGF-β, IL-10, prostaglandin E-2 and PD-1 have been suggested to contribute to poor overall immune surveillance[10]. HCMV has been reported to shift macrophages from an inflammatory M1 state to an immunosuppressive M2 phenotype[78], which is mediated by secretion of GM-CSF, IL4, IL10 and IL13, promoting tumor growth[10]. Given that HCMV is known to establish a latent reservoir within bone marrow-derived monocytes that migrate to GBM[79], it is possible that HCMV gains entry into the tumor via these circulating immune cells, explaining its detection by many in a large proportion of gliomas. Thus, the immunosuppressive TME of GBM may allow HCMV reactivation, further polarizing macrophages toward the M2 phenotype, increasing immunosuppression and potentially aiding the spread of HCMV and supporting tumor progression. Although these mechanisms are plausible, they are largely hypothetical and await validation in human patients and models.

In addition to these effects on innate immune cells, HCMV is known to have a profound impact on the host immune system in general, and is associated with the expansion of T cells that is maintained over a prolonged period of time and is enhanced further during aging[80]: a phenomenon of so-called memory T cell inflation. HCMV proteins targeted by CD4+ and CD8 + T cells and immunodominant epitopes from these proteins have been identified including the HCMV pp65 (UL83), IE1 (UL123) and the 86 kDa immediate-early protein 2 (IE2/UL122)[81]. Additional effects of HCMV within the innate immune system are currently under investigation, including interactions with NK cells[82–84] and chronic enhancement of neutrophil function[85]. HCMV also secretes a homolog of IL10 which could further enhance immune suppression[86,87]. These examples illustrate the broad impact of HCMV on host immunity and suggest that it could have an impact on tumor growth through these effects. Further studies are needed to investigate this in detail.

Immune Response to HCMV

HCMV infection results in a large population of HCMV-specific T cells that are maintained over time, do not display phenotypic traits of exhaustion, and primarily recognize immunodominant viral epitopes from HCMV pp65 (UL83) and HCMV IE proteins[80]. In seropositive adults, approximately 10% of total CD8+ and CD4 + T cells respond to HCMV, however there is significant variability in the activity of these T cells between individuals[88,89]. Effector memory T cells (T_{EM}) are predominantly found in blood and tissues of HCMV positive individuals[80]. Although the number of HCMV-specific T cells in GBM is comparable to healthy patients, these HCMV- specific T cells could be less functional in GBM patients[89]. Currently there is significant interest in enhancing intratumoral CD8 + T cell fitness and infiltration to target GBM[90,91]. These studies have demonstrated that circulating HCMV-specific T cells can be isolated from GBM patients and activated in vitro. These HCMV-specific T cells express the marker CD57, indicating differentiation and activation. Nevertheless, their effector functions appeared limited as evidenced by low production of cytokines and cytolytic activity[89]. Combination therapies that simultaneously stimulate the immune response and target HCMV directly may provide a bystander activation strategy to overcome the immunosuppressive TME of GBM.

HCMV Targeted Therapeutics in GBM

Several clinical trials targeting HCMV in GBM have now been performed, as detailed in Table 1. These fall into two categories: antiviral small molecule therapeutics and HCMV-targeted vaccines, as shown in Fig. 2 and described below.

Antiviral drugs in GBM

The excellent safety profile and modest adverse effects of antiviral drugs position them as promising supplemental therapies for GBM patients. Recently, antiviral drugs have garnered attention as potential treatments for GBM, notably due to encouraging outcomes in clinical trials involving valganciclovir (VGCV) (Fig. 3).

Early clinical trials showed a potential benefit of VGCV therapy when combined with SOC but required further follow-up[92–95]. A study of 102 newly-diagnosed GBM patients who received VGCV alongside SOC demonstrated a significant survival benefit associated with VGCV treatment (OS 24.1 vs 13.3 months)[94]. This effect was notable in individuals with an unmethylated *MGMT* gene promoter status (21.1 months), who are traditionally more resistant to chemoradiotherapy[94]. However, this report used historical controls and lacked critical information including HCMV IgG and IgM status and circulating viral DNA levels (viremia). Despite this limitation, a follow-up multicenter randomized double-blinded clinical trial (NCT04116411) is actively recruiting patients. This trial, which will likely be completed in the coming year, aims to evaluate the efficacy of VGCV as an adjunctive therapy alongside the current SOC for GBM patients.

Other antiviral agents such as brincidofovir (BCV) and cidofovir (CDV) are currently under preclinical investigation for GBM treatment (Fig. 3). CDV displayed antineoplastic activity in GBM cell lines infected with HCMV by impeding HCMV gene expression and triggering cellular apoptosis[76]. In this study, CDV enhanced the DNA-damaging effects of irradiation in GBM cells. When used alone, CDV treatment increased γH2AX phosphorylation, indicating DNA damage. However, combining CDV with irradiation resulted in a more than 20-fold increase in this effect suggesting potent radio-sensitizing effect[76]. Previous reports have also demonstrated that HCMV can cause chromosomal and DNA damage. These discoveries highlight the potential interplay among HCMV, irradiation and antiviral treatments in GBM therapy[43,96].

Brincidofovir (BCV), a lipid-conjugated form of CDV with enhanced cellular uptake, has broad activity against double stranded DNA viruses and is FDA approved for smallpox treatment. Phase I clinical studies assessing BCV pharmacokinetics and safety demonstrated positive results in healthy volunteers[97]. In a separate phase II trial (NTC00942305), BCV treatment in allogenic hematopoietic-cell transplant (HCT) patients reduced plasma HCMV DNA levels (defined as <1000 copies/mL) compared to placebo controls[98]. However, a follow up phase III trial, conducted 24 weeks after HCT, reported that there was no significant difference in HCMV infection between patients receiving BCV and placebo controls. Although BCV led to reduced levels of HCMV DNAemia, the BCV-treated group experienced more frequent adverse events compared to controls[99]. Despite not currently being available for HCMV or GBM treatment, BCV combines potential antiviral properties with radio-sensitizing effects, suggesting its potential as a therapeutic strategy in GBM if it can be delivered without toxicity.

HCMV Vaccines in GBM

Dendritic Cell Vaccines. Dendritic cell (DC) vaccines have been investigated as a novel immunotherapy approach to treat GBM by direct presentation of immunogenic antigens to T cells. Initially, DCs are isolated from patient blood and stimulated in vitro with immunodominant HCMV pp65 (UL83) mRNA, followed by additional activation through cytokines like TNFα[100,101]. These cells are then administered to GBM patients to induce an immune response. In a small randomized trial (NTC00639639), patients who received tetanus-diphtheria Toxoid (Td) preconditioning along with HCMV pp65 (UL83) conditioned DCs and TMZ exhibited improved DC migration and significantly enhanced survival rates[102]. A subset of patients receiving an autologous HCMV DC vaccine containing GM-CSF alongside dose-intensified TMZ showed remarkable long-term benefit with median progression free survival (PFS) of 25.3 months and OS of 41.1 months[100]. This drastic improvement of OS is an exciting finding that warrants further exploration of DC vaccine therapy in GBM. Another clinical trial (NCT00693095) investigating the combined treatment of HCMV pp65 (UL83) specific CD8 T cells and HCMV-DC vaccination resulted in increased cytokine production and improved overall survival[103]. In contrast, another trial (NCT00626483) using similar approach exhibited minimal clinical benefit, as the vaccine failed to induce a robust immune response, although full results have not been formally published[104]. Several other DC vaccine trials (NCT03615404) have not yet published results or were

Table 1 | Clinical trials with cytomegalovirus-based therapies in glioblastoma

	Identifier	Indication	Phase	Study Design	Treatment	Primary Objective	Outcomes
Recruiting	NCT03382977	Recurrent Glioblastoma	I/II	Non-randomized	VBI-1901 (eVLP GBM Vaccine), AS01B Adjuvant	Safety, tolerability, optimal dose	–
	NCT04116411	Glioblastoma	II	Randomized	Valganciclovir, TMZ, RT	Median OS	–
	NCT05698199	Glioblastoma	I	Non-randomized	ITI-1001	DLT, AEs, SAEs	–
Completed	NCT01109095	Glioblastoma	I	Non-randomized	HER.CAR CMV-specific CTLs	DLT, safety of dose escalation	OS 24.8 months, HER2 CMV T cell detection in peripheral blood (12 weeks post infusion)[117]
	NCT00693095	Glioblastoma	I	Randomized	CMV-ALT + CMV-ATCT-DCs/ Saline	Evaluation of vaccine	Vaccine CMV-ATCT-DC enhanced IFNγ +, TNFα + and CCL3+ polyfunctional CMV specific CD8 + T cells, which correlated with OS[103]
	NCT00400322	Glioblastoma	N/A	Randomized	Valganciclovir (Valcyte)	Tumor Size	No difference in OS, PFS or tumor volume – retrospective analysis performed, OS 24.1 months compared to 13.7 months in contemporary controls[92,95]
	NCT01811992	Glioblastoma, Gliosarcoma, Anaplastic ependymoma	I	Non-randomized	Ad-hCMV-TK, Ad-hCMV-Flt3L	DLT, MTD	No DLT, MTD was not reached, median OS 21.3 months[118]
	NCT00626483	Malignant Neoplasms Brain	I	Non-randomized	Basiliximab (RNA-loaded dendritic cell vaccine)	Functional and quantitative analysis (CD4 +, CD25 +, CD127- T cells)	No DLT, PFS 7.7 months, no difference in percent of CD4 + T cells or T-regs after treatment[104]
	NCT03615404	Glioblastoma	I	Non-randomized	CMV-DCs with GM-CSF, Td	Percent patients where 3 or more vaccines can be generated, Toxicity	Not reported
	NCT02661282	Glioblastoma	I/II	Non-randomized	Autologous CMV-specific CTL, TMZ	MTD, tumor tissue immunology, OS, PFS	No DLT, increased number of CD8 + T cells, PFS 1.3 months, median OS 12 months[91]
	NCT00639639	Malignant Neoplasms Brain	I	Randomized	HCMV pp65-LAMP mRNA pulsed DCs +/− ALT, GM-CSF, Additional Arm Td preconditioning and Indium 111-labled DCs	Feasibility and safety	Median PFS 25.3 months, OS 41.1 months, four patients PFS of 59–64 months[100,102]
	NCT02366728	Glioblastoma	II	Randomized	Unpulsed DCs, Td, HCMV pp65-LAMP mRNA-pulsed autologous DCs, 111In-labeled DCs, TMZ, Saline, Basiliximab	OS, percent 111-Indium-labeled DCs	3 year OS in Td-treated 34% compared to 6% in unpulsed DC treated[119]
Terminated/ Withdrawn	NCT02864368	Glioblastoma	I	Randomized	TMZ, PEP-CMV: Component A and Component B, Tetanus-Diphtheria Booster, Tetanus Pre-Conditioning	AEs, toxicity, T cell analysis	Terminated due to manufacturing issues with Component B and hypersensitivity reactions in a subset of patients
	NCT03927222	Glioblastoma	II	Non-randomized	HCMV pp65-LAMP mRNA-pulsed autologous DCs Containing GM CSF, TMZ, Td, GM-CSF, 111-Indium-Labeled Cells	OS	Terminated due to shortage of resources
	NCT01205334	Glioblastoma	I/II	Non-randomized	Autologous CMV-Specific CTL	DLT, MTD,	Terminated due to poor accrual
	NCT00990496	Glioblastoma	I	Non-randomized	Fludarabine, Cyclophosphamide, CMV Specific CTL	Incidence of tumor responses	Terminated; accrual goals not met
	NCT01854099	Glioblastoma	I	Randomized	PEP-CMV	Safety, DLT	Withdrawn by investigator

Abbreviations: eVLP (enveloped virus like particle), TMZ (temozolomide), RT (radiotherapy), OS (overall survival), DLT (dose limiting toxicity), AE (adverse events), SAE (serious adverse events), MTD (maximal tolerated dose), PFS (progression free survival), DCs (dendritic cells), CTL (cytotoxic lymphocytes), ALT (autologous lymphocyte transfer), ATCT (adoptive T cell therapy), Td (tetanus-diphtheria toxoid).
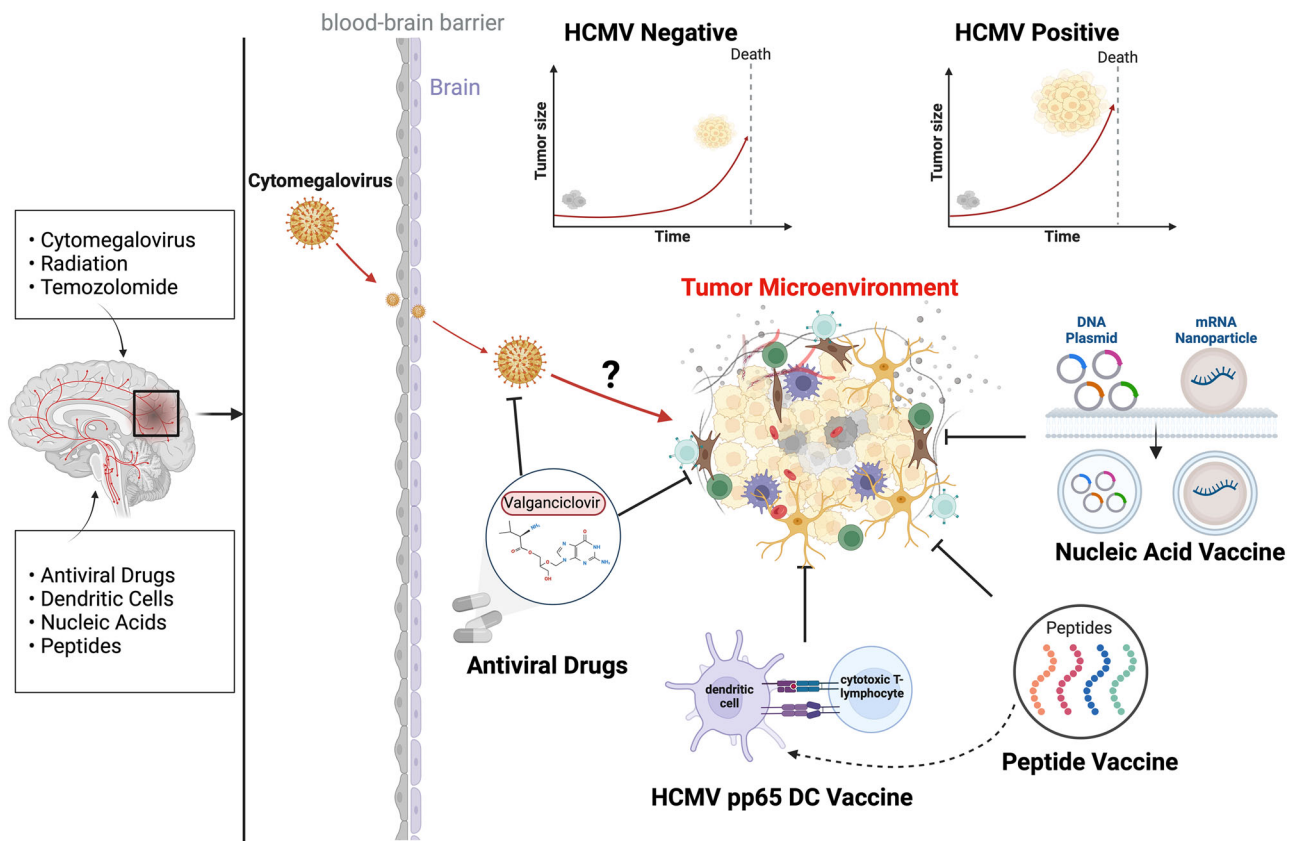
Fig. 2 | Overview of Therapeutic Strategies Targeting HCMV in GBM. HCMV seropositivity and reactivation have been associated with negative outcomes in GBM. Several therapeutic approaches are currently in clinical trials for GBM patients including the antiviral drug valganciclovir, HCMV pp65 pulsed DC vaccine, peptide vaccine PEP-CMV and nucleic acid-based vaccine ITI-1001. Other pre-clinical approaches are also being tested using T cell based immunotherapies.

terminated for various reasons (Table 1). Final results from these trials are awaited and may provide crucial insights into the efficacy and limitations of DC vaccine therapy in GBM. These mixed outcomes across different trials underscore the necessity for larger patient cohorts and the identification of appropriate adjuvant and SOC combinations to understand the full potential of DC vaccines in treating GBM.

Peptide vaccines. Peptide vaccines are typically composed of 8–25 amino acid sequences representing mutated atypically expressed tumor associated proteins[105]. Peptide vaccines offer an effective strategy to elicit antigen-specific and long-lasting anti-tumor T cell responses. In addition they are easy to synthesize, have improved stability and are relatively low cost[105]. Three clinical trials since 2013 have tested HCMV-based peptide vaccines. The first trial in 2013 (NCT01854099) was withdrawn and a second trial in 2016 (NCT02864368) was terminated[106,107]. The main component of these trials was a biological product termed PEP-CMV that was comprised of a HCMV pp65 (UL83) synthetic long peptide (SLP)[106]. The second trial included a neutralizing antibody epitope from HCMV gB (UL55) conjugated to Keyhole Limpet Hemocyanin (KLH) and mixed with GM-CSF adjuvant[107]. The peptide vaccine was discontinued due to manufacturing issues and hypersensitivity reactions in a subset of patients (NCT02864368). The most recent clinical trial using PEP-CMV (NCT03299309) is currently active but not yet recruiting[108]. This trial targets recurrent medulloblastoma and malignant gliomas and only involves the HCMV pp65 (UL83) SLP with tetanus-diphtheria pre-conditioning[109]. Although no definitive results have been reported from these trials, there is potential for future combination therapies that may increase their therapeutic efficacy.

Nucleic acid vaccines. A preclinical study published in 2022 demonstrated that a multi-HCMV antigen DNA based vaccine resulted in 56% cure-rate in tumor bearing mice[110]. In this proof-of-principle experiment murine GBM CT2A cells were engineered to express three HCMV antigens (pp65 (UL83), IE1 (UL123), gB (UL55)) for implantation. The vaccine candidate was termed ITI-1001 and is composed of two plasmids. One plasmid encodes a fusion of HCMV IE1 (UL123) and pp65 (UL83) with lysosomal associated membrane protein (LAMP) and one encodes HCMV gB (UL55) fused with LAMP1 in the NTC8382-VA1 vector[110]. A phase I clinical trial (NCT05698199) will assess the safety and immunogenicity profile of ITI-1001 in GBM patients. The trial employs a prime-boost vaccination strategy in combination with SOC[111]. GBM exhibits frequent mutations in several proteins including EGFR, NF1, PDGFRA, PTEN, TERT, RB1, TP53, IDH1, PIK3CA and PIK3R1, which are heterogenous among tumors[112]. These mutations have the potential to be targeted via nucleic acid- and peptide- based vaccines alongside HCMV targeting strategies and exhibit clinical benefit.

Alternative HCMV immunotherapies. In addition to antivirals, DC, peptide and nucleic acid vaccines, there are alternative avenues to exploit the biological activities of HCMV. Notably, T cell-based therapies and viral-based therapies including adenovirus vector and viral like particle (VLP) therapies present viable options. However, as shown in Table 1, T cell-based therapies have not achieved significant benefit in clinical trials to date, with two trials being withdrawn (NCT01205334 and NCT00990496) and the latter reporting a progression free survival (PFS) of 1.3 months and a median OS of 12 months (NCT02661282). Nonetheless, it is interesting to note that chimeric antigen receptor (CAR) T cell therapy demonstrated improved results with an OS of 24.8 months
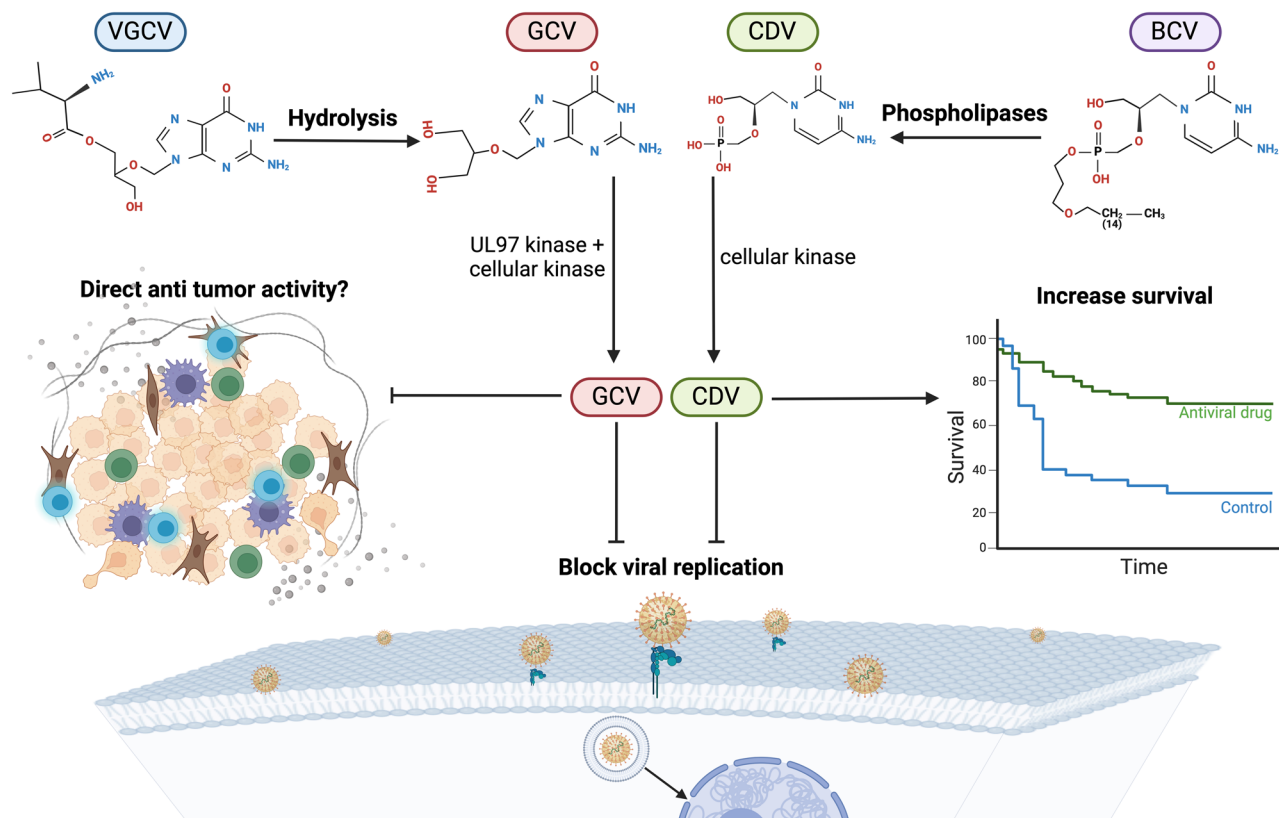
Fig. 3 | Mechanisms of antiviral drugs in GBM. Valganciclovir (VGCV) is converted to ganciclovir (GCV) by hepatic esterases then GCV is intracellularly phosphorylated by HCMV viral protein kinase pUL97 and further downstream phosphorylated by cellular kinases to produce its active form ganciclovir triphosphate, which competitively inhibits deoxyguanosine triphosphate incorporation into DNA and preferentially targets HCMV viral DNA polymerase.

Brincidofovir (BCV) is a lipid conjugate of cidofovir (CDV) with improved cellular uptake. Upon cellular entry, lipases cleave the lipid chain releasing CDV which is then transformed into its active form cidofovir diphosphate by intracellular kinases. CDV is a competitive inhibitor of deoxycytosine triphosphate resulting in viral DNA chain termination.

(NCT01009095). As reported in Table 1, there is a growing interest in the biological exploitation of HCMV in GBM, underscoring the importance of the nuanced relationship between HCMV and GBM.

Discussion

While there have been significant strides in the biological understanding of GBM, new therapies with enhanced clinical efficacy remain elusive. Preclinical studies and clinical trials focused on therapeutics targeting HCMV are generating ongoing interest. However, these approaches are hindered by a lack of understanding of the complex interactions between GBM and HCMV, and the absence of larger, detailed longitudinal studies closely monitoring HCMV.

It may be useful to focus on identifying and standardizing sensitive methods to characterize the presence of HCMV in tumor specimens. In GBM, the detection of HCMV has yielded inconsistent results, possibly due to very low intratumoral levels of the virus, therefore necessitating improved methods for viral detection. The discrepancies in the field have impeded research advancement on the influence of widespread viruses like HCMV in GBM and other tumors, thereby making the issue of intratumoral virus less important. Indeed, HCMV has been reported in tumoral tissues from several cancers beyond GBM, including breast, liver, and cervical cancer[113,114]. Pre-clinical studies modeling HCMV latency have implicated a significant role the virus plays in cancer. HCMV causation in GBM and other cancers has been reported and its impact is clearly important in progression of tumors[40,115].

While direct data in human patients is currently lacking, our emerging understanding of HCMV suggests that there may be a relationship between HCMV and the TME. HCMV is able to evade the host immune system, potentially contributing to immunosenescence and the development of more aggressive phenotypes in cancer[116]. The control and expansion of the TME hinges upon viral suppression and transformation of immune cell responses[114]. Whether this is accomplished through inhibition of anti-tumor effects or promotion of tumor development, HCMV's immunosuppressive abilities play a key role in facilitating cancer hallmarks (as shown in Fig. 1). Better understanding the complexity of HCMV infection and latency in cancer may pave the way for novel therapies targeting modes of oncomodulation and immunosuppression in the TME.

The dynamics of viral latency and reactivation, which have direct associations and negative effects on cancer, should also be further explored. While immunocompetent hosts can dampen down and co-exist with HCMV, immunocompromised individuals are less able to manage the virus. Under immunosuppressive conditions, HCMV reactivation can result in prolonged hospitalization and increased mortality[116]. Given that cancer patients constitute the largest population of immunocompromised individuals, it is essential to understand the impact of HCMV reactivation on GBM disease progression and quality of life. The substantial burden of clinically-relevant virus reactivation among HCMV-positive GBM patients following chemoradiotherapy suggests that prior testing for HCMV IgG serostatus may identify at-risk patients who could benefit from prophylactic antiviral drugs[73,74]. By further understanding the effects of HCMV reactivation, susceptible patient populations may therefore be better protected from potential severe adverse outcomes.

While some clinical trials are currently underway, additional trials are needed to determine the efficacy and tolerability of HCMV-based therapies

in the treatment of GBM. HCMV antivirals such as valganciclovir have shown promise and are currently under further investigation. The effects of these drugs need to be studied to discern how they interact with the current SOC to elicit anti-tumor effects. Exploring additional combination therapies is imperative to address the demand for effective therapies in GBM. Important next steps include analyzing immunological changes within the TME when antivirals are used in combination with SOC. This will allow us to distinguish the oncomodulatory effects of HCMV in GBM and other cancer types. Targeting HCMV in cancer populations may provide increased benefit to patients, especially when used in combination with SOC. Enhancing our understanding of the complex interactions between HCMV and GBM will allow us to design more effective treatment strategies for susceptible patient populations. This may involve employing combination therapies with existing or novel therapeutic approaches.

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Author contributions
N.B.M., J.N.R., and S.E.L. conceptualized and wrote the manuscript. J.K., E.P., and C.H.C. reviewed the manuscript and provided detailed revisions. N.B.M. created the manuscript figures.

Competing interests
Sean E. Lawler received research funding from SymBio Pharmaceuticals. All other authors declare no competing interests.

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