# Effects of valganciclovir as an add-on therapy in patients with cytomegalovirus-positive glioblastoma: A randomized, double-blind, hypothesis-generating study

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Cytomegalovirus is highly prevalent in glioblastomas. In 2006, we initiated a randomized, double-blind, placebo-controlled, hypothesis-generating study to examine the safety and potential efficacy of Valganciclovir as an add-on therapy for glioblastoma. Forty-two glioblastoma patients were randomized in double-blind fashion to receive Valganciclovir or placebo in addition to standard therapy for 6 months. Magnetic resonance images were obtained before and immediately and 3 and 6 months after surgery to evaluate treatment efficacy by measuring contrast enhancing tumor volume (primary end point). Survival data were analyzed for patients and controls in explorative analyses to aid the design of future randomized trials. Trends but no significant differences were observed in tumor volumes in Valganciclovir and placebo patients at 3 (3.58 *vs.* 7.44 cm$^3$, respectively, $p = 0.2881$) and 6 (3.31 *vs.* 13.75 cm$^3$, $p = 0.2120$) months. Median overall survival (OS) was similar in both groups (17.9 *vs.* 17.4 months, $p = 0.430$). Patients could take Valganciclovir for compassionate use after the study phase. Explorative analyses showed an OS of 24.1 months (95% CI, 17.4–40.3) in patients receiving >6 months of Valganciclovir (Val > 6M) versus 13.1 months (95% CI, 7.9–17.7, $p < 0.0001$) in patients receiving Valganciclovir for 0 or <6 months, and 13.7 months (95% CI, 6.9–17.3, $p = 0.0031$) in contemporary controls. OS at 4 years was 27.3% in Val>6M patients versus 5.9% in controls ($p = 0.0466$). Prolonged OS in Val>6M patients suggest that future randomized trials are warranted and should evaluate whether continuous antiviral treatment can improve outcome in glioblastoma patients.

Glioblastoma is the most aggressive malignant primary brain tumor and is not curable. With current standard therapy, median overall survival (OS) is <15 months.[1,2] The 2-year survival rate in patients treated with combined radiotherapy and temozolomide after maximal surgery may reach only 26.5%,[3] and 5-year survival is <10%.[1,4] New strategies

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**What's new?**

An exciting new connection has emerged between human cytomegalovirus infection and glioblastoma multiforme, the most deadly primary brain tumor with a mean overall survival of 15 months. The authors assessed the effect of antiviral Valganciclovir treatment in glioblastoma patients in a randomized hypothesis-generating study. Tumor growth was hampered and survival was longer in patients treated with Valganciclovir for >6 months. Because Valganciclovir is not curative and antiviral treatment during the study was only transient, the authors propose larger randomised trials to evaluate the effect of long-term Valganciclovir treatment in glioblastoma patients.

that improve the prognosis for these patients are urgently needed.

Human cytomegalovirus (HCMV) infection may contribute to the establishment and progression of certain tumors, including glioblastomas.[4–9] HCMV has been detected in 90–100% of glioblastomas, medulloblastomas, and cancers of the colon, breast, prostate, and salivary gland.[4,6–12] Although nearly 100% of glioblastomas express HCMV protein,[4,13,14] noncancerous cells near the tumor are consistently HCMV-negative. We recently found that survival >18 months in glioblastoma patients was associated with low-grade HCMV infection in their tumors at diagnosis.[13] Such patients also had a higher median OS after surgery than those with a high-grade HCMV infection (33 *vs.* 13 months, $p = 0.036$).[15] Moreover, anti-HCMV treatment reduced the growth of HCMV-positive medulloblastomas by 72% in an animal model.[9] This finding suggests that anti-HCMV therapy hampers tumor growth.

Because HCMV may be pathogenetic in glioblastoma and medulloblastoma and may be a target for therapy, it is imperative to establish whether treatment for HCMV improves the prognosis for patients with these tumors. Therefore, we initiated a hypothesis-generating study in adult patients with glioblastoma.

## Material and Methods

### Study design

The valganciclovir treatment in glioblastoma patients in Sweden (VIGAS) study was a randomized, double-blind, placebo-controlled trial to assess the safety and potential efficacy of Valganciclovir as an add-on therapy for 6 months in patients with glioblastoma. Forty-two patients with glioblastoma (WHO grade IV) were randomized 1:1 to receive Valganciclovir or placebo (two 450-mg tablets twice daily for 3 weeks and then one tablet twice daily until week 24) in addition to standard therapy for 6 months. The study was conceived as a hypothesis-generating, explorative, phase I/II trial; a power calculation of sample size was not performed. The study was registered at the Swedish medical agency (Eudra number 2006–002022-29) and at ClincalTrials.gov (Identifier NCT00400322), and approved by the Karolinska ethics committee (2006/755-31).

The study duration was 24 months. The primary end points were differences in tumor volume between treatment and placebo groups at 3 and 6 months after surgery, as assessed by neuroimaging. Secondary end points were progression-free survival and OS at 6, 12, 18, and 24 months. Valganciclovir (900 mg daily) could be prescribed to patients who wished to be treated with this drug after exclusion from the study because the treatment failed or after 6 months, when primary end point data had been collected. All patients who chose this option did so when the randomization codes were still sealed. The recursive partition analysis (RPA) scores for patients prescribed Valganciclovir are described in Supporting Information Table 1.

### Patients

Patients were eligible for the study if they were at least 18 years of age, had a glioblastoma (WHO grade IV), underwent surgical removal of at least 90% of the contrast-enhancing tumor mass, and had a histologically verified HCMV infection in the tumor. Patients were recruited at Karolinska University Hospital, Stockholm ($n = 38$) and Umeå University Hospital ($n = 4$). All patients provided informed consent. An RPA class was allocated for each patient (Table 1). Contemporary control patients ($n = 34$) for explorative analyses consisted of all glioblastoma patients who underwent surgery during 2006 at the Karolinska University Hospital except one, who was the first patient included in the VIGAS trial; all these patients also had more than 90% surgical removal of contrast-enhancing tumor and received a similar standard of care by the same surgeons and physicians at Karolinska University Hospital as the study group.

### Standard of care treatment

The standard of care for glioblastoma patients at Karolinska University Hospital after surgery is fractionated radiation therapy (60 Gy, 2 Gy fractions) with concomitant temozolomide. Patients over age 70 are seldom treated with radiation therapy at our institution. There is no standard of care for recurrent glioblastoma; therefore, therapy at relapse was at the discretion of the treating physician and included surgery, gamma knife, chemotherapy, or bevacizumab (Table 2), but no another experimental treatment at recurrence.

### HCMV analysis

The presence of HCMV proteins in tumor tissue was determined by immunohistochemistry for HCMV immediate-early antigen and late antigens. Biopsy samples were graded from 1–4+ according to the estimated percentage of tumor cells that were positive for HCMV.[13] HCMV RNA and DNA in blood cells were analyzed by PCR.[16] HCMV-specific IgG

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**Table 1.** Patient and disease characteristics at baseline (ITT)

| Characteristics | Placebo (n = 20) | Valganciclovir (n = 22) |
|---|---|---|
| Age (years) | | |
| Mean (range) | 59·0 (33–75) | 61·1 (33–78) |
| ≥50 years | 16 (80%) | 19 (86%) |
| Sex | | |
| No. of men | 11 (55%) | 18 (82%) |
| No. of women | 9 (45%) | 4 (18%) |
| No. of Caucasian | 19 (95%) | 21 (95%) |
| Grade of HCMV infection in tumour[1] | | |
| 1 | 2 (10%) | 1 (4.5%) |
| 2 | 1 (5%) | 0 (05) |
| 3 | 9 (45%) | 8 (36%) |
| 4 | 8 (40%) | 13 (59%) |
| Initial tumor volume (pre-op; $cm^3$) | | |
| Mean | 37.6 | 33.2 |
| Median (range) | 26 (54–148[2]) | 29 (3–81) |
| Inter-quartile range | 17–51 | 17–46 |
| RPA (regression partition analysis) grade (%) | | |
| IV | 5 (25%) | 4 (18%) |
| V | 13 (65%) | 13 (59%) |
| VI | 2 (10%) | 5 (23%) |
| Tumor localization | | |
| Temporal | 10 (50%) | 12 (55%) |
| Frontal | 6 (30%) | 6 (27%) |
| Others (parietal/occipital) | 4 (20%) | 4 (18%) |

[1]Determined by immunohistochemistry for HCMV immediate-early antigen.
[2]If one outlier is excluded, the range would be = 5·4–71.

antibodies in serum were analyzed with a commercial ELISA kit (Dade Behring, Marburg, Germany).

**Analysis of MGMT methylation**
Total DNA was extracted from paraffin-embedded tissue sections obtained from all patients with a QIAamp DNA FFPE tissue kit (Qiagen). Extracted DNA was bisulfite converted with the EpiTect Bisulfite kit (Qiagen) and used for Pyro sequencing, as recommended by the manufacturer (MGMT Pyro kit, Qiagen). Samples with >25% methylation in the MGMT promoter were considered positive.

**Analyses of plasma VEGF levels**
Plasma samples obtained before Valganciclovir treatment at $T = 0$ ($n = 41$) and at $T = 6$ months ($n = 29$) were analyzed for VEGF levels in plasma with a kit from R&D Systems.

**Efficacy and safety assessments**
The contrast-enhancing tumor volume was calculated as $V = 4/3$ л a/2·b/2·c/2, where $a$ is the longest diameter, $b$ and $c$ are the

**Table 2.** Baseline treatment and treatment at recurrence

| Base line treatment | Placebo (n = 20); No. (%) | Valganciclovir (n = 22); No. (%) |
|---|---|---|
| Extent of resection | | |
| 100% | 7 (35%) | 15 (68%) |
| ≥95% to <100% | 12 (60%) | 5 (23%) |
| ≥90% to <95% | 1 (5%) | 2 (9%) |
| <90% | 0 (0%) | 0 (0%) |
| Radiotherapy | 16 (82%) | 18 (82%) |
| Chemotherapy (temozolomide) | 17 (85%) | 18 (82%) |
| Concomitant (radiotherapy and chemotherapy) | 13 (65%) | 15 (68%) |
| Steroids <14 days (excluding perioperative use) | 18 (90%) | 21 (95%) |
| Treatment at recurrence | | |
| Chemotherapy (CCNU) | 9 (45%) | 11 (50%) |
| Bevacizumab | 4 (20%) | 6 (27%) |
| Re-operation | 4 (20%) | 0 (0%) |
| Gamma-knife treatment | 6 (30%) | 6 (27%) |

diameters perpendicular to this along the $x$, $y$ and $z$ axes ($V \approx$ a·b·c/2); in most patients, these measurements were obtained by MRI.[17] Progressive disease was defined as any new measurable lesion or a ≥40% increase in volume compared with baseline tumor volume calculated after the initial surgery or surgery for tumor growth before week 24. Changes that did not meet the criteria for progressive disease was considered as stable disease. Safety was evaluated descriptively by summarizing adverse events and serious adverse events, physical exams, and laboratory data.

**Analysis populations**
The database was locked and the study code was broken for primary end point analyses when all patients had passed 6 months of follow up. A number of patients had been prescribed Valganciclovir before the study code was broken. Survival data were analyzed at 12 and 18 months and at 24 months and was therefore influenced by this fact. The intent-to-treat (ITT) population consisted of all patients who were randomized and received at least one administration of study medication. In explorative analyses after 24 months, we analyzed patients for actual Valganciclovir intake and performed subgroup analyses of patients randomized to or treated with Valganciclovir for ≥6 or <6 months compared with controls. Patients who were randomized to placebo or Valganciclovir and after exclusion from the study prescribed Valganciclovir (at failure or at 6 months in the randomized protocol if they completed the study), were also analyzed separately.

**Statistical analysis**
The primary end point analyses at 3 and 6 months investigated tumor size compared to baseline. OS data are presented

in graphs as Kaplan–Meier estimates, calculated from the time of informed consent. Secondary end points measured as the time to an event were summarized with life-table methods and Kaplan–Meier curves and were tested for significance with the two-sided log-rank test. For patients who did not experience a particular event, the censoring date was the last known assessment date for the event, plus 1 day. All analyses were according to treatment group. Continuous variables were analyzed with descriptive statistics (*e.g.*, mean, median, standard deviation). Categorical variables were analyzed by using frequency tables with numbers and percentages of patients. All statistical hypotheses were two-sided, with a significance level of 5%. Events with a reasonably high frequency were analyzed with a chi-square test without continuity correction. A nonparametric one-way ANOVA test was used for VEGF analyses; A Cox regression analysis was used to investigate the influence of MGMT promoter methylation and tumor resection grade independently in comparison to overall survival. $p < 0.05$ was considered significant.

## Results
### Patient characteristics
Between December 20, 2006, and June 3, 2008, 42 patients were enrolled in the VIGAS study; 22 were randomized to Valganciclovir and 20 to placebo during the 24-week blinded phase of the study (6 months) (Fig. 1). All patients performed written informed consent for the VIGAS study, but in one case, the patient who agreed to participate in the study had frontal apraxia and could not write so written consent was obtained from a proxy. Patient demographics are summarized in Table 1. In the placebo group, the mean age at inclusion was 59 years, and 55% of the patients were men. In the Valganciclovir group, the mean age was 61.1 years, and 82% of the patients were men. The proportions of patients in RPA classes IV–V were similar in both groups; however, 23% of Valganciclovir patients, but only 10% of placebo patients, were classified as RPA class VI (Table 1). The treatment regimen was also similar in both groups (Table 2). Eight patients in the placebo group and four in the Valganciclovir group had tumor recurrence, infection, or side effects and were unable to complete the study. Notably, although all patients had HCMV-protein-positive tumor cells in the brain and were positive for HCMV DNA and 63% were positive for HCMV-RNA in blood cells, only 71% had HCMV-specific IgG antibodies in the serum (data not shown).

### Safety
In glioblastoma patients receiving temozolomide and radiation therapy, Valganciclovir was safe and well tolerated. A drug safety monitoring board performed three safety evaluations during the blinded study phase. Treatment-related adverse events were generally mild to moderate. The most common hematological events were thrombocytopenia and
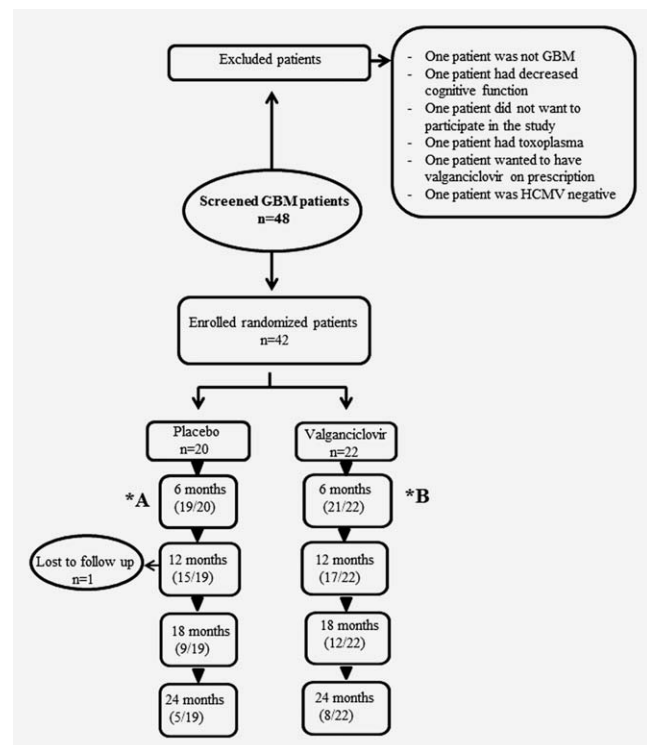


**Figure 1.** Consort diagram. The study flow indicates number of patients who were screened, randomized to placebo or Valganciclovir, and alive at the indicated time points. (*A*) Number of patients randomized to placebo who were prescribed Valganciclovir at failure or at study completion at 6 months. (*B*) Number of patients randomized to Valganciclovir who were prescribed Valganciclovir at failure or at study completion at 6 months. All patients who chose this option did so when the randomization codes were still sealed.

leucopenia. The most common nonhematologic events were nausea and diarrhea (Supporting Information Table 2).

### Efficacy
At 3 months, the mean increase in tumor volume calculated from baseline was 3.58 cm$^3$ in the Valganciclovir group and 7.44 cm$^3$ in the placebo group ($p = 0.2881$ *vs.* baseline). At 6 months, the mean increases from base line were 3.31 and 13.75 cm$^3$, respectively ($p = 0.2120$) (Fig. 2*a*, ITT populations), which suggest a trend for hampered tumor growth in Valganciclovir-treated patients. Secondary end point analyses included progression-free survival at 3 and 6 months and overall survival (up to 24 months). At 3 and 6 months, similar proportions of patients in the two groups were alive and had stable disease. However, at 12, 18, and 24 months, more patients were alive with stable disease in the Valganciclovir group (Supporting Information Table 3), but we did not observe a statistically significant difference in progression free survival between patients randomized to placebo or valganciclovir (Fig. 2*b*, ITT populations). The PFS estimates were 5.6 months (95% CI, 3.0–10.0 months) for Valganciclovir treated patients and 5.5 months (95% CI, 2.9–6.7 months, $p = 0.30$
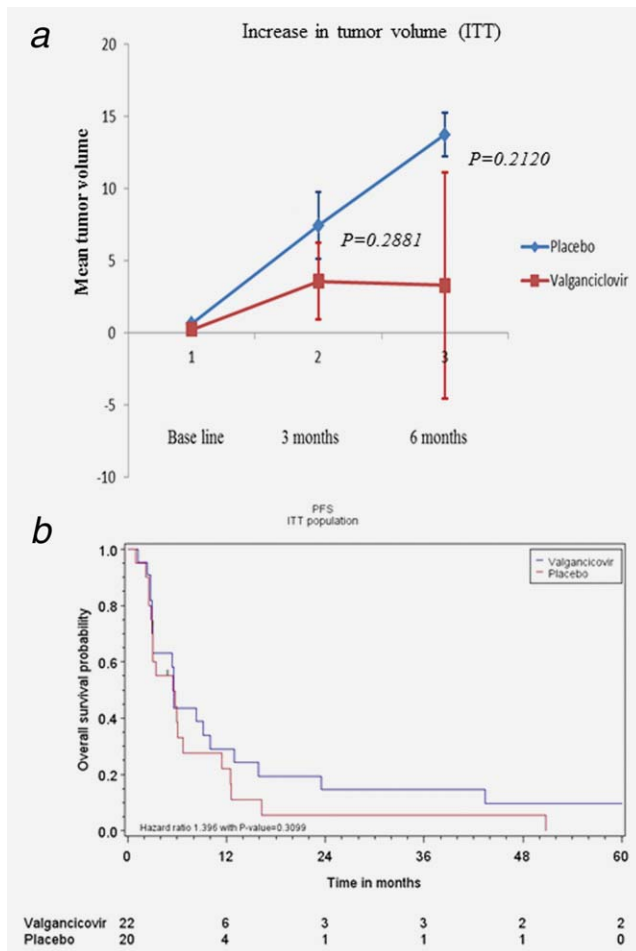
Figure 2. (*a*) Mean tumor volume calculated from MRI measurements at baseline and after 3 and 6 months of treatment and data represent increased tumor volumes. Values are baseline ± SEM). (*b*) Kaplan–Meier estimates of the progression free survival according to initial allocation to Valganciclovir or placebo (ITT population), calculated from day of inclusion. As patients could be prescribed Valganciclovir for compassionate use after 6 months, this fact will influence the interpretation of these data after this time point.

by log-rank test) for placebo treated patients. Kaplan–Meier analyses showed no difference in OS between the Valganciclovir and placebo groups (ITT populations, Fig. 3*a*). The median survival estimates were 17.9 months (95% CI, 12.7–23.7 months) for the Valganciclovir group versus 17.4 months (95% CI, 11.7–22.5 months) for the placebo group ($p = 0.4302$ by log-rank test, Fig. 3*a*), which is somewhat higher than expected in a glioblastoma patient cohort. Radical surgery did not affect survival (HR = 0.58, CI: 0.24–1.46, $p = 0.346$), regardless of treatment group. The median estimated OS also tended to be longer for the VIGAS patient cohort (17.7 months; 95% CI, 14.3–21.2) than for contemporary control patients (13.7 months; 95% CI, 6.7–17.3) ($p = 0.0261$ Wilcoxon, $p = 0.0934$ log-rank test, Fig. 3*b*).

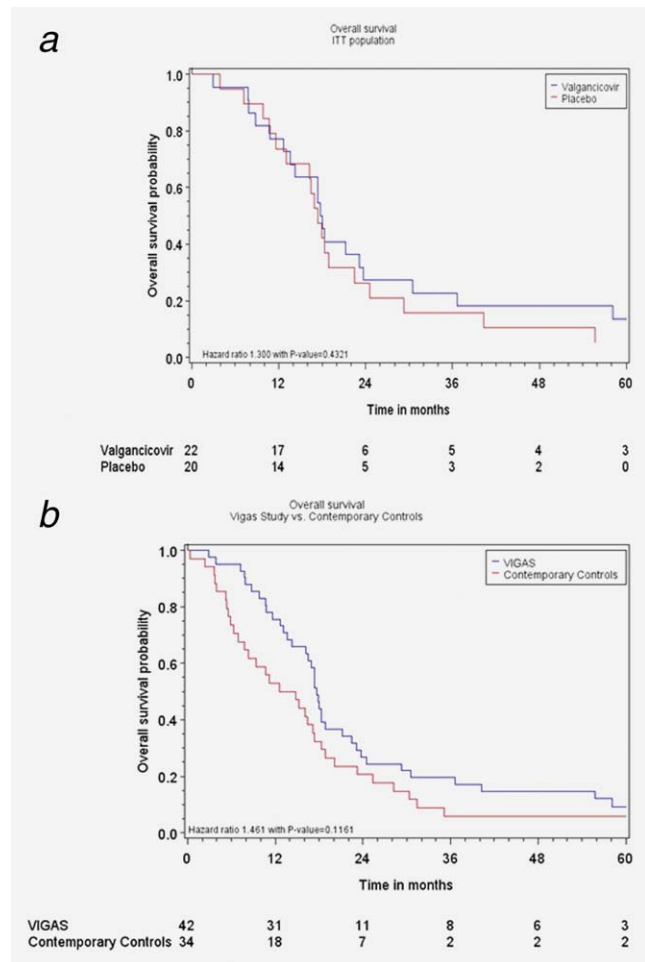Previous studies have suggested that methylation of MGMT is a positive prognostic factor for outcome of



Figure 3. (*a*) Kaplan–Meier estimates of overall survival according to initial allocation to Valganciclovir or placebo (ITT population), calculated from day of inclusion. (*b*) Kaplan–Meier estimates of OS for the VIGAS cohort and contemporary controls. As patients could be prescribed Valganciclovir for compassionate used after 6 months, this fact will influence the interpretation of these data after this time point.

glioblastoma patients treated with temozolomide.[1,2,18] We therefore analyzed 37 VIGAS patients for methylation of the MGMT promoter (DNA from five patients was not of sufficient quality for pyrosequencing). Eight (22%) had a methylated MGMT promoter, and 29 (78%) did not (data not shown). Among VIGAS patients, MGMT methylation did not affect survival (HR 0.60, CI 0.24–1.46, $p = 0.260$), independent of treatment with placebo or Valganciclovir.

**Explorative analyses of actual Valganciclovir intake demonstrate improved survival for patients receiving at least 6 months of Valganciclovir treatment**

VIGAS was a hypothesis-generating study aimed to aid in the design of a well-powered future clinical trial. Because patients could elect to receive Valganciclovir at treatment failure or after 6 months, it is possible that antiviral treatment could affect tumor progression regardless of when

treatment was given. When the study code was broken, 12 of 20 placebo patients had been prescribed Valganciclovir after the blinded phase, and seven of them took Valganciclovir for >6 months. Seven patients who were randomized to Valganciclovir elected to receive Valganciclovir at treatment failure or after the 6 months study phase. The RPA and Karnofsky performance scores (KPS) for all patients prescribed Valganciclovir >6 months are included in Supporting Information Table 1. Overall, the patients who opted for Valganciclovir treatment for compassionate use were doing well at prescription, with a stable or better KPS than at the time of inclusion.

We next analyzed whether antiviral treatment, prescribed postoperatively or later, was associated with improved outcome. None of the patients who received Valganciclovir treatment died within the first year after diagnosis, which is unusual. When analysing patients for actual Valganciclovir intake, we further observed that survival correlated with the duration of Valganciclovir treatment ($r = 0.815$, $p < 0.0001$, Fig. 4*a*). In contrast, survival did not correlate with the number of chemotherapy cycles ($r = 0.042$, $p = 0.8041$, Fig. 4*b*). The mean number of chemotherapy cycles for patients randomized to Valganciclovir was 7.8, and 8.7 for placebo patients (data not shown and Fig. 4*b*).

The estimated mean OS was longer in patients who received Valganciclovir for at least 6 months (24.1 months (95% CI, 17.4–40.3) than in patients receiving short-term or no Valganciclovir treatment (13.1 months; 95% CI, 7.9–17.7, $p < 0.0001$, Fig. 5*a*) or contemporary controls (13.7 months; 95% CI, 6.9–17.3, $p = 0.0031$, Fig. 5*b*). Among patients treated with Valganciclovir for at least 6 months, as many as 50% were alive at 24 months, 27.3% were alive at 4 years, and 13.6% at 5 years (Fig. 5*b*). Among contemporary controls, 20.6% were alive at 24 months and 5.9% at 4 and 5 years, respectively ($p = 0.0466$, 4 years, Fig. 5*b*). These survival rates compare well to those of glioblastoma patients with similar RPA and MGM methylation status at other centers. Eleven patients survived over 24 months and were doing well during the treatment of Valganciclovir; two of these had a methylated MGMT promoter (Supporting Information Table 4).

To determine whether survival differed in patients receiving early or later Valganciclovir treatment, we performed separate analyses of OS for patients randomized to placebo or Valganciclovir who had been prescribed Valganciclovir for at least 6 months. The number of patients in each of these groups was small; however, survival was improved in patients randomized to Valganciclovir (Fig. 5*c*) or placebo (Fig. 5*d*). Thus, the benefit of Valganciclovir is not limited only to patients who receive treatment upon diagnosis.

## Discussion

Although standard multimodality treatment for glioblastoma has improved survival, OS is still only 12–15 months and less than 10% of patients survive over 5 years.[1] Therefore new strategies must be explored. Current data suggest that
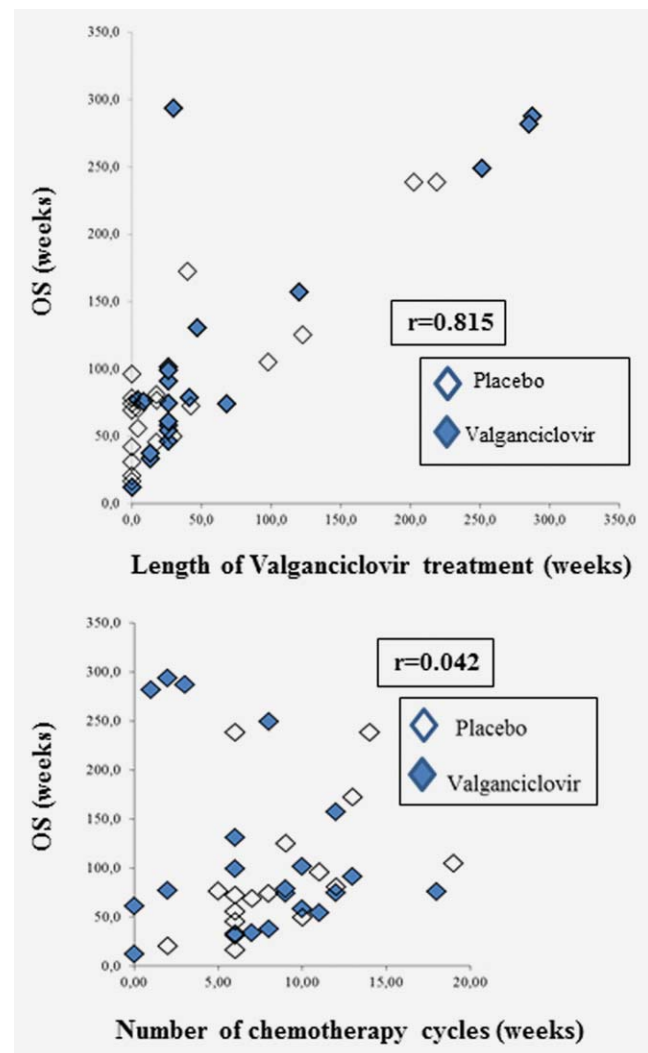


**Figure 4.** (*a*) Duration of Valganciclovir treatment (weeks) in VIGAS patients versus survival time (weeks). (*b*) Number of chemotherapy cycles VIGAS patients versus survival time (weeks). Open diamonds indicate patients randomized to placebo; closed diamonds, indicate patients randomized to Valganciclovir.

HCMV infection is highly prevalent, approaching 100% in glioblastoma.[4,13,14] The viral infection is confined to the tumor and can affect numerous tumor biological pathways and therefore is a potential therapeutic target for both medical intervention and immunotherapy. Indeed, we recently showed that Valganciclovir significantly reduces the growth of HCMV-positive medulloblastoma in a mouse model.[9] This finding supports the concept of using antiviral therapy to prevent the growth of HCMV-positive tumors.[9] Two recently published studies demonstrated the feasibility of generating HCMV-specific T cells for adoptive therapy in glioblastoma patients and the ability of these cells to kill glioblastoma cells. Thus, HCMV is also a potential target for immunotherapy in glioblastoma patients.[19,20]

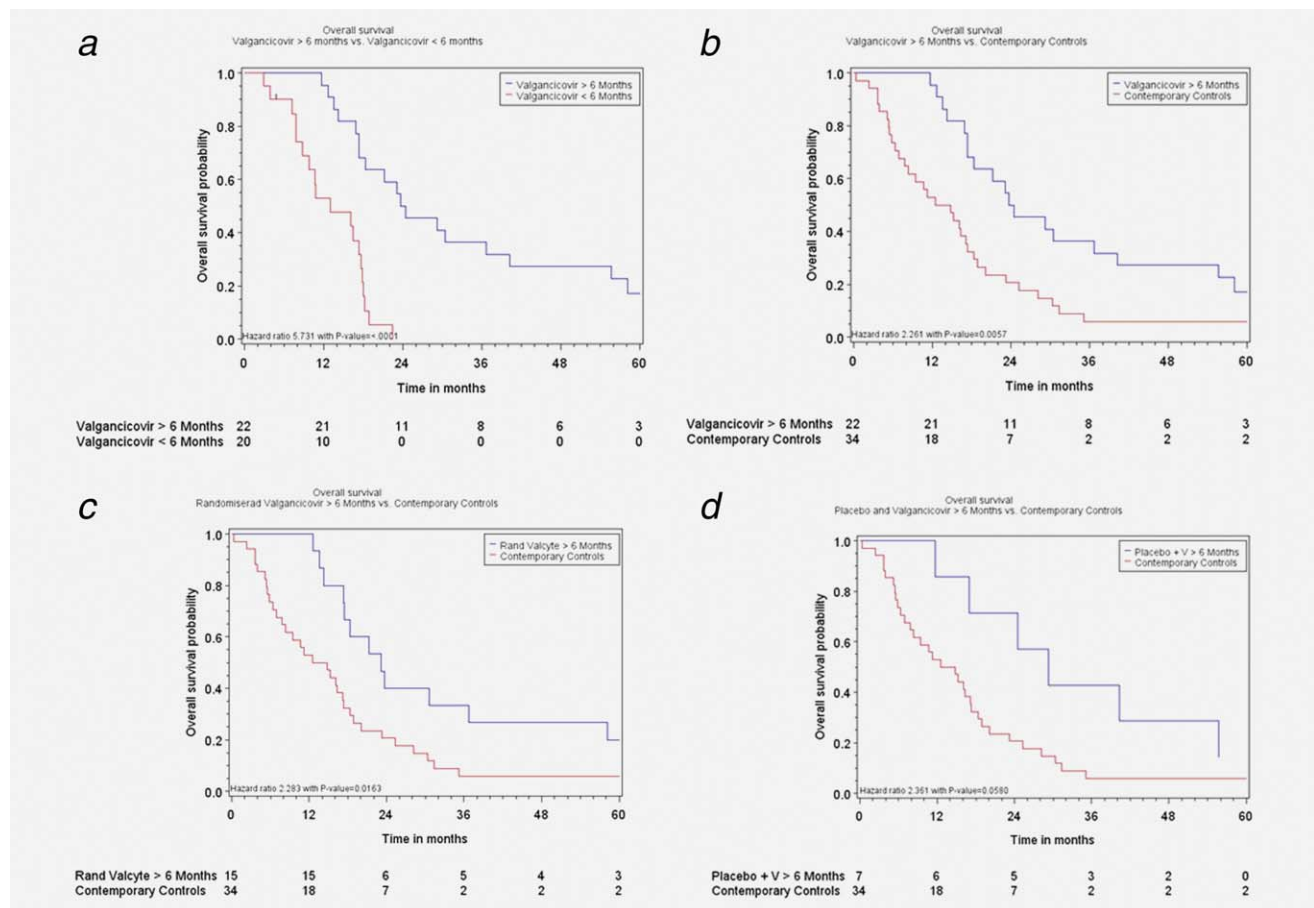The VIGAS study is the first clinical trial of an anti-HCMV therapy (Valganciclovir) in patients with cancer, and

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**Figure 5.** (*a*) Kaplan–Meier estimates of OS for VIGAS patients treated with Valganciclovir for at least 6 months versus <6 months. (*b*) Kaplan–Meier estimates of OS for patients treated with Valganciclovir for at least 6 months versus contemporary controls. (*c*) Kaplan–Meier estimates of OS for patients randomized to Valganciclovir and treated with Valganciclovir > 6 months versus contemporary controls. (*d*) Kaplan–Meier estimates of OS for patients randomized to Placebo and treated with Valganciclovir > 6 months for compassionate use versus contemporary controls.

was conceived as an explorative phase I/II study to assess safety issues, search for potential efficacy of Valganciclovir in glioblastoma patients, and to aid in the design of future randomized well-powered trials.

The add-on treatment of Valganciclovir was safe and well tolerated in patients receiving both temozolomide and radiation therapy. The VIGAS study failed in its primary end point—demonstrating trends but no statistically significant differences in tumor volumes at 3 and 6 months for Valganciclovir- versus placebo-treated patients. This may be caused by the low number of patients in this study. However, the explorative analyses of actual Valganciclovir intake support the hypothesis that prolonged Valganciclovir treatment may be beneficial in glioblastoma patients. The decision to prescribe this drug was taken during the blinded phase (and was not influenced by the clinical status of the patients or the caring physician), and 12 of 20 placebo patients had taken Valganciclovir on prescription (some over 5 years). Therefore, we investigated whether actual Valganciclovir intake influenced survival. Interestingly, no patient who received

>6 months of Valganciclovir treatment died within the first year after diagnosis; this is unusual. Moreover, prolonged survival correlated with longer Valganciclovir intake (Fig. 4*a*) but not with the number of chemotherapy cycles (Fig. 4*b*). We observed that long-term survivors had received relatively few chemotherapy cycles, which indicates no residual or stable disease. Most interestingly, patients taking Valganciclovir for at least 6 months had an OS of 24.1 months (mean age 59.6 years, range 33–75) (Fig. 5), which compares favorably with most published survival rates for patients in the same age group with similar RPA who underwent radical surgery and chemoradiotherapy. The increased OS among Valganciclovir-treated patients was not due to a selection of patients with methylated MGMT, as only two of 11 (18.2%) patients who survived over 24 months had a methylated MGMT promoter (Supporting Information Table 4).

In the phase III EORTC-NCIC study, radiotherapy and temozolomide improved survival compared to radiotherapy alone; the median OS was 18.8 months with combined therapy and complete resection in all patients, but only

13.6 months for patients above 50 years.[1] VIGAS patients who did not take Valganciclovir and contemporary control patients at our own hospital had comparable OS (13.1 and 13.7 months, respectively). Among patients who received combined RT and temozolomide therapy in the EORTC-NCIC study, 27.2% were alive at 24 months, 12.1% were alive at 4 years, and 9.8% at 5 years.[1] Among VIGAS patients, 50% of those who received Valganciclovir for at least 6 months were alive at 24 months and 27.3% were alive at 4 years, 13.6% at 5 years. These rates are higher than those in other studies of newly diagnosed glioblastoma patients of the same age and similar MGMT methylation status. Other studies showed OS of 14.6 months for patients receiving serial temozolomide cycles,[3] 13.7 or 18 months for those receiving carmustine polymers (Gliadel wafers), and 19.6 months for those receiving bevacizumab, radiation, and temozolomide (a phase II study).[21–23]

A robust randomized trial is needed to determine whether long-term Valganciclovir treatment for glioblastoma improves survival. Its potential effect could be mediated by different modes of action, for example by suppressing HCMV activity or by acting as a nucleoside analogue with chemotherapeutic effect. Valganciclovir is phosphorylated mainly by the HCMV-encoded protein UL97, but also to some extent by cellular kinases; the resultant ganciclovir triphosphate form acts mainly by competitively inhibiting HCMV DNA polymerase and thus HCMV replication.[24] Because Valganciclovir is likely activated preferentially in HCMV-infected cells, the observed potential survival benefit of taking Valganciclovir for >6 months could be explained by suppression of virus-mediated, tumor-promoting mechanisms specifically in infected cells. This possibility is supported by our previous finding that Valganciclovir inhibits the growth of HCMV-positive medulloblastoma but does not prevent the growth of HCMV-negative tumors in an animal model or affect the growth of normal cells.[9] Thus, Valganciclovir mainly targets HCMV-positive tumor cells and hence may be virus specific.

Valganciclovir can suppress HCMV replication but cannot eradicate the virus from its host or from infected tumor cells. Therefore, it is plausible that only long-term treatment maintains this suppressive effect on the chronic HCMV infection in glioblastoma and prevents tumor recurrence and progression. Short-term treatment would only affect tumor growth during the limited treatment phase, after which HCMV replication may be induced along with virus-mediated oncomodulatory effects. It is also possible that the failure of Valganciclovir in some patients affected our results. The UL97 gene is the most frequently identified mutant gene providing ganciclovir resistance in HCMV strains.[25] Sequence variations exist in the UL97 gene in the HCMV genome sequenced in glioblastoma tissue samples.[14] However, it is not known whether these UL97 mutations observed in glioblastoma patients provide resistance against ganciclovir.

Theoretically, another potential benefit of anti-HCMV treatment may be mediated through an HCMV-induced decrease in the production of vascular endothelial growth factor (VEGF); the VEGF inhibitor bevacizumab has a similar mode of action.[26] The HCMV protein US28 upregulates VEGF production,[27] which may be relevant to the progression of HCMV-positive glioblastomas. We therefore measured VEGF levels in plasma samples from 41 VIGAS patients at study initiation and 29 patients at 6 months. VEGF levels decreased sharply between $T = 0$ and $T = 6$ months in patients randomized to placebo ($p = 0.0008$) or Valganciclovir ($p = 0.0024$) treatment, but there was no difference between these groups ($p = 0.2926$). Thus, the higher levels of VEGF in glioblastoma patients at diagnosis were associated with the tumor burden and decreased in patients after successful treatment with surgery and chemoradiotherapy; this was not influenced by Valganciclovir treatment.

We cannot exclude the possibility that Valganciclovir acts on other viruses potentially present in glioblastoma. Valganciclovir has antiviral activity not only against HCMV, but also against herpes simplex virus (HSV) 1 and HSV-2, human herpesvirus (HHV) 6, HHV-7 and HHV-8, Epstein-Barr-virus (EBV), varicella-zoster-virus, and hepatitis B virus.[28] We previously examined glioblastoma tissue samples for HSV-1, HSV-2, EBV, and HHV-6 and only observed very few (single cells positive) for EBV and HHV-6 in <10% of samples examined, and none were positive for HSV-1 or HSV-2.[15] These findings suggest that the effect of valganciclovir in glioblastoma is HCMV specific. Previously, we showed that Valganciclovir inhibits the growth of HCMV-positive medulloblastomas but not HCMV-negative tumors—providing further evidence that the antitumor effect of Valganciclovir is HCMV specific.[9]

Whether HCMV truly plays a role in tumor initiation and has oncomodulatory functions that enhance progression or is merely an epiphenomenon is currently under debate.[5] Through its effects on cellular and immunological functions, HCMV has oncogenic, oncomodulatory, and immunomodulatory effects that facilitate tumor development. The HCMV protein US28 is a chemokine receptor homologue that confers oncogenic properties in mouse models. Expression of US28 in cells increases expression of COX-2, production of VEGF, phosphorylation of STAT3 and production of interleukin-6 and enhances cellular migration and tumor formation.[27,29–32] Transgenic mice with US28 expression targeted to the intestinal epithelium develop adenomas and adenocarcinomas.[33] Other HCMV proteins block cellular differentiation, control cell cycle regulation (through interactions with p53, Rb and p21), block apoptosis, and induce chromosomal instability, oncogene expression and mutations, angiogenesis, migration and invasiveness (as reviewed in Refs. 34 and [35]). Infection of murine salivary gland explants results in dysplasia and activation of the COX-2/EGFR, ERK, and amphiregulin pathways linked to tumor development.[36] Mouse CMV infection of Trp53 heterozygous new-born mice results in rhabdomyosarcoma containing HCMV DNA, RNA and proteins.[37] The chronic and incurable nature of HCMV infection

would imply that long-term Valganciclovir treatment may be required for a clinical effect on outcome. This hypothesis is supported by our data from the explorative analyses of an unexpectedly high OS of 24.2 months in patients only on long-term Valganciclovir treatment.

### Limitations of the study

In retrospect, the design of the VIGAS study in 2005, which aimed to compare tumor volume differences at 3 and 6 months, does not match current knowledge of HCMV biology in tumors or the notion that survival is the optimal primary end point of clinical trials of treatments for glioblastoma. Therefore, we conclude that the VIGAS study was not designed optimally to evaluate the potential efficacy of Valganciclovir in glioblastoma patients; it did not have sufficient power to determine outcome differences in this small population of patients. However, despite its limitations—including a small sample size, lack of stratification for significant prognostic factors and allowance of active drug intake among all patients in the study—VIGAS is the first study of this new concept and it supports the feasibility and safety of further clinical trials of Valganciclovir for glioblastoma. Furthermore, regardless of these limitations, it is very rare that glioblastoma patients survive 4 years after their diagnosis; six of 22 (27.3%) VIGAS patients receiving at least 6 months of Valganciclovir treatment were alive and well at 4 years after their diagnosis. Their mean age at diagnosis was 63.5 years (4 men and 2 women, range 55–75 years). After their surgery, five of the six received both radiotherapy and temozolomide, one elderly patient received only chemotherapy after surgery and two had a methylated MGMT promoter. It is unlikely that any biased selection of these patients could influence this high survival rate and that the data would only be anecdotal. The study was continuously monitored by Karolinska Trial Alliance, and the eCRF data were independently monitored by Quintiles at study closure. In the remonitoring phase, Quintiles discovered one error in a tumor volume calculation (1.1 instead of 1.3 mm$^3$). In addition, we discovered that a baseline tumor volume for one patient was measured at 3 weeks instead of immediately postoperatively. Neither of these errors altered the conclusions. The corrected numbers are reported here. Thus, long-term Valganciclovir treatment may curb the rapid growth of glioblastoma, and the potential efficacy of prolonged treatment with Valganciclovir in glioblastoma patients merits further investigation in a robust, well-powered clinical trial.

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