

# Cytomegalovirus and Cancer: A Long and Winding Road to Conclusive Evidence

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It has long been known that collecting convincing evidence relating a certain virus to the formation of one or more cancer type(s) is a tedious task. In 1911, Peyton Rous, being aware of similar previous experiments on the transmission of leukaemias between chicken by Vilhelm Ellerman and Olaf Bang [1], had shown that cell-free extracts from chicken sarcomas induced sarcomas in healthy chickens [2]. He concluded that a “minute parasitic organism” or a “chemical stimulant, elaborated by the neoplastic cells” must be responsible for sarcoma formation [2]. Later, the causative agent was identified to be a retrovirus and called the Rous sarcoma virus (RSV) [3]. However, it took 55 years and the discovery of the Epstein-Barr virus, the first human tumour virus [3,4], before Peyton Rouse eventually received the Nobel Prize for his work in 1966.

Since 1972, human cytomegalovirus (HCMV) has been associated with cancer in various ways [5-7]. In contrast to the eight viruses that are known to contribute to carcinogenesis in humans (Epstein-Barr virus, hepatitis B virus, human T-lymphotropic virus-I, high risk human papilloma viruses, hepatitis C virus, Kaposi's sarcoma herpesvirus, Merkel cell polyomavirus) [8], HCMV is not regarded as a tumour virus because evidence that it can transform human cells is lacking [6,7].

To describe a possible interaction of cytomegalovirus (and potentially other viruses) with cancer cells, the concept of oncomodulation was developed based on experimental findings [9,10] and a neuroblastoma patient with therapy-refractory disease who had developed HCMV disease and in whom antiviral therapy with ganciclovir not only suppressed HCMV replication but also resulted in resensitisation of the tumour to anti-cancer therapy [7].

Oncomodulation means that a virus enhances the malignancy of established cancer cells independently of its carcinogenic potential [6,7,9,10]. Oncomodulatory effects are also exerted by tumour viruses, but HCMV turned out to be an ideally suited model virus to study oncomodulation by viruses because it does not transform cells. A number of different groups determined oncomodulatory effects by HCMV in a wide range of experimental systems [6,7,11-14].

Interest into the clinical role of HCMV in cancer sharply increased from 2002 onwards, after the group of Charles Cobbs had detected HCMV at very high frequencies in cancer tissues from glioma patients [15], colorectal cancer patients [16], and prostate cancer patients [17] by so-called ‘highly sensitive’ detection methods [18,19]. A couple of groups confirmed these findings, and to date the presence of HCMV has been reported in very large fractions of investigated glioma, colorectal cancer, prostate cancer, breast cancer, neuroblastoma, medulloblastoma, rhabdomyosarcoma, and hepatocellular cancer tissues [6,7,14,20-27]. With regard to the investigation of HCMV in cancer, glioma has been the most intensively studied cancer entity [6,7,14,20]. This includes

studies that not only indicate the presence of HCMV DNA, RNA, and/or proteins in cancer cells but also HCMV-induced signalling events and correlations suggesting that higher levels of HCMV infection of tumour tissues are associated with advanced disease stages and poorer outcomes [6,7,14,24,26,28,29]. Moreover, HCMV pp65-specific T-cells were reported to kill autologous primary glioblastoma cells, and dendritic cells pulsed with tumour RNA were described to cause an expansion of HCMV pp65-specific T-cells [30].

However, a significant number of other research groups failed to detect HCMV in cancers including glioma, neuroblastoma, childhood brain tumours, breast cancer, colorectal cancer, cervical cancer, gastric cancer, head and neck cancer, renal cancer, leukaemias, liver cancer, ovarian cancer, prostate cancer, and skin cancer [6,7,31-42]. Most strikingly, no deep sequencing study has been able to detect HCMV in cancer so far [34,36,37,42]. Thus, there is an ongoing dispute whether HCMV is actually present in cancer tissues or not.

A concern with regard to the presence of HCMV in glioma is that there is no consistent relationship between the HCMV seroprevalence and the glioma incidence. Gliomas are not overrepresented among HCMV-seropositive individuals, and the prognosis does not appear to differ between HCMV-seropositive and -seronegative glioma patients [35,43,44]. One study had reported a significant proportion of patients (80%) with newly diagnosed glioblastoma to have detectable HCMV DNA in their peripheral blood, while sero-positive normal donors and other surgical patients did not exhibit detectable virus [45]. The authors interpreted this as either being a systemic reactivation of HCMV within glioblastoma patients or virus shedding from infected cancer cells [45]. However, others failed to detect enhanced HCMV DNA levels in glioblastoma patients [46,47].

Notably, the ‘highly sensitive methods’ detect HCMV in tumour tissues from HCMV-seropositive and -seronegative patients [48-50]. The authors of one study concluded that HCMV seropositivity is not indicative for the presence of HCMV in tumour tissues [48]. Indeed, one study correlated high anti-HCMV IgG with a low glioblastoma risk and low anti-HCMV IgG with a high glioblastoma risk [51]. However, this appears to be in conflict with findings showing that there is no difference in disease outcome between HCMV-seropositive and HCMV-seronegative glioblastoma patients [35,43,44]. It remains also unclear how HCMV should reach tumour tissues without inducing an immune response. Serology was criticised to be a ‘poor test’ for detecting HCMV in glioblastoma patients [50]. However, the currently applied serological methods are highly reliable in identifying the (HCMV-positive) immunocompromised patients that are at risk of HCMV disease [52].

Despite these controversies, clinical trials have started that aim to

exploit HCMV as a therapeutic target for the treatment of glioblastoma patients. Initial trials on HCMV-directed immunotherapies for glioblastoma patients indicated these approaches to be safe but efficacy data is not available, yet [53,54]. A randomised trial investigating the anti-HCMV drug valganciclovir in glioblastoma patients did not demonstrate any benefit over placebo [55]. Subsequent re-analysis of parts of this study in combination with some additional cases, however, demonstrated a survival advantage for glioblastoma patients treated with valganciclovir [56,57]. However, this approach was strongly criticised as being flawed [58-61]. It is possible that a compound like ganciclovir, the active principle of the prodrug valganciclovir, exerts HCMV-independent anti-cancer effects. The anti-HCMV drug cidofovir was demonstrated to exert anti-glioblastoma effects in non-HCMV infected cancer cells [62] and similar unpublished results have been reported for ganciclovir [63].

Taken together, the question whether HCMV may play a role in cancer has been around since the 1970s [6] the concept of oncomodulation was proposed for the first time almost 20 years ago [9,10], the presence of HCMV has been suggested in large fraction of tumours from various cancer types since 2002 [14,15]. We have been confronted with requests from desperate cancer patients who wanted to know whether they should be treated with antiviral drugs for almost two decades now, and we still do not have an answer to this question. This is very frustrating. Arguably, the situation may be even more complex. For example, although we initially assumed that oncomodulation by HCMV depended on the presence of HCMV (antigens) in the tumour tissue [6], our later experimental findings indicated that HCMV can irreversibly increase cancer cell malignancy [64]. Also, HCMV-induced  $\gamma\delta$  T cells were described to exert anti-cancer effects [65,66]. However, today we are still not even able to answer with certainty the relative easy question whether HCMV is present in patient tumour tissues. This should be a wake-up call.

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