Valganciclovir as Add-on to Standard Therapy in Glioblastoma Patients

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ABSTRACT

Purpose: Several groups have reported a prevalence of human cytomegalovirus (CMV) in glioblastoma close to 100%. Previously, we reported that treatment with the antiviral drug valganciclovir as an add-on to standard therapy significantly prolonged survival in 50 patients with glioblastoma. Here, we present an updated retrospective analysis that includes an additional 52 patients.

Experimental Design: From December 2006 to November 2019, 102 patients with newly diagnosed glioblastoma received valganciclovir as an add-on to standard therapy. No additional toxicity was observed. Contemporary controls were 231 patients with glioblastoma who received similar baseline therapy.

Results: Patients with newly diagnosed glioblastoma receiving valganciclovir had longer median overall survival (OS 24.1 vs. 13.3 months, $P < 0.0001$) and a 2-year survival rate (49.8% vs. 17.3%) than controls. Median time-to-tumor progression was also longer than in controls; 9.9 (0.7–67.5 months) versus 7.3 (1.2–49 months), $P = 0.0003$. Valganciclovir improved survival in patients with radical or partial resection and an unmethylated or methylated *MGMT* promoter gene.

Conclusions: Valganciclovir prolonged median OS of patients with newly diagnosed glioblastoma (with methylated or unmethylated *MGMT* promoter gene) and was safe to use.

Introduction

Despite increased understanding of their molecular phenotypes, glioblastomas remain incurable and have a dismal prognosis. Total surgical resection provides better outcomes than partial resection (1). With standard treatment, maximal surgical resection followed by concomitant chemoradiotherapy with temozolomide, median 2-year survival of up to 26% has been reported in controlled trials (2, 3), but seldom exceeds 15% to 20% in daily practice. Moreover, due to an unmethylated *MGMT* promoter gene in the tumor of the majority of . patients, they may not benefit from temozolomide therapy. They have an expected 2-year survival rate of only 13.8% (4).

Human cytomegalovirus (CMV) nucleic acids and proteins are found in 90% to 100% of glioblastomas (5–8). CMV is also present in solid cancers, including cancer of the breast (9), colon (10), prostate (11), and ovaries (12, 13), medulloblastoma (14), neuroblastoma (15), and rhabdomyosarcoma (16). Present mainly in tumor cells, the virus has oncomodulatory effects (5), can cause all the hallmarks of cancer (17), and promotes tumor growth in glioblastoma mouse models (18). Some evidence suggests that certain CMV strains can be oncogenic; two clinical strains were so far independently shown to transform cells *in vitro* (19) and established tumors in immunodeficient mice (19, 20).

In a case–control study, we found that patients with low-grade CMV infection had longer median overall survival (OS) than those with high-grade infection (33 vs. 13 months, $P = 0.036$) and a higher 2-year survival rate (63.6% vs. 17.2%, $P = 0.003$; refs. 21, 22). A low-grade CMV infection was also associated with longer survival of patients with breast (23, 24), colon (24), and ovarian cancer (12, 25). These observations suggest that CMV affects tumor progression and may be a target of therapy, and support the hypothesis that antiviral therapy for CMV may slow tumor progression. In an animal model, we showed that anti-CMV treatment with valganciclovir and celecoxib reduced medulloblastoma growth by 72% (14). In a xenograft model, valganciclovir reduced neuroblastoma growth by 40% (15). The anti-CMV drug cidofovir (18, 26) and nanobodies to the CMV protein US28 also significantly reduced glioblastoma growth in mouse models (27).

To determine whether antiviral therapy for CMV improves the outcome of patients with glioblastoma, we enrolled 42 patients in a double-blind clinical phase I/II hypothesis generating trial to assess the safety and potential efficacy of valganciclovir treatment in patients with glioblastoma [the VIGAS study - Efficacy and Safety of Valcyte as an Add-on Therapy in Patients With Malignant Glioblastoma and Cytomegalovirus (CMV) Infection, NCT00400322; ref. 28]. Patients received a full dose of valganciclovir (900 mg) or placebo twice daily for 3 weeks followed by a maintenance dose of 450 mg twice daily for 21 weeks. The study was underpowered and failed its primary endpoint of reduced tumor growth by MRI at 6 months. However, in exploratory analyses of VIGAS patients with active valganciclovir intake, we observed that 50% of patients were alive at 2 years compared with 18% of contemporary control patients receiving the same baseline therapy. The median OS was also longer in valganciclovir-treated patients than in controls (24.1 vs. 13.1 months; refs. 28, 29). CMV is also evaluated as target in immunotherapy approaches. Dendritic cells pulsed with pp65 mRNA have been used to vaccinate patients with glioblastoma (30, 31). A larger clinical trial is currently ongoing to evaluate this therapeutic strategy. Adoptive transfer of CMV-specific T cells is also a promising approach (32) and vaccination studies are ongoing.

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Translational Relevance

Cytomegalovirus presence in the majority of glioblastoma may represent a therapeutic target. We performed a retrospective analysis of survival data of 102 patients with glioblastoma receiving the antiviral drug valganciclovir as an add-on to standard therapy and of 231 controls treated at our institution. Valganciclovir improved survival of both newly diagnosed patients with glioblastoma who underwent complete or partial resection, with unmethylated and methylated *MGMT* promoter status. Given the discouraging results from drug intervention studies for this patient group, and an almost doubled life expectancy in valganciclovir-treated patients with glioblastoma, our data suggest that this drug has a biological effect on glioblastoma rather than representing a random event or patient selection bias. This could open for a new understanding of glioblastoma pathogenesis. A multicenter double-blind randomized trial to evaluate the treatment effect of valganciclovir was recently initiated to confirm or dismiss these promising treatment results.

For several years, we have tried to obtain funding for a randomized clinical trial to confirm or disprove the hypothesis that valganciclovir as an add-on to standard therapy improves the prognosis of patients with glioblastoma. During this time, our cohort of patients with newly diagnosed glioblastomas treated with valganciclovir has increased to 102 patients. In a letter to New England Journal of Medicine in 2013, we reported an updated analysis of OS for 50 valganciclovir-treated patients, who had a median OS of 25 months versus 13.5 in controls ($P < 0.001$). Here, we present an updated, in-depth retrospective analysis of this accrued patient cohort.

Materials and Methods

Study design

This is a retrospective study of 102 patients with newly diagnosed glioblastoma that received valganciclovir as an add-on therapy to standard treatment between December 12, 2006, and November 10, 2019, at Karolinska University Hospital (Stockholm, Sweden). The diagnosis of primary glioblastoma was confirmed pathologically. The primary endpoint was OS. The study was conducted in accordance to the declaration of Helsinki and approved by the regional ethics committee in Stockholm (Dnr: 2016/1426–31/1). We were not required to take informed consent from the living patients or from relatives of deceased patients for this study. Patient data are in a data file that can be connected to the patient with a code key. The authors followed all patients throughout the study. The controls were selected among patients with pathologically confirmed primary glioblastoma treated at our institution between December 2006 and November 2019. These contemporary controls were identified by manual search of patient charts and the list of multidisciplinary tumor board conferences. The control patients that were excluded are those that were enrolled in other trials during the same period (a total of 30 patients, from the neoadjuvat temozolomide trial, the Nordic trial, a trial of denditic cell therapy and the EF-14 TTF trial). Control patients may have been missed with this method, but no active selection has taken place.

Standard-of-care treatment

Patients' demographic and clinical characteristics are summarized in **Table 1**. Standard care for newly diagnosed patients after surgery at Karolinska University Hospital is fractionated radiotherapy (60 Gy in 2-Gy fractions) with concomitant temozolomide. As patients over age 70 are often not suitable candidates for full 60-Gy radiotherapy, some elderly patients received hypofractionated radiotherapy (3.4 Gy × 10 days) in addition to chemotherapy (5 valganciclovir-treated and 11 controls) or only chemotherapy (12 valganciclovir-treated patients received only temozolomide and among controls 3 received CCNU and 57 received only temozolomide). Valganciclovir was given at the standard recommended dose: 900 mg twice daily for 3 weeks followed by 900 mg daily until disease progression ($n = 12$ cases) or palliative status. Severe signs of intolerance were not observed.

Table 1. Demographic and clinical characteristic.

Characteristics	Primary disease	
	Controls[a] ($n = 231$)	Valganciclovir ($n = 102$)
Age, years		
Median	63	59
Range	24–85	22–76
Sex		
Women, *n*	86 (37.2%)	30 (29.4%)
Men, *n*	145 (62.8%)	72 (70.6%)
Race		
Caucasian	100%	100%
MGMT promoter status		
Methylated	50 (45.0%)	39 (44.3%)
Unmethylated	61 (55.0%)	49 (55.7%)
IDH status		
Wild type	30	55
Mutated	2	1
Tumor location		
Temporal	84 (36.4%)	42 (41.2%)
Frontal	69 (29.9%)	31 (30.4%)
Parietal	51 (22.1%)	19 (18.6%)
Occipital	10 (4.3%)	5 (4.9%)
Other	17 (7.3%)	5 (4.9%)
Treatment, *n* (%)		
Surgery		
Radical resection	164 (71.0%)	79 (77.5%)
Partial resection or biopsy	67 (29.0%)	23 (22.5%)
Hypofractionated RT and chemotherapy	11	5
Chemotherapy only	60	12
Concomitant full dose RT and chemotherapy	157	77
KPS after surgery		
≤80	141	40
≥80	83	58

Abbreviation: RT, radiotherapy.
[a]Controls received standard-of-care treatment.

Postoperative Karnofsky performance score (KPS) was determined as a routine.

The methylation status of the O^6-methylguanine DNA methyltransferase (*MGMT*) promoter gene (available for 88 newly diagnosed patients with glioblastoma treated with valganciclovir and for 111 controls) was determined by pyrosequencing at the Karolinska University Hospital's pathology department, as a routine from March 2015, or in our laboratory. In the latter case, total DNA was extracted from paraffin-embedded tissue sections with QIAamp DNA FFPE Tissue Kit (Qiagen), bisulfite converted with the EpiTect Bisulfite Kit (Qiagen), and used for pyrosequencing (*MGMT* Pyro Kit, Qiagen). Samples with >25% methylation in the *MGMT* promoter gene were

considered positive. Analysis for *IDH* mutations was introduced at our hospital in 2016. Only one of 54 valganciclovir-treated patients and two of 32 controls had an *IDH* mutation.

Statistical analysis

Patients were analyzed for median OS, 2-year survival, median time-to-tumor progression (TTP), effects of the duration of valganciclovir treatment and when it was initiated, and extent of resection. Other variables considered were age, sex, KPS, *MGMT* status, and *IDH* mutation. Survival data are presented in graphs as Kaplan–Meier estimates, calculated from the time of surgery. Correlation among the mentioned variables inrelation to survival was calculated by nonparametric Spearman correlation and multiple Cox regression. Cox regression analyses with treatment status as a time-dependent covariate was also performed in order to investigate whether "immortal time bias" affected the data. All statistical hypotheses were two-sided, with a significance level of 5%. Significance was determined with log-rank test; $P < 0.05$ was considered statistically significant. GraphPad Prism (version 8.3) and SAS were used for statistical analyses.

Results

Between December 12, 2006, and November 10, 2019, 102 patients with newly diagnosed glioblastomas received valganciclovir in addition to standard therapy. Most of the patients received long-term therapy: median time of valganciclovir therapy was 15.6 months (0.7–149 months), with only 16 patients receiving 6 months of therapy or less. Valganciclovir treatment was interrupted because of hematologic toxicity in 6 patients, skin rash in 2, myocardial ischemia in 1 (determined not to be related to valganciclovir), infection in 3, and diarrhea in 1. No additional toxicity was observed. One patient wanted to stop treatment. Contemporary controls were 231 newly diagnosed patients with glioblastoma and they were all treated at our institution. Patient characteristics are summarized in **Table 1**.

Valganciclovir is associated with improved survival of newly diagnosed patients with glioblastoma

The median age of 102 newly diagnosed patients with glioblastoma was 59 years (range, 22–76 years), and 70.6% were men. The median KPS value was 90. Of 79 patients (77.5%) who underwent radical surgery, 68 received optimal therapy with concomitant radiotherapy + temozolomide. The median duration of valganciclovir therapy was 15.6 months (range, 0.7–149 months). The median time between surgery and the start of valganciclovir treatment was 2.7 months (range, 5 days–16.9 months). The median age of the 231 controls was 63 years (range, 24–85 years), and 62.8% were men. The median KPS value was 80. Of the 164 controls who underwent radical surgery, 127 received concomitant radiotherapy + temozolomide. TTP, defined as the interval of time between primary therapeutic intervention and a diagnose of disease progression according to RANO criteria, was recorded for 179 controls (77.5%) and 44 (43.1%) valganciclovir-treated patients. *IDH* mutation status was available from clinical records in 56 of 102 valganciclovir-treated patients; only one was positive for *IDH1* mutation. Among 32 of 231 controls, one was positive for *IDH1* and one other for *IDH2* mutation. Given the small number of patients with an *IDH* mutation, we assumed that it would not be a relevant variable in this study.

In valganciclovir-treated patients, the median OS was longer (24.1 vs. 13.3 months, $P < 0.0001$; **Fig. 1A**), and the 2-year survival rate was higher (49.8% vs. 17.3%, $P < 0.0001$) than in controls. Among all patients who had a radical resection ($n = 243$), OS was also longer in valganciclovir-treated patients than in controls (28.7 vs. 15 months, $P < 0.0001$; **Fig. 1B**). OS also improved in valganciclovir-treated patients with nonradical resection (18.4 vs. 10.6 months, $P = 0.0010$; **Fig. 1C**). Radical resection provided significant survival advantage among the controls and the valganciclovir-treated patients ($P < 0.0001$). Among the optimally treated patients, those who had complete resection and received concomitant radiotherapy + temozolomide, OS was also longer in valganciclovir-treated patients (29.7 vs. 17.0 months, $P < 0.0001$; **Fig. 2A**), and the 2-year survival rate was higher (63.9% vs. 27.6%, $P < 0.0001$) than in controls.

Among the 90 patients not undergoing complete resection, 16 of 23 patients in the valganciclovir-treated group and 30 of 67 patients in the control group received 6 weeks of temozolomide and full dose of radiotherapy (60 Gy). Patients receiving valganciclovir had a higher OS than controls (18.4 vs. 14.35 months, $P = 0.0278$) and a 2-year survival rate of 20.0% versus 10.0% in the control group ($P = 0.0580$).

Next, we compared outcomes in patients who received valganciclovir within 2 months ($n = 41$) or more than 2 months ($n = 61$) after surgery. OS was 21.4 and 25.9 months, respectively, and the difference was not statistically significant ($P = 0.9442$). Both groups had longer OS than controls ($P < 0.0001$; **Fig. 2B**). Similarly, the 86 patients who received valganciclovir for longer than 6 months showed a longer OS than the 16 patients that received valganciclovir for 6 months or less (25.8 vs. 20.6 months), but the difference was not significant ($P = 0.5760$). Both groups had longer OS than controls (**Fig. 2C**).

MGMT promoter methylation status was available for 88 (86.3%) valganciclovir-treated patients and 111 (48.0%) controls. The *MGMT* promoter was unmethylated in 49 (55.7%) and methylated in 39 (44.3%) patients who received valganciclovir. Among controls, the *MGMT* promoter was unmethylated in 61 (55.0%) and methylated in 50 (45.0%) patients. OS was longer in 39 valganciclovir-treated patients with a methylated MGMT promoter than in 49 matched controls (32.1 vs. 15.15 months; $P = 0.0006$; **Fig. 3B**). OS was also longer in the 49 treated patients with unmethylated *MGMT* promoter than in 61 matched controls (21.1 vs. 11.6 months; $P < 0.0001$; **Fig. 3A**). Both among controls and valganciclovir-treated patients, OS was longer in patients with methylated *MGMT* promoter than unmethylated ($P = 0.0189$ and $P = 0.0048$, respectively).

Spearman correlation analysis also revealed that OS in patients with newly diagnosed glioblastoma (treated and controls) positively correlated with valganciclovir therapy ($P < 0.0001$), high KPS ($P < 0.0001$), methylated *MGMT* promoter ($P < 0.0001$), complete resection ($P < 0.0001$), 6-week cycle of radiotherapy + temozolomide ($P < 0.0001$) and duration of valganciclovir therapy ($P < 0.0001$). A negative correlation with OS incurred with age ($P < 0.0001$) and an unmethylated *MGMT* promoter ($P < 0.0001$). Sex ($P = 0.6333$) and delay of valganciclovir therapy ($P = 0.1975$) did not correlate with OS.

Multiple Cox regression analyses showed that valganciclovir treatment was the most significant variable positively associated with OS ($P < 0.0001$) followed by complete resection ($P < 0.0001$), methylated *MGMT* promoter status ($P = 0.0028$), and older age ($P = 0.0019$). Among these patients, 6-week cycle of radiotherapy + temozolomide, KPS, and gender did not correlate with survival ($P = 0.6451$, $P = 0.1446$, and $P = 0.1480$, respectively).

To assess whether an immortal time bias could explain the increased survival of valganciclovir-treated patients (33), we further analyzed our data using Cox regression with treatment status as a time-dependent covariate as described previously (34). Yielded HRs between the models (time from surgery to start of treatment in comparison with untreated vs. original data) were not significantly

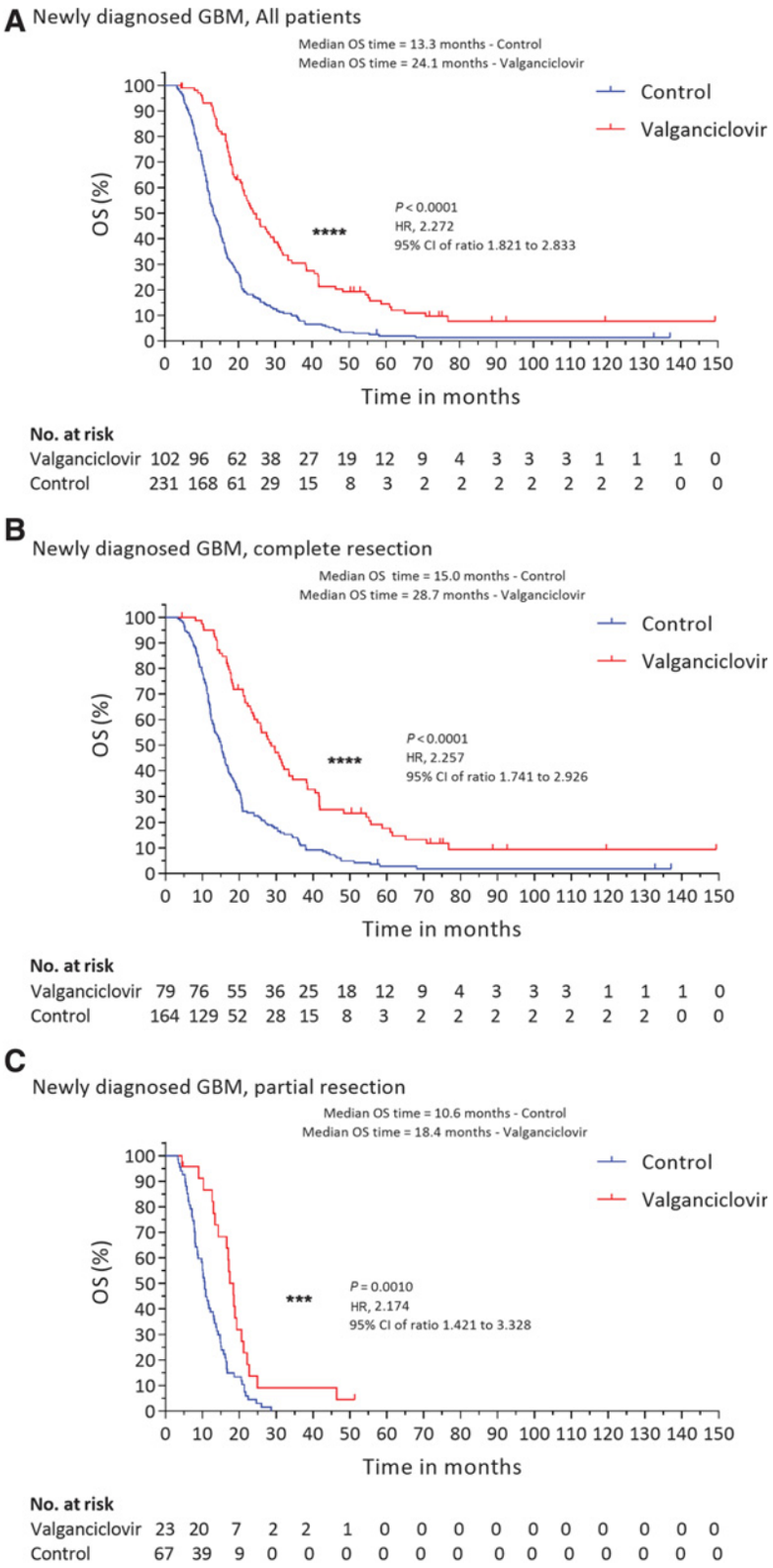

Figure 1.

Kaplan–Meier estimates of OS in patients with newly diagnosed primary glioblastoma treated with valganciclovir. Estimated median OS for all 102 patients with newly diagnosed primary glioblastoma who received valganciclovir therapy and for 231 contemporary controls who received similar baseline therapy (**A**), patients who had complete resection (**B**), and patients who had a partial resection or biopsy (**C**).

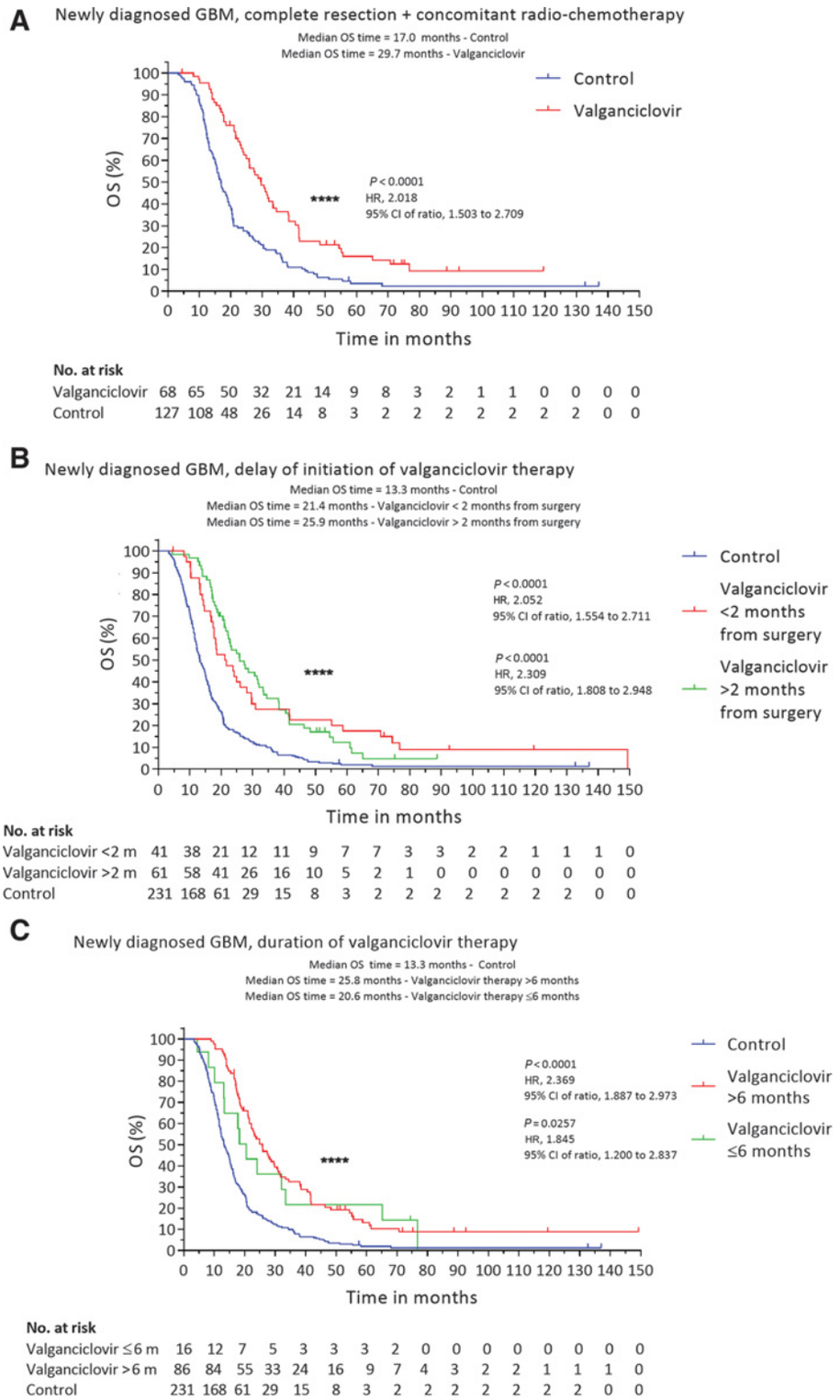

Figure 2.

Kaplan–Meier estimates of OS in patients with newly diagnosed primary glioblastoma treated with valganciclovir in whom the timing or duration of treatment differed. **A,** Estimated median OS of 68 patients with newly diagnosed primary glioblastoma who received both optimal treatment (complete resection and concomitant radiotherapy + chemotherapy) and valganciclovir therapy and for 127 contemporary controls with glioblastoma who received optimal treatment only. **B,** Estimated median OS of 41 patients who started valganciclovir within 2 months after diagnosis, 61 patients who started valganciclovir more than 2 months after diagnosis, and 231 contemporary controls. **C,** Estimated median OS of 16 patients who received valganciclovir for up to 6 months after diagnosis, 86 patients who received valganciclovir for more than 6 months, and 231 contemporary controls.

different [HR = 2.238; 95% confidence interval (CI), 1.736–2.884 in vs. HR = 2.334; 95% CI, 1.813–3.003], implying that immortal time bias does not explain the increased survival in patients who received valganciclovir treatment.

Because older age was associated with shorter OS according to Spearman correlation analysis, we identified 50 years of age as an appropriate cutoff, as previously suggested by Thakkar and colleagues (35) and compared median OS in valganciclovir-treated

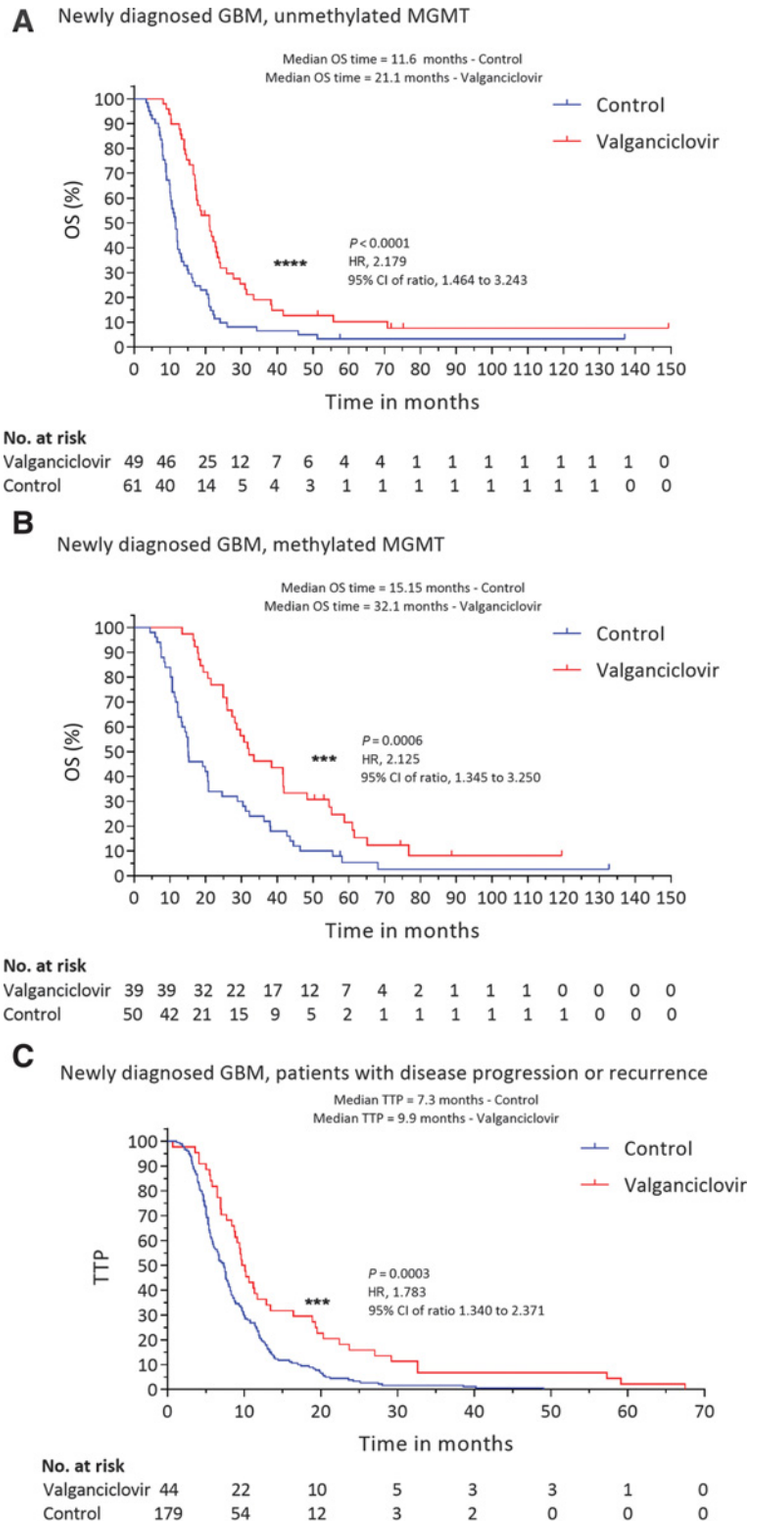

Figure 3.

Kaplan–Meier estimates of OS in patients with newly diagnosed primary glioblastoma according to *MGMT* status and estimated survival after glioblastoma diagnosis in patients with secondary glioblastoma treated with valganciclovir. **A,** Estimated median OS of 49 patients with newly diagnosed primary glioblastoma with an unmethylated *MGMT* promoter who received valganciclovir therapy and of 61 contemporary controls who received similar baseline therapy. **B,** Estimated median OS of 39 patients with newly diagnosed primary glioblastoma with a methylated *MGMT* promoter who received valganciclovir therapy and of 50 contemporary controls who received similar baseline therapy. **C,** Estimated median TTP of patients who developed disease progression or recurrence in valganciclovir treated group and in controls.

patients younger or older than 50, to respective controls. Patients younger than 50 receiving valganciclovir ($n = 23$, median age = 45) survived 27.4 months compared with 16.4 months in the younger controls with standard-of-care treatment ($n = 28$, median age = 44; $P = 0.0018$). The 2-year survival was 62% and 21.4%, respectively ($P = 0.0019$). Patients older than 50 years receiving valganciclovir ($n = 79$, median age = 61) survived 23.3 months compared with 13.1 months in the controls ($n = 203$, median

age = 65; $P < 0.0001$). The 2-year survival was 47.1% and 16.7%, respectively ($P < 0.0001$).

Finally, because a low KPS was associated with shorter median OS according to Spearman correlation analyses, we sorted the patients in two groups: those with KPS ≤ 80 and those with KPS > 80 and compared OS between valganciclovir-treated patients and controls. Patients with KPS ≤ 80 ($n = 40$) survived 18.4 months compared with 12.0 months in the controls with KPS ≤ 80 receiving standard-of-care treatment ($n = 141$, $P = 0.0001$). The 2-year survival rate was 28.2% and 9.9%, respectively ($P = 0.0001$). Patients with KPS > 80 receiving valganciclovir ($n = 58$) survived 29.6 months compared with 16.7 months in the controls ($n = 83$, $P < 0.0001$). The 2-year survival was 64.7% and 27.7%, respectively ($P < 0.0001$).

Progression of disease was more frequent among control patients (77.5% vs. 43.1%) and TTP was also shorter in the control group 7.3 (1.2–49 months) versus 9.9 (0.7–67.5 months), $P = 0.0003$ (**Fig. 3C**). Patients in the control group who developed disease progression or recurrent disease had a median age of 62 at diagnosis and 39 of 179 (21.8%) patients had undergone partial resection, which was comparable with valganciclovir-treated patients whose median age was 61 and 18.2% (8/44 patients) had a partial resection.

Discussion

This retrospective study shows that among patients with newly diagnosed glioblastoma who received standard care at a single institution, those treated with valganciclovir had higher 2-year survival rates and longer median OS than contemporary controls who did not receive valganciclovir. Valganciclovir had a positive effect on patient outcome regardless of the *MGMT* promoter gene status. Among patients whose tumors had an unmethylated *MGMT* promoter, a group that responds poorly to therapy and has limited treatment options, valganciclovir treatment increased both median OS (21.1 vs. 11.6 months in controls; $P < 0.0001$) and 2-year survival rate (34% vs. 9.8% in controls, $P < 0.0001$). Thus, valganciclovir treatment in addition to standard therapy may be a promising new therapeutic option for patients with glioblastoma, in particular for those with an unmethylated *MGMT* promoter. As expected, survival was greater after radical surgical resection than partial resection both in controls and valganciclovir-treated patients. However, valganciclovir improved survival regardless of whether it was given early or late after surgery. TTP was longer in patients treated with valganciclovir (9.9 months vs. 7.3 in controls, $P = 0.0003$). In our previous VIGAS study, we observed a nonsignificant trend for smaller tumor volume at 6 months (mean 3.3 cm^3 in valganciclovir vs. mean 13.75 cm^3 in placebo-treated patients; refs. 28, 29).

As in any retrospective study, patient selection criteria could affect the treatment results. In patients with glioblastoma, these factors include better performance status, radical surgery, a methylated *MGMT* promoter, *IDH* mutation status, and age. The percentage of patients with a methylated *MGMT* promoter did not differ between valganciclovir-treated patients (44%) and controls (46%) and was comparable with that in other studies (4). *IDH* mutation status was known for 88 patients and only 3 were positive. In patients with newly diagnosed glioblastomas, the extent of resection affected survival, which was further improved by valganciclovir treatment. Valganciclovir-treated patients were younger than controls (59 vs. 63 years) and more likely to be men (70.6% vs. 62.8%). However, in our study, patients younger than 50 years receiving valganciclovir survived significantly longer compared with younger controls, 27.4 months compared with 16.5 months ($P = 0.0018$), with a 2-year survival rate of 62% and 21.4%, respectively ($P = 0.0019$).

Median OS after complete resection was greater in valganciclovir-treated patients than in controls (28.7 vs. 15 months). After partial resection or biopsy, OS was also greater in valganciclovir-treated patients than in controls (18.4 vs. 10.65 months). The 2-year survival rate for all treated patients with newly diagnosed glioblastoma was 49.8%, which compares favorably to that in the EORTC-NCIC study (26.5%; ref. 2). Survival was highest in patients who received optimal therapy, radical resection and concomitant temozolomide and radiotherapy. In our cohort of valganciclovir-treated patients who received similar optimal therapy (median age, 56 years), OS was 29.7 months and the 2-year survival rate was 63.9%. In contemporary control patients who received optimal treatment in our study (median age, 60 years), median OS was 17.0 months and the 2-year survival rate was 27.6%. In the EORTC-NCIC study, OS after radical surgery and combined therapy was 18.8 months, but only 13.3 months for patients older than 50 years of age (2). Likewise, our controls over 50 years had a median OS of 13.1 months, but if they were treated with valganciclovir, their median OS was 23.3 months ($P < 0.0001$).

Another potential confounding factor is the time between diagnosis and the start of treatment. If this time delay is long, patients with a good survival rate even without additional treatment may have been selected, a phenomenon referred to as "immortal time bias" (33). We therefore used Cox regression with treatment status as a time-dependent covariate as described previously (34) to study this aspect in further depth. We found that immortal time bias did not seem to explain the high survival among patients with glioblastoma who received valganciclovir treatment as an add-on to standard therapy. In our study, the median time between diagnosis and the start of valganciclovir was 2.7 months (range, 5 days–16.9 months). Valganciclovir was beneficial regardless of whether treatment was started within 2 months or more than 2 months after surgery; an early start of therapy trended positive but this effect was not statistically significant. Furthermore, we found that the length of treatment positively correlated with survival, as was reported by us earlier (29). In addition, we also found that patients receiving less than 6 months of valganciclovir therapy (median time, 3.9 months) benefitted from valganciclovir therapy. Three weeks of valganciclovir therapy reduced 80% of virus levels in plasma of transplant patients (36), which implies that even short-time valganciclovir therapy may reduce CMV load in the patients, aid the immune system to take control over the virus, and reduce CMV's effects on the tumor cells with a potential impact on long-term survival. It is also possible that valganciclovir treatment protects patients from developing viremia, encephalopathy, and sudden decline in neurologic status with enhanced mortality risk within the first month after radiotherapy, as was reported among 27% elderly patients with glioblastoma (37).

Our observations suggest that valganciclovir increases survival rates in a wide time frame in newly diagnosed glioblastoma. In contrast, bevacuzimab (38), dendritic cell vaccination (39), *EGFR*–targeted immunotherapy (40), and integrin or mTOR inhibitors (41) do not improve the survival of patients with glioblastoma. Survival of these patients is currently only increased by the tumor-treating fields (Optune; 2-year survival 43% vs. 31% in controls; ref. 42), which is increasingly adopted as a standard treatment in several countries since 2019. We speculate that valganciclovir affects the pathogenesis of glioblastoma by suppressing CMV and consequently dampening the activation of oncomodulatory pathways caused by this virus in the tumors and potentially life-threatening CMV encephalopathy, although off-target effects cannot be excluded. In a mouse model, we

showed that valganciclovir inhibited the growth of CMV-positive medulloblastoma xenografts but did not affect the growth of CMV-negative xenografts (14). Thus, valganciclovir's effect on the tumor likely involves an antiviral effect.

Conclusions

Our study shows that valganciclovir improves survival in patients with newly diagnosed glioblastoma, even in those with an unmethylated *MGMT* promoter gene, and this drug appeared to suppress tumor growth and prolong survival regardless of when given after surgery. The data are very consistent with earlier reports on fewer patients and taken together, these observations suggest that valganciclovir provides a true biological effect on glioblastoma rather than representing a random event or a selection of patients. Given the consistent discouraging results of most recent studies of patients with glioblastoma, our findings should prompt new efforts to understand the role of CMV in the pathogenesis of glioblastoma. Although it will take time to dissect the biological effect of CMV in tumors, studies investigating anti-CMV therapies in patients with glioblastoma are warranted more urgently. Only a randomized trial can establish whether anti-CMV treatment improves the outcome of patients with glioblastoma. A randomized international multicenter trial evaluating anti-CMV therapy in 220 patients with glioblastoma was initiated on September 4, 2019, to further evaluate this promising treatment approach (ClinicalTrials.gov Identifier: NCT04116411) and is actively recruiting patients.

Disclosure of Potential Conflicts of Interest

C. Söderberg-Naucler holds an unlicensed patent on diagnostics and treatment of a variant CMV strain highly associated with cancer titled "Genetic variant of cytomegalovirus (CMV)." No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): G. Stragliotto, M.R. Pantalone, A. Rahbar, J. Bartek, C. Söderberg-Naucler
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