Molecular Therapy
Oncolytics
Review

Tumor Control by Cytomegalovirus: A Door Open for Oncolytic Virotherapy?

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Belonging to the herpesviridae family, human cytomegalovirus (HCMV) is a well-known ubiquitous pathogen that establishes a lifelong infection in humans. Recently, a beneficial tumor-cytoreductive role of CMV infection has been defined in human and animal models. Described as a potential anti-tumoral activity, HCMV modulates the tumor microenvironment mainly by inducing cell death through apoptosis and prompting a robust stimulatory effect on the immune cells infiltrating the tumor tissue. However, major current limitations embrace transient protective effect and a viral dissemination potential in immunosuppressed hosts. The latter could be counteracted through direct viral intratumoral delivery, use of non-human strains, or even defective CMV vectors to ascertain transformed cells-selective tropism. This potential oncolytic activity could be complemented by tackling further platforms, namely combination with immune checkpoint inhibitors or epigenetic therapy, as well as the use of second-generation chimeric oncovirus, for instance HCMV/HSV-1 oncolytic virus. Overall, preliminary data support the use of CMV in viral oncolytic therapy as a viable option, establishing thus a potential new modality, where further assessment through extensive basic research armed by molecular biotechnology is compulsory.

Human cytomegalovirus (HCMV) or human herpesvirus-5 (HHV-5) is a ubiquitous opportunistic species-specific herpesvirus that infects a large proportion of the population worldwide. Even though HCMV infection often results in an asymptomatic latent infection in healthy individuals, it largely engenders significant mortality and morbidity in immunosuppressed patients.[1,2] Alongside, HCMV establishes a latent reservoir in the $CD34^+$ hematopoietic progenitor cells resident in the bone marrow, as well as in peripheral monocytes.[3,4] In contrast to the described "oncomodulatory" effect of HCMV in favor of cancer progression, the potential of CMV to counteract tumor growth in both human and animal models has been recently highlighted.[5–8] For instance, early HCMV reactivation reduced the relapse rate of acute myeloid leukemia (AML) and non-Hodgkin's lymphoma in patients enduring allogeneic stem cell transplantation.[9–16] In line with this, viral reactivation after kidney transplantation has been linked to a reduced risk of skin cancer.[17] Furthermore, murine cytomegalovirus (MCMV) showed tumor control in a model of bone marrow transplantation and acute liver-infiltrating B cell lymphoma,[18,19] as well as after intratumoral injection of MCMV in context of melanoma.[20–22] Likewise, systemic MCMV infection not only inhibited the growth of murine carcinomas but also decreased human colon carcinoma development *in vivo*.[23] This reported antitumor activity could be actively exploited to shed the light on CMV as a potential oncolytic virus against a wide variety of tumors. This is encouraged by the rapid expansion of numerous oncolytic viruses as a promising therapeutic modalities in cancer treatment.[24] Those encompass naturally occurring non-human pathogenic viruses exemplified by parvoviruses, myxoma virus, Newcastle disease virus, reovirus, and Seneca valley virus, in addition to the genetically-manipulated vaccine vectors comprising measles virus, poliovirus, and vaccinia virus, with a special emphasis on the genetically-engineered adenovirus, herpes simplex virus, vesicular stomatitis virus, and poxvirus.[25–27] It is worthy to mention that a wide array of oncolytic viruses are currently in different stages of preclinical testing and in clinical trials with the most prominent example the FDA approved T-VEC, a recombinant human herpes simplex virus (HSV) type 1 for the local treatment of unresectable lesions in patients with recurrent melanoma.[28,29]

CMV and Tumor Micro-environment Modulation: Different Mechanisms, One Outcome

Several pathophysiological mechanisms could explain the role of CMV in modulating the tumor micro-environment in favor of tumor remission or ablation, including among others cytopathogenic infection of tumor cells, induction of cell death, stimulation of inhibitory cytokines, interference with tumor cell extravasation or tumor vascularization, or bystander stimulation of an antitumoral immune response. Those multi-modal mechanisms are reviewed in Figure 1, where CMV is approached as a potential oncolytic virus. First, CMV might directly kill the infected tumor cells. In fact, it has been demonstrated that clearance of well-established tumors in a mouse melanoma model was obtained after injecting CMV into the growing tumor.[21,22] A direct pro-apoptotic effect of HCMV on acute myeloid leukemia (AML) cell lines Kasumi-1 and SD-1 (BCR-ABL-positive acute lymphoblastic leukemia, ALL) has been shown. This caspase-dependent anti-leukemic effect could explain, at least in

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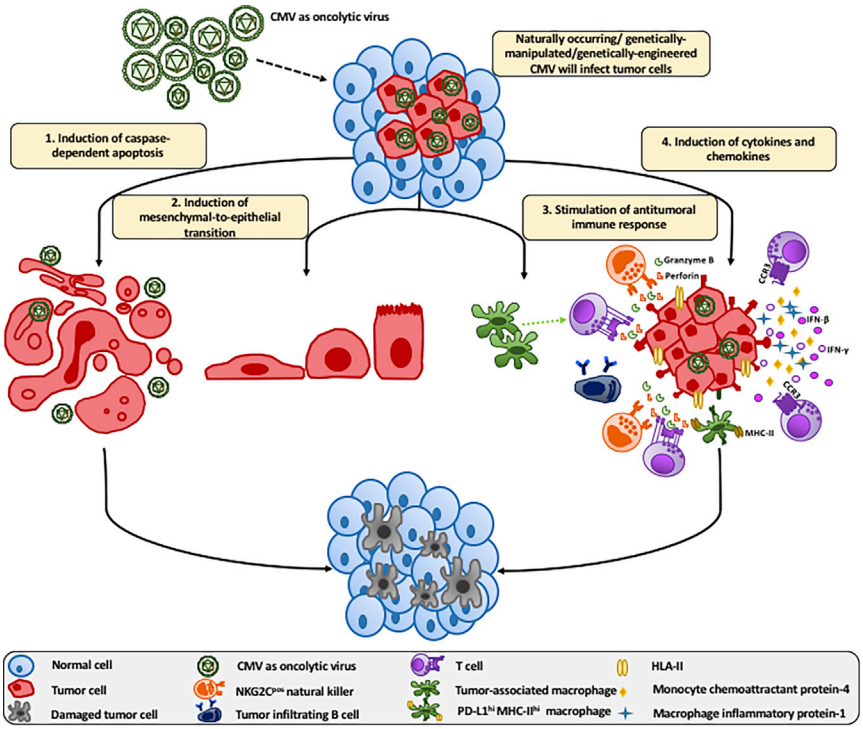

Figure 1. Physiopathological Mechanisms Illustrating Tumor Control following CMV Infection and Its Potential Consideration as an Oncolytic Virus

A magnitude of multi-modal mechanism of action can explain the modulation of tumor micro-environment by CMV in favor of remission or ablation, while sparing normal healthy cells. In addition to targeting cancer cells through the induction of caspase-dependent apoptosis, CMV was shown to stimulate mesenchymal-to-epithelial transition, reverting thus the transformation process. In addition, CMV could induce an upregulation of HLA-class-II-molecules on tumor cells and augment the host antitumor immune response through the viral-stimulated $V\delta2^{neg}\gamma9$ T cells and $NKG2C^{pos}$ natural killer (NK) cytotoxic effectors and the release of perforin and granzyme B, as well as the tumor infiltrating B lymphocytes (TIB), the tumor-associated macrophages and activated macrophage population expressing high levels of PD-439 L1 and MHC-II. Lastly, cytokines such as IFN-β, IFN-γ, and chemokines, namely the macrophage inflammatory protein-1 and monocyte chemoattractant protein-4, are also shown to be actively involved in tumor clearance.

part, the decreased leukemic relapse rate in AML patients with HCMV reactivation.[30] Correspondingly, a cytoreductive potential of HCMV infection in a murine model of human hepatocellular carcinoma (HCC) was observed, where viral infection delayed the development of HCC tumors due to restricted cellular proliferation and increased caspase-dependent apoptosis of tumoral cells.[31] In line with this, HCMV infection of Kasumi-1 cells and the promyeloid leukemia cell line NB4 not only inhibited cellular proliferation, but also induced apoptosis in those AML cells. This was correlated with a substantial upregulation of histocompatibility leukocyte antigen (HLA)-class-II-molecules, accompanied by an increase granzyme B, perforin, and interferon-γ (IFN-γ) secretion upon coculture with peripheral blood mononuclear cells (PBMCs). This implies that alloreactivity of PBMCs against cancer cells could be enhanced by HCMV infection possibly through a synergy between CMV-specific T cells from one side, and natural killer (NK) cells and NKG2 epitopes considered as potential alternative costimulatory molecules from the other side, widening thus the spectrum of cellular immunity to be assessed in order to prevent leukemic relapse in patients after allogenic hematopoietic stem cell transplantation (alloHCT).[32] Second, recent reports point toward the ability of the virus to revert the transformation process in cancer cells. For instance, HCMV induced a mesenchymal-to-epithelial transition (MET) with inhibition of the migratory capacity of the mesenchymal breast cancer lines MDA-MB-231 and SUM1315, thus establishing an epithelium-like cellular environment upon expression of early viral gene.[33] Third, CMV could increase the antitumoral effect of immune cells present in the tumor microenvironment. The mechanism underpinning this beneficial HCMV effect was suggested to rely on the reported recognition of cancer cells by donor-derived HCMV-stimulated $V\delta2^{neg}\gamma9$ T cells[34–38] and $NKG2C^{pos}$ natural killer (NK) cytotoxic effectors (for reviews, see Litjens et al.[39] and Bigley et al.[40]) $V\delta2^{neg}\gamma\delta$ T cell expansion has been reported in CMV infection and has demonstrated its ability to kill both CMV-infected cells and carcinoma cells *in vitro* due to shared reactivity of $V\delta2^{neg}\gamma\delta$ T cells against CMV-infected cells and tumor intestinal epithelial cells.[34] In agreement with their *in vitro* antitumoral activity, $V\delta2^{neg}\gamma\delta$ T cells are associated with reduced cancer risk in CMV-infected kidney transplant recipients.[41] Worth emphasizing is the influence of the polymorphism of the major histocompatibility complex class I chain-related gene A (MICA), a ligand of the natural killer receptor NKG2D on CMV infection and CMV-induced disease in the setting of alloHCT, where the weak NKG2D receptor binding affinity genotype MICA-129 V/V was linked to a higher risk of CMV infection and disease.[42] In addition, tumor infiltrating lymphocytes (TIL), especially tumor infiltrating B lymphocytes (TIB) respond to CMV peptides, as well as TIB-derived CMV-specific immunoglobulin G (IgG). This could be considered as an indicative of "cross-reacting" antibodies recognizing tumor-associated targets as suggested by the improved survival of patients with pancreatic cancer or glioblastoma.[43,44] Attractively, transfer of enriched IFN-γ-secreting CMV-specific T cells induced CMV-specific responses of both $CD4^+$ and $CD8^+$ T lymphocytes in the setting of peripheral blood stem cell transplantation (PBSCT),[45] pinpointing toward a possible combination between adoptive T cell therapy and virotherapy. Beside adoptive T cell therapy, another cancer immunotherapy perspective highlighting the role of $CD8^+$ T cells is the use

of tumor-targeting antibody conjugated to CMV-derived epitopes to retarget CMV-specific $CD8^+$ T cells against tumors by viral antigen presentation by HLA-I. Taking advantage of the CMV memory inflation and the abundance of circulating CMV-CTLs in the peripheral blood, this approach demonstrated a redirection of the pre-existing CMV immunity in tumor models both *in vitro* and *in vivo*.[46] To further emphasize on the role of NK cells, it has been shown that the expansion of a specialized subset of adaptive human NK cells with a $CD56^{dim}CD57^+NKG2C^+$ phenotype in response to CMV reactivation is linked to a reduced leukemia relapse in patients undergoing allogeneic hematopoietic cell transplantation.[47] Not limited to the previously mentioned immune players, the observed CMV-induced immune modulating effects could be also mediated by the activity of tumor-associated macrophages (TAMs). This is reinforced by the fact that in a mouse model of melanoma, and following injection of MCMV into the tumor, the only infected cells were the macrophages.[22] Indeed, intratumoral delivery of MCMV alters TAMs in such a way as to boost CD8 T cells' responses,[21,22] suggesting that beside the direct antitumoral CMV effect, viral infection might be considered as an adjuvant approach to precipitate an anti-tumoral immune infiltration into the immunologically "cold" tumors by recruiting and polarizing TAMs.[48] Parallel to this, intratumoral injection of MCMV in the setting of a B16-F0 melanoma model modulated the tumor microenvironment by recruiting macrophages to the tumor via the virus-encoded 41 chemokine MCK2, inducing a transient increase in the inflammatory cytokines inducible nitric oxide synthase (iNOS), tumor necrosis factor alpha (TNF-α), and interleukin-1β (IL−1β) production and resulting in the emergence of a distinct $F4/80^{hi}Ly6C^{int}$ activated macrophage population expressing high levels of PD-439 L1 and MHC-II.[49] In fact, beside antitumoral cellular response, cytokines and chemokines elicited by CMV infection could be actively involved in tumor clearance. IFN-β, produced after infection of human foreskin fibroblasts (HFF) with HCMV, showed a potent antiproliferative activity against most human tumor cells *in vitro*, inhibited tumor growth, and favored tumor regression in immunodeficient mice. This was probably mediated through several mechanisms including the induction of caspase-dependent apoptosis, blockage in the transition into G2/M phase of the cell cycle, and an expressed anti-angiogenic activity.[50,51] On the other hand, injection of $V\delta2^{neg}\gamma\delta$ T cell clones in a generated hypodermal human colon adenocarcinoma HT29 tumor in immunodeficient mice resulted in the expression of chemokine C-C motif receptor 3 (CCR3) by those clones that migrated *in vitro* in response to chemokines secreted by HT29 cells, the latter including CCR3 ligands macrophage inflammatory protein-1 delta and monocyte chemoattractant protein-4.[52] As an emphasis of the critical role of chemokines in tumor control, the *in vivo* anti-tumoral activity observed following $\gamma\delta$ T cell passive immunotherapy can be regressed by addition of a blocking anti-CCR3 antibody.[52] Thus, by way of conclusion, several physiopathological mechanisms could explain tumor control following viral infection (summarized in Table 1). A profounder conception of this multimodal activity of CMV is indispensable to advantageously translate this anti-tumoral activity into a potentially promising oncolytic virotherapy.[53]

The Antitumoral Activity of CMV: A Long Stride toward Oncolytic Virotherapy

Although the antitumoral effect of CMV infection has been demonstrated in several malignancies and diseases, with the most striking example being the delayed relapse in leukemia and lymphoma, potential use of oncolytic CMV therapy is currently limited by several restrictions that should be taken into account (Table 1). First, the reduced leukemic relapse after CMV reactivation is transient and not sustained for a long period of time.[10] This is in line with the transient anti-tumor effect of intratumoral MCMV injection in the context of poorly immunogenic B16 melanomas where a positive correlation was reported between tumor regrowth and loss of viral activity in the tumor.[49] Second, it is of high importance to limit viral dissemination in immunosuppressed patients treated with oncolytic CMV therapy, as active infection in this population subset could have deleterious outcomes. Interestingly, CMV replication was described as limited and/or abortive in cancer cells, suggesting the use of CMV intratumoral injection as a potential safe therapeutic approach. In fact, infection of HepG2 cells with HCMV results in restricted viral growth,[54] similar to the stalled HCMV replication cycle reported in infected MDA-MB-231 and SUM1315 breast cancer cells.[33] Indeed, the virus cannot productively infect cancer cell lines, a limitation elucidated by the presence of multiple restrictions to HCMV replication in cells expressing oncogenic alleles. This was demonstrated by the restrictive effect of the expression of the oncogenic alleles simian virus 40 (SV40) T antigen (TAg) and H-Ras on the viral entry, DNA replication, and expression of immediate early genes upon infection with the laboratory strains TB40E and BADwt, a strain derived from a clone of AD169.[55] In agreement with the intracellular viral block in cancer cells, HCMV genome was undetectable in tumor tissue of mice xenografted with HCMV-infected HepG2 cells several weeks post-infection.[31] This was also reported by a previous study where undetectable HCMV level was noted few weeks post-infection in a murine model.[56] Thus, although HCMV replicates efficiently in non-cancerous cells, its replication is very limited in transformed cells, suggesting that intratumoral delivery of HCMV might be used as a potentially safe therapeutic strategy. Another approach to avoid the potential dissemination risk following viral intratumoral injection is the use of CMV strains from non-human species, such as MCMV. This might allow not only the control of tumor growth, but also the containment of viral replication due to the CMV inter-species block.[57] This is based on the fact that a strict host restriction for MCMV has been demonstrated with several human cell lines.[58,59] While typical cytopathic effects were observed following infection of human diploid cells WI-38 with MCMV, no efficient synthesis of infectious virions was detected.[60] Even though MCMV transcription, translation, DNA replication, or virus production, along with the expression of immediate-early (IE1), early (E1), early-late (M44), and late (gB) gene can be detected in infected human cells,[57,61] the probability of MCMV production by infected cells or reinfection of neighboring cells is low. This is due to a significant degradation of newly replicated MCMV DNA[61] or due to the activation of caspase-9, triggering thus the intrinsic apoptosis pathway and activating the innate immune defense.[57] Hence, an advantage of a potential MCMV therapy (versus HCMV therapy) is the restriction of viral dissemination especially in high-risk patients.

Table 1. Use of CMV as a Potential Oncolytic Virus

Cellular/Animal Model	Outcome	Limitation	Solution
Mouse melanoma model	induction of virus-specific $CD8^+$ T cells slowing tumor growth[21,22]	potential viral dissemination in immunosuppressed patients treated with oncolytic CMV therapy	● intratumoral delivery of CMV[21,54] ● use of CMV strains from non-human species, for instance MCMV[57] ● use of defective CMV vectors[48]
Human MC38 colon cancer cells	reduction of carcinoma development *in vivo* and induction of IFN-β and ND10 expression[23]		
Acute leukemia cell lines (Kasumi-1 and SD-1)	anti-leukemic pro-apoptotic effect[30]		
Mouse model of human hepatocellular carcinoma (HCC)	absence of tumor or limited tumor growth[31]		
Acute leukemia cell line (Kasumi-1) and promyeloid leukemia cell line (NB4)	inhibition of cellular proliferation and induction of apoptosis[32]		
Mesenchymal breast cancer lines (MDA-MB-231 and SUM1315)	induction of a mesenchymal-to-epithelial transition[33]		
Astrocytoma cells, primary foreskin fibroblasts, and tumor intestinal epithelial cells	expansion of $V\delta2^{neg}\gamma\delta$ T cell capable of killing CMV-infected cells and inhibiting viral propagation *in vitro*[34]	transient and no sustained reduction in leukemic relapse after CMV reactivation[10]	● combination with anti-PD-L1 therapy, which promoted tumor clearance and long-term protection[20] ● combination with adoptive transfer of enriched IFN-γ-secreting CMV-specific T cells, which induced CMV-specific responses of both CD4+ and CD8+ T lymphocytes[45] ● use of chimeric HCMV/HSV-1 oncolytic virus to improve intratumoral viral replication without restoring the neurovirulence, which resulted in enhanced tumor reduction and prolonged survival[77–79]
Mouse melanoma model	boosting CD8 T cells responses by tumor-associated macrophages[22]		
Human recipients after hematopoietic cell transplantation	expansion of a specialized subset of adaptive human NK cells with a $CD56^{dim}CD57^+NKG2C^+$ phenotype and reduction in leukemia relapse[47]		
B16-F0 melanoma model	recruitment of a distinct $F4/80^{hi}Ly6C^{int}$ activated macrophage and induction of iNOS, TNF-α, and IL-1β production[49]		
Human foreskin fibroblasts, immunodeficient mouse model	potent antiproliferative activity *in vitro*, inhibition of tumor growth, and favoring tumor regression in immunodeficient mice[50,51]		
Mouse xenograft tumor model	expression of chemokine C-C motif receptor 3[52]		

This was reinforced in a recent report where MCMV was able to infect human MC38 colon cancer cells, reduce carcinoma development *in vivo*, and mount the expression of IFN-β and ND10, an effect exclusively seen for MCMV compared with HCMV,[23] highlighting the selective advantage of MCMV over HCMV to control human colon cancer. Interestingly, and in the context of CMV-based vaccine vectors, the use of defective CMV vectors could enhance the safety margin of such preparations. In fact, it has been recently reported that the lack of the M94 gene limited the spread of MCMV beyond the first cells infected.[62] This was in line with the MCMV vector lacking glycoprotein L, which rendered it safe in a mouse model of severe combined immune deficiency (SCID).[63,64] Another approach is the attachment of the degradation domain of FK506 binding protein to the murine M79 protein, resulting in the restriction of viral replication to a single cycle.[48] This highlights the possibility of improving the safety profile while maintaining or even enhancing efficacy.

Oncolytic CMV Therapy: Possible Combinations and Future Perspectives

The combination of oncolytic CMV therapy with other anti-cancer drugs could further enhance the control of tumor growth. For instance, intratumoral delivery of MCMV synergized with PD-1/PD-L1 checkpoint inhibitor lead to the clearance of melanoma lesions and long-term protection in 60% of mice,[20] which further consolidates the fact that the use of intratumoral CMV injection along with treatments targeting the oncogenesis pathways could be highly efficient. In a general sense, oncolytic immunotherapy consists of combining immune checkpoint inhibitors (ICI) (anti-CTLA4, anti-PD-1…) along with oncolytic viruses. Whether those viruses are naturally replicating, such as parvoviruses, or genetically manipulated for use as vaccines or vectors, like vaccinia virus (VACV) and HSV, they increase the immunogenicity of the local tumor microenvironment, optimizing thus the function of ICI. It is worthy to note that multiple clinical trials are currently ongoing to assess this sort of combination therapy.[53] Another strategy could be coupling oncolytic CMV therapy with epigenetic therapy. In support of such hypothesis, histone deacetylase (HDAC) inhibitors recently emerged as enhancers of oncolytic virotherapy, as exemplified with the oncolytic HSV, adenovirus, and measles virus, raising the concept of "epi-virotherapeutic treatment."[65–68] By way of illustration, combining resminostat, an orally bioavailable HDACs inhibitor, with the oncolytic measles vaccine virus effectively killed HCC *in vitro*.[69] This approach

could be of prodigious interest in the context of CMV as the use of HDAC inhibitors enhances CMV replication and activation.[70,71] For instance, Trichostatin A (TSA), a specific inhibitor of class I and II HDACs, significantly enhanced viral replication.[72] Additionally, treatment with the class II-specific HDAC inhibitor MC1568 induced a robust induction of IE lytic gene expression without full virus reactivation, indicating that this endorsed expression of IE antigens could be prime targets for the well-established immunodominant IE-specific cytotoxic T cells.[73] Other epigenetic modifiers such as demethylating drugs could be of use in conjunction with oncolytic therapy, given the fact that most viral promoters including the major immediate early promoter (MIEP) of CMV are potential sites for silencing through methylation.[74] As a proof of concept, the use of 5-Aza and decitabine in glioma models enhanced HSV gene expression and replication *in vitro* in addition to increasing the survival of mice bearing orthotropic human gliomas *in vivo* upon treatment with oncolytic HSV.[75] Correspondingly, the use of demethylating agents enhances CMV replication, where treatment with 5-Aza activated CMV promoter-controlled reporter gene expression in human neuroblastoma cell line U87[71] and significantly increased the gene transcription and protein expression of HCMV-IE and HCMV-gB in human medulloblastoma cell line D342,[76] which could potentially optimize oncolytic CMV therapy. On the other hand, a HCMV/HSV-1 oncolytic virus has been produced with disruption of the HSV $\gamma_1$34.5 neurovirulence gene to eliminate its ability to cause encephalitis, along with human CMV protein kinase R (PKR) evasion gene IRS1 under control of the HCMV IE promoter in the U_L3/U_L4 intergenic region to enhance anti-tumor activity.[77–79] In concept, HSV-1 $\gamma_1$34.5-encoded protein is a multifunctional protein implicated in neurovirulence due to viral replication in post mitotic neuronal cells[80] and in conserving late viral protein synthesis in infected cells. This later function is conserved through inhibiting PKR-mediated phosphorylation a subunit of eukaryotic initiation factor (eIF-2a), opening the door toward continued viral protein synthesis.[81] Although recombinant HSV-1 viruses lacking the $\gamma_1$34.5 gene do not precipitate encephalitis and were deemed safe, they exhibited reduced viral replication, which resulted in reduction of efficacy and tumor lysis.[82–84] Advantageously, CMV PKR-evasion gene IRS1 could complement HSV-1 viruses lacking the $\gamma_1$34.5 gene by restoring late viral protein synthesis through PKR disruption without reinstating neurovirulence.[78] Besides, HCMV IRS1 restore an additional $\gamma_1$34.5 gene function characterized by evasion of IFN-inducible PKR, which further enhances late viral protein synthesis.[85] Added to the direct anti-tumor activity caused by viral replication and lysis in infected cells, HCMV/HSV-1 oncolytic virotherapy elicited an immune response that contributed further to its anti-tumor activity in glioblastoma multiform *in vivo*. This was illustrated by significant induction CD8 T lymphocytes, but not CD4 T cells. Interestingly, this model revealed that a circulating memory immune response against tumors was established in long-term survivors and that repeated administration of the HCMV/HSV-1 oncolytic virus could extend its anti-tumor effects.[79] Another advantage for the HCMV/HSV-1 virus over the $\gamma_1$34.5-deleted viruses is their ability to induce infectivity and cytotoxicity in adult and pediatric patient-derived glioblastoma xenografts under hypoxia,[86] a pathophysiological marker of high-grade gliomas, positively correlated with tumor development, angiogenesis, and therapy resistance.[87] Altogether, the use of oncolytic CMV therapy in combination with anti-cancer drugs, epigenetics therapy, or as HCMV/HSV-1 oncolytic virus could constitute a promising approach as those elucidate direct and indirect mechanisms of antitumoral activity.

Taken together, it is indeed obvious that CMV can modulate the tumor microenvironment toward an anti-tumoral state. Despite the previously discussed limitations, CMV offers several advantages that make it an attractive exploitable platform.[88] First, it was shown that CMV can induce a strong, long-lasting $CD8^+$ T cell response despite the presence of preexisting anti-CMV immunity, suggesting its use as a therapeutic, as well as a prophylactic, agent in the context of melanoma, even proving superior to the commonly used vesicular stomatitis virus vector.[89] This is favored by a well-known CMV property termed as memory inflation, a state characterized by a longitudinal expansion of stable $CD8^+$ T memory pools that constitute approximately 5%–10% of the total $CD8^+$ T cells in healthy individuals, pointing toward a continued host immunity boost.[90] This is enhanced by occasional viral reactivation from latency or even superinfection.[33] Second, HCMV has the largest genome in the HHV family, thus offering a large podium to improve safety and/or efficacy through genetic engineering without affecting viral replication.[33,91] In addition, several CMV genomes have been propagated in bacterial artificial chromosomes, which further permits complex and precise genetic manipulation.[92] However, although controversial, it is ultimately crucial to mention the potential tumorigenic ability of CMV.[93] Although not recognized as an oncogenic virus, CMV could favor the progression and the spread of the tumor, a paradigm termed oncomodulation.[8] This is supported by the activation of various prooncogenic pathways by CMV proteins, illustrated by tumor suppressor evasion, genome instability induction, cell survival enhancement, cellular migration promotion, and others.[5,8] Hence, it is indispensable to experimentally exploit the interplay between the triad of CMV, the tumor, and the tumor microenvironment. Besides, important questions in the stride of CMV toward virotherapy are still unanswered, some of which are the ideal route of introducing such virus into the tumors or the preferences of selecting laboratory strains versus clinical strains in such preparations. Acknowledging those points could pave the road toward the establishment of new innovative therapeutic platform against various types of cancers, a success conditioned by further assessment and continued exploration.

CONFLICTS OF INTEREST

The authors declare no competing interests.

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