Can cytomegalovirus protect us against brain tumours? A novel global correlation and survey of evidence

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Abstract: The aetiology of human cytomegalovirus (CMV) in tumorigenesis is still a matter of debate. Mounting evidence suggests that the pathogen may offer a degree of protection against various malignancies. The association between CMV and brain/central nervous system (CNS) tumours has yet to be fully elucidated, and studies inquiring into this issue from a global standpoint are lacking. We have investigated the relationship between CMV and CNS tumours the world-over and reviewed relevant literature published so far. Works inquiring into the interplay between CMV and CNS neoplasia published by November 2022 were reviewed through the PubMed® database. We also analysed the correlation between available country-specific CMV seroprevalence and the age-standardized CNS tumour incidence rates for the year 2020 for 73 countries using the data provided by the International Agency for Research on Cancer (IARC) of the World Health Organization. Statistical assessment was done using Spearman's correlation. The survey of literature yielded conflicting opinions on the aetiology of CMV in CNS malignancies. Oncomodulation as a mechanism of pathogenesis would seem to prevail over frank oncogenesis. On the contrary, analysis of global data revealed a significant negative correlation (p=0.001, Spearman's ρ=-0.541) between CMV seroprevalence and the age-standardized incidence of brain tumours. Interestingly, the inverse association holds for incidence rates of all cancers combined as well (p=0.001, Spearman's ρ=-0.732). A number of studies speak in favour of an immune-mediated anti-oncogenic effect against brain tumours in CMV-infected individuals. This is supported by recent success of CMV-derived therapies against gliomas. Our study offers novel data hinting at an oncoprotective capacity of CMV the world-over, which corroborates experimental research done so far. Extensive exploration of the molecular arsenal of CMV, host-pathogen interactions and CMV seroepidemiology are warranted in order to fully clarify this pathogen's role in CNS tumour dynamics.

Keywords: cytomegalovirus; glioblastoma; oncoprotection; oncomodulation; T-cell vaccines.

1. Introduction

The human cytomegalovirus (CMV) belongs to the family *Herpesviridae*, a well-known group of pathogens that cause persistent infection within the host. A ubiquitous and metropolitan agent of infection, the CMV can reach a pervasiveness of nearly 100% in some areas of the world (Zuhair *et al.*, 2019). It is mostly considered an innocuous pathogen in the healthy population. Conversely, in an immunocompromised setting, CMV can cause severe disease and lead to a fatal outcome.

The interaction between CMV and its host is a complex and manifold one, and the potential oncogenic capacity of the virus has been extensively debated. Cytomegalovirus has been associated with a vast spectrum of malignancies, including neoplasms of the brain, breast and the hematopoietic cell lineage (Stangherlin *et al.,* 2016; Geisler *et al.,* 2019; Francis *et al.*, 2017; Wiemels *et al.*, 2019). The faculties of the virus that speak in favour of oncogenicity, or at least oncomodulation (Herbein, 2018), seem to be ample. However, a number of studies imply an anti-tumour effect of CMV (Herbein, 2018; Kumar *et al.*, 2016; Jurak & Brune 2006; Erkes *et al.*, 2017). Finally, in recent years, mounting evidence supports a T-cell mediated host anti-cancer response targeting CMV molecules expressed on transformed cells (Cf. Discussion - The T-cell approach – from oncoprotection to a CMV-specific immunotherapy).

The most studied of CNS neoplasia in this regard is the glioblastoma multiforme (GBM); gliomas

indeed account for nigh on 80% of all primary brain neoplasia (Schwartzbaum *et al.*, 2006). Notoriously, GBM is one of the most lethal tumours with a median survival of less than 15 months, even when the full therapeutic panoply of surgical intervention, radiation and chemotherapy is deployed against it (Imperato *et al.*, 1990; Strupp *et al.*, 2005). The first description of CMV in malignant glioma tissue was reported in 2002 by Cobbs and co-workers (Cobbs *et al.*, 2002). Information about CMV presence in glioblastoma is still conflicting, and various opinions exist on the tumour/virus association.

Hitherto, we are yet to arrive at a categorical conclusion on the interplay between CMV and brain cancer, be it GBM or any other. Furthermore, most of the research done so far are single-centre studies employing *in vitro* or *in vivo* methodologies. Conflicting results also undermine the potential value of an immunotherapeutic approach. Additional molecular investigation into the CMV- and host-associated minutiae are certainly warranted. Finally, a global approach across a broad demographic swathe is lacking.

We inquired into the link between CMV pervasiveness and brain/CNS tumour incidences drawing from available population-wide data the world over. Therefore, the aim of this study was twofold: 1) to investigate the role CMV may play in CNS tumours from a global standpoint and 2) to summarize all hitherto published evidence from literature pertaining to CMV/CNS tumour/host interplay in a single comprehensive survey.

2. Materials and Methods

2.1. CMV and brain tumours – Global statistical analysis

For information on global cancer statistics for malignancies of the central nervous system, we accessed the World Health Organization Global Cancer Observatory (International Agency for Research on Cancer [IARC], 2020). All cancer histologies in the grouping "Cancer sites" were combined under an umbrella term "Brain, central nervous system" (B/CNS), and will be referred to as such from this point on, unless specified otherwise. The data was presented as age-adjusted annual incidence rates (per 10^5 persons) for both genders, encompassing the broadest age-range (0-85+ years) available. Incidences were observed for B/CNS malignancies monitored in 185 countries by the International Agency for Research on Cancer (IARC). As a control groups, we have chosen the incidences of Kaposi's sarcoma (as the tumour is decidedly known to be caused by another pathogen, the Kaposi's

sarcoma-associated herpesvirus, or KSHV) and of all cancers combined. Cytomegalovirus pervasiveness was assessed based on the seroprevalence of the pathogen in 73 countries, as reported by Zuhair et al (2019). In order to inquire for potential association between CMV and CNS malignancies, the mentioned incidences and seroprevalences were subsequently compared by means of the Spearman's rank correlation test. The same analysis was performed for KSHV and all cancers. All p-values <0.05 were considered significant.

2.2. CMV and brain tumours – Survey of published evidence

The PubMed® search engine was used in order to obtain all relevant literature. Studies listed in this manner were further interrogated for relevant publications, i.e., those that pertain to the underlying issue. Furthermore, works referenced within the acquired studies were additionally scoured for pertinent information and included herein. Original papers and reviews in the English language were utilized, along with papers with abstracts as the only available section of the work.

3. Results

The Spearman's rank correlation test yielded a highly significant and inverse correlation between country-specific cancer incidences for B/CNV tumours and CMV seroprevalences (p=0.001, Spearman's ρ=-0.541). The same significance and reverse association were attained when taking into account global incidence rates for all cancers combined (p=0.001, Spearman's ρ=-0.732). Control dataset of KSHV incidences did not demonstrate statistical significance under the same circumstances (p=0.953, Spearman's ρ=-0.007). It may be worthwhile noting that, while indeed no significance was observed, the correlation coefficient did likewise show an inverse association. Results of all three calculations are visually represented in Figure 1.

As for the survey of published evidence, the search utilizing the PubMed® engine yielded 270 results, with possible overlap due to repeating keywords. The results of the search are shown in Table 1.

4. Discussion

4.1. Cytomegalovirus – local infection, global protection?

The connection between CMV and tumours in general is still a matter of some debate. Although it certainly possesses an oncogenic panoply within its genome – and so far, frank induction of tumour

Fig. 1-A

Fig. 1-B

Fig. 1-C
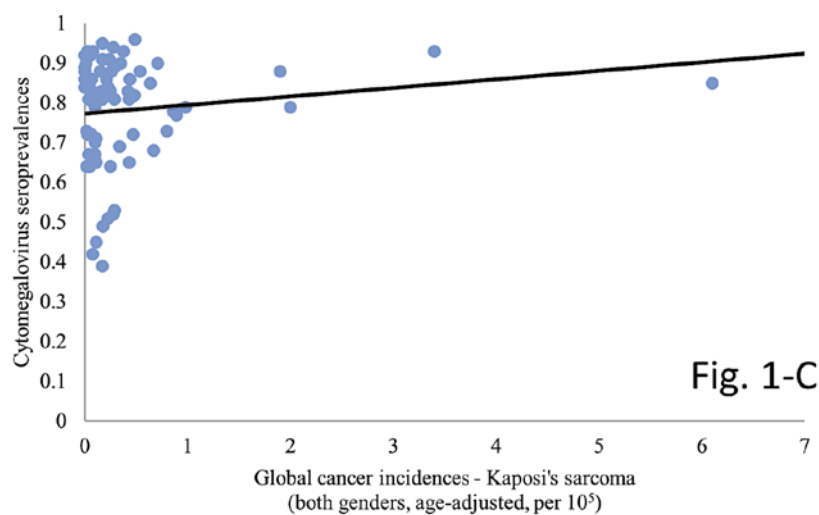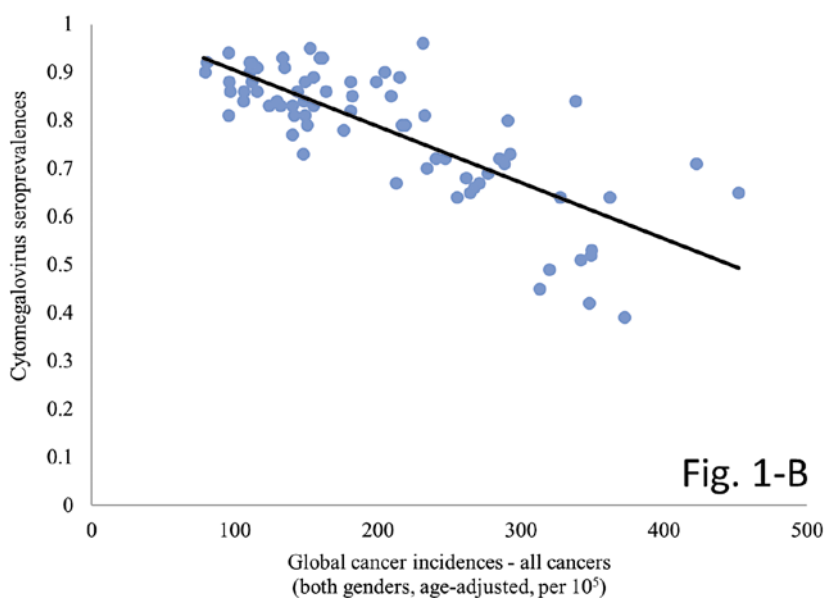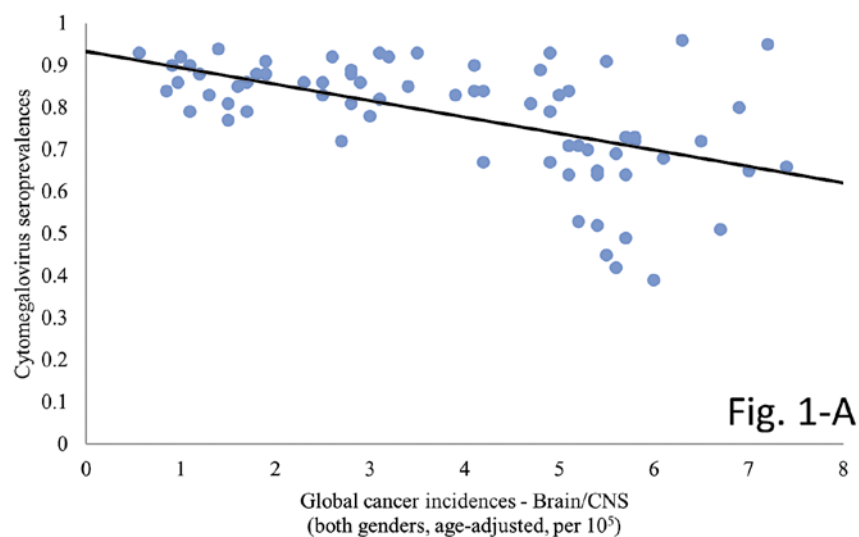
Figure 1. The graphs demonstrate the correlation between country-specific CMV prevalence and corresponding incidence rates for A) B/CNS tumours (p=0.001, Spearman's ρ=-0.541), B) all cancers (p=0.001, Spearman's ρ=-0.732) and C) Kaposi's sarcoma (p=0.953, Spearman's ρ=-0.007). Note the conspicuous lack of association for the control tumour incidences on graph C.

Keywords	Filter	Search engine	Results (no.)	Period†	Date of search
cytomegalovirus, brain	Title	PubMed®	135	1978-2022	18 Nov 2022
cmv, brain	Title	PubMed®	12	1989-2022	18 Nov 2022
cytomegalovirus, glioblast*	Title	PubMed®	87	1994-2022	18 Nov 2022
cmv, glioblast*	Title	PubMed®	13	2010-2022	18 Nov 2022
cytomegalovirus, brain tumo*	Title	PubMed®	12	2005-2022	18 Nov 2022
cmv, brain tumo*	Title	PubMed®	1	2008	18 Nov 2022
cytomegalovirus, brain cance*	Title	PubMed®	2	2018-2022	18 Nov 2022
cmv, brain cance*	Title	PubMed®	0	N/A	18 Nov 2022
cytomegalovirus, brain cancer	Title	PubMed®	2	2018-2022	18 Nov 2022
cmv, brain cancer	Title	PubMed®	0	N/A	18 Nov 2022
cytomegalovirus, CNS	Title	PubMed®	4	1980-2022	18 Nov 2022
cmv, CNS	Title	PubMed®	2	1993-2022	18 Nov 2022
Total	**N/A**	**N/A**	**270**	**1978-2022**	**N/A**

Table 1. The survey of literature as addressed by PubMed® search.
†The "Period" column indicates the time span in years during which all of the studies were published;
N/A – not applicable.

formation cannot be unconditionally excluded – it would seem more probable that CMV plays an oncomodulatory role in cancer pathogenesis. Conversely, evidence exists for an oncoprotective role of CMV in certain cases (Jankovic *et al.*, 2022). Finally, in the last couple of decades, efforts to harness CMV for immunotherapeutic purposes have been underway – the notion of T-cell based cancer therapies that would home on CMV-derived epitopes has been meticulously explored.

As for the enigmatic affiliation between CMV and brain malignancies, the scientific limelight has so far predominantly focused on the CMV/GBM interplay; other tumours are mentioned only scarcely. The studies hitherto carried out are single-centre and *in vitro* studies. Additionally, a number of vaccine trials based on T-cells and dendritic cells have either been carried out, or are under way (Ahn *et al.*, 2022).

This work aimed to summarize the existing knowledge on the interaction between the human CMV and brain tumours. Special attention will be given to glioblastomas, as the vast majority of research up to now concentrates on this entity. Moreover, we are the first to report on a highly significant association between CMV and B/CNS tumours the world over which supports a potential oncoprotective capacity of the pathogen. So far, no study investigated the possible association that might become apparent only at across multiple

country-wide landscapes. Our results speak in favour of an oncoprotective effect of CMV – as virus seroprevalences got higher, B/CNS incidences got significantly lower (p=0.001, Spearman's ρ=-0.541). Interestingly, we have observed the same significant and inverse correlation across a wide spectrum (approximately 75%) of cancer histologies monitored by the World Health Organization (WHO) and IARC (author's unpublished results, available upon reasonable request). The significance held when comparing incidences for all cancers combined (p=0.001, Spearman's ρ=-0.732), as seen in Figure 1. The latter result would suggest a wide-ranging oncoprotective effect of CMV, not exclusive to brain tumours.

Unexpectedly, the lack of association between CMV and KSHV would also seem to speak in favour of a possible anti-tumour influence. Namely, a known mechanism by which CMV aids in eliminating tumour cells is via T-cell mediated cancer cell destruction. Kaposi's sarcoma (KS) is rare and usually manifests in immunodeficiency, such as that caused by HIV. This tumour is the most frequent neoplasm in AIDS patients (Mercader *et al.*, 2000), a known population which has a compromised T-cell immunity and diminished T-cell numbers. In this setting of a severely impaired cellular immunity, the reduced T-cell pool fails to control or eliminate KS – hence the lack in statistical reinforcement of the correlation.

4.2. Hidden, or in plain sight?

The ongoing discussion concerning the association between CMV and glioblastoma tumours is a polarized one. The very presence of CMV in glioblastoma is still a matter of some debate. The pervasiveness of viral infection has been widely deliberated, with the pathogen detected anywhere from almost 100% of samples, to the complete absence of CMV in neoplastic tissue. Hitherto, rate of CMV infection in glioblastoma is contentious and a categorical conclusion is so far lacking (Yang *et al.*, 2022).

A comprehensive approach by Holdhoff and co-workers (Holdhoff *et al.*, 2017) in which they utilized an array of highly sensitive detection methods demonstrated the utter lack of CMV in high grade glioblastoma tissues. Their conclusions are in concert with several other studies (Lau *et al.*, 2005; Tang *et al.*, 2013; Baumgarten *et al.*, 2014; Yamashita *et al.*, 2014; Tang *et al.*, 2015; Strong *et al.*, 2016; Lin *et al.*, 2016; Garcia-Martinez *et al.*, 2017; Taha *et al.*, 2016), which also did not identify CMV in these malignancies. Recent and comprehensive investigation into the virus/tumour interplay did not detect CMV across several CNS tumour histologies (medulloblastomas, pilocytic astrocytoma, glioblastomas and oligodendrogliomas) (Zapatka *et al.*, 2020). A study on 42 FFPE samples of tumour tissue from patients with GBM identified CMV in only 3 (7.1%) cases; furthermore, the patient group did not statistically differ from the control group that involved samples from subjects without brain disease (Ghaffari *et al.*, 2021). Notably, EBV was herein significantly associated with GBM. Deep-coverage whole-genome sequencing was used in another study of tumours, where CMV DNA was not detected in 34 glioblastomas (Tang *et al.*, 2015). Research aiming to detect CMV in high grade gliomas and other paediatric brain tumours demonstrated a clear lack of viral material in neoplastic cells of the examined cohort, be it by PCR or immunohistochemistry (Sardi *et al.*, 2015).

The absence of CMV in GBM may stem from a number of reasons, not least of which is the methodology used in virus detection or the specific test used, as a number of methods have been employed for viral detection (Ahn *et al.*, 2022). False positives due to antibody cross-reactivity and non-specific glial cell staining have been proposed (Korbecki *et al.*, 2018). It may be argued as well that false negative results were obtained in some of these laboratory studies. However, in the study by Holdhoff *et al.* (2017) a number of rigorous methodologies were used, leaving the supposition of a laboratory error an unlikely explanation for the lack of CMV in glioblastoma

tissues. The discrepancy in CMV detection from one laboratory setting to another could be due to variances in control specimens, sample preparations and method sensitivity. Interestingly, disparate to other methods, all studies using next generation sequencing (NGS) did not demonstrate the occurrence of CMV. One positive case was noted, however, but it could have resulted from contamination (Ahn *et al.*, 2022). Achieving inter-laboratory uniformity in this regard could yield more precise results. Finally, the absence of CMV in glioblastoma may also be a consequence of the affinity of the virus towards a certain subtype of the malignancy, as there are four of those (Lehrer *et al.*, 2011).

In contrast to research done in patients with glioblastoma that did not find CMV in tissue samples, stands a variety of studies reporting on the detection of CMV in the majority of samples analysed (Cobbs *et al.*, 2002; Rahbar *et al.*, 2013; Stangherlin *et al.*, 2016; Mitchell *et al.*, 2008; Scheurer *et al.*, 2008; Ranganathan *et al.*, 2012; Libard *et al.*, 2014; Shamran *et al.*, 2015; Bhattacharjee *et al.*, 2012). A recent systematic review of literature concluded that glioblastoma tissue is highly pervaded with CMV and that optimal immunohistochemistry is mandated in order to detect presence of the pathogen (Peredo-Harvey *et al.*, 2021). In a study by Zavala-Vega *et al.* (2017), which aimed at detecting herpes simplex virus (HSV) and EBV in addition to CMV in brain tumours, the authors concluded that the viruses were found frequently in a highly seropositive population. It is interesting to note a work by Rahbar and colleagues (2013), which describes serology as an untrustworthy test for investigating the presence of prior or active CMV infection. Therein, the authors describe the presence of CMV in all tumours – however, 29% of the patients were IgG negative. This in itself might imply that CMV pervasiveness is greater in patients with glioblastoma than expected, which may contradict our results attained from 73 countries worldwide. The research was done on only 42 patients, however, and larger studies are warranted to substantiate the authors' results of serology being an unreliable method. It has been suggested that, in order to remedy for the inconsistencies in CMV detection among studies, a standardized protocol for viral detection likely using more than one method needs to be developed (Hochhalter *et al.*, 2017).

4.3. Oncogenesis, oncomodulation or both?

So far, a division in opinions precludes a decided conclusion about the oncogenicity of CMV in B/CNS cancers. Literature provides leverage for both pro-oncogenic and anti-oncogenic capabilities of CMV.

Nevertheless, a third option exists, where CMV can alter the course of an already established malignancy – a property termed "oncomodulation".

A review by Solomon *et al.* states that there does not seem to be enough evidence for CMV as an agent of oncogenesis – rather, data would support an oncomodulatory role of the virus, excluding direct malignant transformation (Solomon *et al.*, 2014). The infection with CMV may well be a double-edged sword – conferring protection early on in life, but harmful if it occurs later on in life. Namely, there is evidence for a temporal effect, where the pathogen may safeguard against glioblastoma multiforme in early childhood, while infection in later childhood and afterwards could pose as a causative factor (Lehrer, 2012).

Resent research employing single-cell RNA sequencing concluded that there was no decisive indication of full viral transcripts in analysed tumour tissue and cell lines (Johnson *et al.*, 2017); low-abundance reads aligned across all tumours were also recognized. In a study of 116 Taiwanese subjects, researchers could not conclude an association between the virus and glioblastoma (Yang *et al.*, 2017). The results from a study by Habibi and coworkers (2021) also could not support the role of CMV in non-glioblastoma multiforme infantile brain tumours. In a population of Japanese patients suffering from GBM, the authors did not find a connection between CMV and the cancer (Hashida *et al.*, 2015), and a similar conclusion was reached elsewhere (Loit *et al.*, 2019). Interestingly, CMV seroprevalence rates are significantly lower in men than in women, even though glioblastoma is more common in men (Lehrer *et al.*, 2015). This would suggest a potential oncoprotective capacity of CMV, which is in accordance with the results of our study.

The use of oncolytic viruses to destroy neoplastic tissue is not a new concept (Hawkins & Croul, 2011). The CMV itself can cause direct cell death, along with "bystander" apoptosis; the brain pathology can even manifest via T-cell independent apoptosis of meningeal, glial and neuronal cells; this cell die-out was, however, demonstrated only in an immunodeficient mouse model (Reuter, 2005).

In another mouse model of glioma, the authors dispute the causal role of CMV as an agent of gliomagenesis; instead, they argue for an oncomodulatory effect of the virus, which leads to tumour-suppressor loss, thereby accelerating glioma formation/proliferation (Price *et al.*, 2013). An oncomodulatory role of CMV has been proposed in brain malignancies including gliomas, medulloblastomas and neuroblastomas (El Baba & Herbein 2021). Oncomodulatory effects are most often explained as those that contribute to the increase of a tumour's malignancy (Michaelis *et al.*, 2009). We would be remiss not to mention, among others, the self-sufficiency in growth signals, insensitivity to anti-growth signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis, deregulation of cellular energetics, avoiding immune destruction, tumour-promoting inflammation, and genome instability and mutation – all specific onco-modulatory roles of CMV-infected glioma proposed by several investigations (El Baba & Herbein 2021; Herbein, 2018). The virus can also promote tumorigenesis and tumour invasion by creating oncomodulatory proteins that interact with cancer cell pathways (Daei Sorkhabi *et al.*, 2022). However, oncomodulation may not strictly imply an onco-promoting effect. Namely, *in vitro* research on glioblastoma multiforme cell lines by Dos Santos and colleagues concludes that the oncomodulatory potential of CMV also comprises a spectrum of hostile effects on tumour cell proliferation or survival, and is not restricted solely to cancer-promotion (Dos Santos *et al.*, 2018). Cytomegalovirus can also manifest an inhibiting effect on breast cancer cell migration by means of the virus glycoprotein B (Yang *et al.*, 2018).

Korbecki *et al.* (2018) note in their work that there seems to be a poor correlation between CMV infection and glioblastoma multiforme epidemiology, as latent infection is present in over half the population, but the incidence of GBM is low (3/10000 persons/year). We offer a possible explanation for this phenomenon in our study; therein, changes in brain tumour incidences and CMV seroprevalence strongly and inversely associate the world over. Moreover, the authors' have noted the high-CMV/low-GBM phenomenon, which speaks in favour of our conclusions that CMV might offers a degree of oncoprotection. CMV is significantly less pervasive whites than in blacks or Hispanics (Mexican Americans), while glioblastoma incidence is higher in whites than in blacks or Hispanics (Lehrer *et al.*, 2015). This is in line with our findings, as CMV is associated with a lower socioeconomic status (SES) – a social index unfortunately often found lower in blacks and Hispanics alike. Supporting evidence hails from a Swedish study, which reports on increased odds of glioma in persons with a higher family income (adjustments were made age, sex and geographic region (Wigertz *et al.*, 2010). Similar results are reported for those residing in areas with high SES, even after statistical adjustment for confounding factors – again, higher rates for glioblastoma were demonstrated in this group (Chakrabarti *et al.*, 2005). Having all else in mind, as this effect was observed around the world, we postulate that this protection is mediated via the

T-cell CMV-aimed immune response at the very least.

Contrary to the potential oncoprotective capacity conceivably tied with CMV, the virus proteins could induce telomerase activity and angiogenesis; they could also control the cell cycle, inhibit apoptosis, activate the migration of cells and metastasis; finally, they could avoid immune-mediated obliteration, intensify genome instability, and endorse stemness via the obstruction of the differentiation of cells (Dziurzynski *et al.*, 2012; Söderberg-Nauclér & Johnsen, 2015). Therefore, the pathogen's occurrence in glioblastoma would uphold tumour progression.

An *in vitro* investigation done on U373MG cells (human glioblastoma cell line) aimed to study the effect of the CMV IE1 protein on gene expression: the authors concluded that the IE1 gene product could modify the expression of genes that might be important actors in the pathogenesis of glial tumours. Notably, the expression of glial fibrillary acidic protein (GFAP), thrombospondin-1 (TSP-1), and p53 mRNA was decreased in cells expressing the aforementioned viral protein (Lee *et al.*, 2005). An approach similar to our own was used by Lehrer *et al.* where the authors compared CMV seroprevalences and glioblastoma incidence rates in the USA. Contrary to our findings, no consistent association between CMV and glioblastoma incidence was found (Lehrer *et al.*, 2012). We postulate that this divergence may result from different statistical approaches to available data. Moreover, while our results included all CNS tumours, the previous study was done solely considering patients with glioblastoma. Finally, the global aspects in our study may simply not be apparent on a state-wide level.

Since the first description of CMV oncomodulation by Cinatl and colleagues nigh on 30 years ago (Cinatl *et al.*, 1996a; Cinatl *et al.*, 1996b), this capacity of CMV has been extensively studied, along with the pros and cons for potential CMV tumorigenic effects. Holdhoff and colleagues (2017) have performed PCR on a portion of their patients, and the results were consistent with the rates of CMV seropositivity in the general population. This does not speak in favour of a potential oncoprotective effect of CMV in glioblastomas. It may be, however, that this anti-oncogenic property manifests in other types of tumours, or perhaps only in only a fraction of the patients with high grade glioma.

Interestingly, in a report from 2012 (Dziurzynski *et al.*, 2012), it was summarized from the conclusions from The human cytomegalovirus (CMV) and glioma symposium that "*existing evidence supports an oncomodulatory role for CMV in malignant gliomas, but future studies need to focus on determining the*

role of CMV as a glioma-initiating event", as well as that "*there is sufficient evidence to conclude that CMV sequences and viral gene expression exist in most, if not all, malignant gliomas…*" – a stark contrast to a later research (Holdhoff *et al.*, 2017). It seems there exists a close association between inflammation and tumour formation; viruses by themselves could induce an inflammatory environment, with emerging data pinning a pathogenic role on CMV in epithelial and neuronal malignancies (Söderberg-Nauclér & Johnsen, 2015). Another study speaks of CMV oncomodulation (and not necessarily oncogenesis) in which infection by the virus promoted epithelial-to-mesenchymal transition in glioblastoma cells and strengthened the invasiveness of glioma cells (Zhu *et al.*, 2020).

An investigation into the molecular panoply of CMV has yielded conclusions that the virus induces the upregulation of transcripts of the MET oncogene (linked with a subset of glioblastoma multiforme patients) which may be a mechanism included in glioblastoma multiforme growth (Krenzlin *et al.*, 2021). A significant increase in tumour growth was observed in mice infected with murine CMV, and the authors conclude that the virus potentiates glioblastoma growth by increasing pericyte recruitment and angiogenesis. This is, in itself, an oncomodulating capacity of the murine CMV, and does not necessarily imply oncogenesis (Krenzlin *et al.*, 2019). Matlaf and colleagues have suggested that the pp71 protein of CMV, previously demonstrated to promote cell cycle progression, can possibly be conducive to the aggressive phenotype of the glioblastoma multiforme (Matlaf *et al.*, 2013). Finally, direct evidence for CMV oncogenicity – albeit in a mouse model – was demonstrated by Price and Chiocca (2015). To date, there is no decisive evidence to support this concept in humans.

4.4. The T-cell approach – from oncoprotection to CMV-specific immunotherapy

The last decade and a half saw a rise in interest for immunotherapy using T-cells that target GBM neoplasia (Ahn *et al.*, 2022). T-cells are known to eliminate cancers existing in the immunologically privileged environs of the central nervous system. Viral antigens that are present in malignant cells have been investigated as potential immunotherapeutic targets for quite some time, including cytotoxic T-lymphocyte (CTL) or dendritic cell (DC)-based vaccines (Ahn *et al.*, 2022). The idea of CMV presenting as a valid target for this therapeutic modality has also been discussed (Ahn *et al.*, 2022). Cytomegalovirus antigens seem as a conspicuous bull's eye for cellu-

lar immunotherapies in that they could present more potent tumour recognition sites than tumour-derived antigens themselves (Schuessler *et al.*, 2014a). The assumption that CMV may act a target for cancer treatment is not a novel one; with CMV being localized within tumour cells, and healthy cells in close proximity remaining CMV-negative, it was proposed that the virus itself may prove as a fresh mark for therapeutic regimens against malignancy (Söderberg-Nauclér & Johnsen, 2012).

As the notion of CMV epitopes as viable targets for the host immune system in tumour eradication seem to gain momentum, it is conceivable that the T-cells might "enforce" homeostasis more strongly in CMV infected individuals by eliminating GBM cells as they develop – an action we would call oncoprevention. This shielding effect conferred by "natural immunization" is supported by our findings of a global significant and inverse association between CMV seroprevalence and B/CNS tumour incidences.

The literature, again, extends conflicting opinions, although it would seem that the majority of studies speak in favour of an efficient T-cell mediated destruction of tumour tissue guided by CMV molecules expressed with cancer cells; CMV is already widely accepted as a promising target for various immunotherapeutic approaches. The selective tropism of CMV for glial cells as an immunotherapeutic bull's eye in patients suffering from glioblastoma was proposed some 15 years ago by Prins and colleagues (2008). The authors recognized the significance and simplicity of galvanizing a T-cell mediated anti-viral (and, by extension, anti-tumour) response against pathogenic, foreign epitopes instead of "self" tumour antigens. It was also proposed that the improvement in the immunotherapeutic strategies against GBM be made by combining used endogenous with CMV-specific antigens expressed on tumour cells (Duinkerken *et al.*, 2016). In a recent in-human trial of the therapeutic potential of cytomegalovirus-specific adoptive cell therapy in participants with primary GBM, *in vitro*–expanded autologous CMV-specific T-cells were safely utilized as an adjuvant therapy (Smith *et al.*, 2020). Of note, the authors concluded that overall survival may improve in these patients if the therapy was offered before recurrence. Another earlier study suggested that a therapeutic regimen combining autologous CMV-specific T-cells and chemotherapy may offer clinical advantage in subjects afflicted by recurrent GBM (Schuessler *et al.*, 2014b). Both research hint at a successful T-cell-vs-tumour phenomenon based on CMV molecules as targets – a possible mechanism that could be substantiated by the results from our investigation. Immunologic targeting of CMV infected cells is further

supported by the notion that it can be achieved even with low levels of CMV gene expression (Prins *et al.*, 2008); furthermore, destruction of infected cells by cytotoxic T-lymphocytes can happen with as few as 3 antigenic peptides on the cell's surface (Purbhoo *et al.*, 2004). In human models, it is elsewhere also indicated that antibody and T-cell reactivity to CMV (as well as Epstein-Barr virus) epitopes in those afflicted with glioblastoma or pancreatic cancer point to the antiviral immune response constituting an essential component of the in situ host protection against malignancies (Meng *et al.*, 2018).

The acuteness of a T-cell mediated tumoricidal activity is underscored by Sampson and Mitchell (2011); the authors postulate that if CD133+ glioma stem cells do favourably express CMV antigens *in vivo*, therapies that mark CMV as a target may exert a high degree of inhibition on malignant growth by exterminating this tumour-propagating group (Sampson & Mitchell, 2011). In a mouse model, Brizic and colleagues (2018) demonstrated that adoptively transferred murine CMV-specific CD8+ T-cells provided protection to newborn mice against primary infection with this pathogen as well as reduced brain pathology. These tissue-resident memory T-cells controlled the latent murine CMV; with their depletion, the virus subsequently reactivated and led to heightened inflammation in the brain. This animal model would seem to advance the notion of T-cell importance in controlling CMV in the brain – and, by extension, perhaps brain tumours as well. Anti-CMV immunotherapy may owe its efficiency to 1) the directing of the immune response towards virus protein-exhibiting cells that drive tumor growth, 2) activation of other immune cells that elicit further destruction of cells naïve for CMV, or 3) cross-priming after killing of cancer cells invaded by CMV (Rahman *et al.*, 2018).

An additional argument may well be that early-stage immunotherapy-oriented trials with CMV as a target have yielded encouraging results (Lawler, 2015). It has been also suggested that new modalities using antiviral and immunotherapeutic approaches may have a role in combating the disease. Another study supports the rationale behind CMV-driven immunotherapy by demonstrating, among other conclusions, that CMV pp65-specific T-cells identify and destroy autologous glioblastoma multiforme cells (Nair *et al.*, 2014a).

In a cohort of 49 newly diagnosed GBMs, another group of researchers have found the tell-tale viral proteins pp65 and pIE1 in roughly 50% of samples; the authors further speculate that cells positive for the virus can be recognized by T-cells specific for pp65/IE1 (Lucas *et al.*, 2011). Recent research into

pp65-specific cellular responses elicited in a regimen of dose-intensified temozolomide (TMZ) therapy accompanied by pp65-dendritic cells showed long-term progression-free survival (PFS) and overall survival (OS), lending further credence to studies targeting CMV in GBM (Batich *et al.*, 2017). The median PFS and OS were 25.3 and 41.1 months, which is an encouraging time-span for a disease with a <15-month median survival regardless of surgical resection, high-dose radiation and TMZ. It was also shown that CMV-specific T-cells that recognized pp65- and IE1-expressing cells subsequently destroyed GBM cells infected with CMV. The authors of the study suggest that an infusion of these virus-aimed T-cell lines may be beneficial in patients with CMV-positive GBMs (Ghazi *et al.*, 2012). The virus was also proposed as a therapeutic target in a cohort of 25 serially diagnosed paediatric glioblastoma multiforme patients, as the expression of pp65 and IE1-72 CMV antigens was present in the majority of cancer tissues (Wakefield *et al.*, 2015). Although US28 of the CMV was described as contributing to the detrimental effects of GBM, the authors do suggest that the protein itself may prove a valid target in GMB treatment (Soroceanu *et al.*, 2011).

An interesting find is described in a work by Crough *et al.* (2012); the researchers report on the status of CMV-specific CD8+ T-cells in GBM patients already pervaded by the virus. The functionality of these cells in ex vivo analyses was found to be impaired; however, their faculties were re-established *in vitro* using CMV peptide epitopes and IL-2. This report on a rebound in T-cell functionality when exposed to CMV molecules also speaks in favour of the virus being a possibly potent cancer-destroying adjunct to our own immune system, as well as to T-cell based therapies.

Studies testing dendritic cell therapies also seem to be promising; two papers report on more frequent occurrence of CMV-specific T-cells, along with better survival outcome, when using a CMV pp65-based dendritic cell vaccine (Mitchell *et al.*, 2015; Reap *et al.*, 2018). A promising nearly one third of patients which have undergone dendritic cell vaccine therapy in a report by Batich *et al.* have achieved exceptionally long survival (Batich *et al.*, 2020). A recent work by Nair and colleagues endeavoured to stimulate T-cells from patients afflicted with GBM with autologous dendritic cells pulsed with viral RNA coding for the pp65, a known CMV protein. The researchers measured the function of the effector CMV pp65-specific T cells, and conclude that "CMV-specific T-cells can effectively target glioblastoma tumour cells for immunologic killing and support the rationale for the development of CMV-directed immunotherapy in patients with GBM" (Nair *et al.*, 2014b).

There are studies, however, that present evidence against T-cell mediated cancer-suppression facilitated by CMV. Cancer associated environs might curb the usefulness of antiviral T-cells inside the tumour itself (Schuessler *et al.*, 2014a). The tumour cellular composition prior to treatment may influence the effect of adoptive T-cell therapy (Walker *et al.*, 2019). An experimental riposte to the supposition of T-cell mediated elimination of CMV-infected tumour cells comes from a study of Vδ2neg γδ T cells, which are important effectors against CMV. Herein, the authors show that the largest subset of these, the Vδ1+ γδ T cells, expanded/activated ex vivo, successfully recognize and eliminate established glioblastoma multiforme cell lines as well as and primary tumour-derived glioblastoma multiforme cells irrespective of CMV infection (Knight *et al.*, 2013). Moreover, the authors note that the pathogen may well augment the resistance GBM cell lines to innate recognition. A study of 65 glioblastoma patients used highly-functional autologous polyclonal CMV pp65-specific T-cells (Weathers *et al.*, 2020). The authors conclude that prior contact with CMV (i.e. seropositivity) does not vouch for the neoplasm to be susceptible to CMV-specific T-cells. This is hypothesised to be a consequence of heterogeneity in viral antigen expression; notably, the T-cell effector function was decreased. Furthermore, CMV itself has multiple ways to cause immune evasion. This is particularly apparent in glioblastoma stem cells (Schneider *et al.*, 2016). It disrupts major histocompatibility complex (MHC) I: human leukocyte antigen (HLA)-A, HLA-B and HLA-C, the expression of which thwarts the recognition of the altered antigens on neoplastic cells (Trgovcich *et al.*, 2006; Jackson *et al.*, 2011; Noriega *et al.*, 2012; Barel *et al.*, 2003; Rölle & Olweus, 2009). Moreover, the pathogen can decrease the surface and increase the soluble HLA-G fraction (Onno *et al.*, 2000; Pizzato *et al.*, 2003) and produce the viral interleukin-10 (vIL-10) with an immunosuppressive property (Korbecki *et al.*, 2018). These are only some of the ways CMV may aid in fending off the host's immune arsenal. Moreover, the variable success of the T-cell based CMV-aimed immune response in patients with GBM may be attributed to an inconstant permissiveness of glioblastoma multiforme cells to CMV infection, as demonstrated *in vitro* (Dos Santos *et al.*, 2018).

Finally, there are a number of unpublished trials that use the CMV-based approach, most of which did not, to our knowledge, made public their results (Ahn *et al.*, 2022; Hochhalter *et al.*, 2017). Overall, there now exists a substantial foundation for T-cell

based vaccine approaches using CMV in cancer therapy. The results of our study are in line with these approaches and further support their rationale.

5. Conclusions

So far, the association between CMV and B/CNS tumours was broadly discussed. The literature is heavily reliant on CMV in glioblastoma, with other tumours mentioned only seldom. In this study, we have tried to recapitulate the sum of existing knowledge regarding this issue, and interpret our own results vis-à-vis the literature surveyed. Hitherto, there have been four hypotheses on the association between CMV and GBM, as elegantly summarized by Hochhalter and colleagues (2017):

1. CMV is a *bona fide* causal agent of GBM. So far, except for a study in mice, no other evidence supports this claim;
2. CMV may be oncomodulatory. As discussed above, oncomodulation is mostly equated with augmenting cancer progression, although there is evidence of oncomodulation that would benefit the patient. Studies advocating for this property of CMV abound;
3. CMV is present in GBM because of the immunosuppressive tumour microenvironment;
4. The detection of CMV in GBM is merely a laboratory artefact, a consequence of various methodological glitches.

However, mounting evidence would support an anti-cancer role of CMV in general. As regards GBM, this is reflected in the success of CMV-based T-cell therapies, our most recent results that hint at CMV-related oncoprotection in B/CNS tumours, as well as other research mentioned above (Cf. Discussion). Having all this in mind, we find it prudent to put forward a fifth premise:

5. CMV may offer a degree of oncoprotection and/or oncoprevention agent against GBM.

Up until now there hasn't been any conclusive correlation between CMV seropositivity and glioblastoma incidence. Furthermore, the literature is still conflicting on the presence of CMV in glioblastoma cells, which may limit the potential usefulness of CMV-based therapeutic approaches. Our study is the first to hint at a phenomenon of CMV protection against B/CNS tumours on a global scale, and corroborates all similar experimental results reported elsewhere. Correlation does not have to infer causation, however, and these results should be interpreted with caution – much still remains obscure in the domain of CMV oncogenicity.

A complete understanding of the interplay between CMV and glioblastoma – and brain tumours in general – still eludes us. The novel evidence emergent from a global scale that CMV potentially may be oncoprotective speak in favour of the importance of T-cell mediated immunotherapies. More comprehensive molecular studies investigating host-pathogen interactions, as well as large multicentre studies and broad-swathed epidemiological inquiries are needed to corroborate our results and hopefully finally untie a Gordian knot of a longstanding debate.

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