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Use of Cox regression with treatment status as a time-dependent covariate to re-analyze survival benefit excludes immortal time bias effect in patients with glioblastoma who received prolonged adjuvant treatment with valganciclovir

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In a letter to the Editor, Liu and Hu[1] commented on our studies demonstrating a potential survival benefit in glioblastoma patients who received prolonged treatment with valganciclovir for cytomegalovirus (CMV) infection in their tumor[2,3] as add on to their standard oncology treatment. Liu and Hu raised the question of whether so-called immortal-time bias could explain the highly improved survival observed in treated patients. Indeed, with a retrospective design and a definition of exposure that requires a minimum time of exposure, there is a risk of immortal time bias, since those qualifying for exposure by definition are given a certain degree of immortal time.[4] Hence, Liu and Hu's call for re-analysis of our data using Cox regression with treatment status as a time-dependent covariate is justified. Here we present the outcome of that re-analysis.

First, we reanalyzed our data by splitting the follow-up time for the treated group into treated and untreated follow-up time. A Cox regression model allowing for delayed entry in the treatment group yielded an overall hazard ratio (HR) of 0.41 (95% confidence interval [CI]: 0.28–0.61) for treated versus untreated patients. Figure 1, to be compared with graph A in the original letter,[2] shows that the effect of immortal time introduced in the previous analysis is relatively small (note that for reasons of comparison the HR of untreated versus treated patients is presented in Fig. 1). The main reason for the limited effect of immortal time bias is that treatment in most cases started relatively soon after operation (median, 53 days; lower quartile, 21 days; upper quartile, 154 days) and that relatively few deaths occurred in controls or treated patients within the range of time before treatment was initiated. When estimating the HR for patients starting treatment before and after 53 days after operation (*i.e.*, median) the HRs were 0.42 (95% CI: 0.26–0.70) and 0.40 (95% CI: 0.23–0.69), respectively. Thus, timing of treatment start did not substantially modify the HR, further limiting the potential effect of immortal time bias.

When analyzing the treatment association as function of time since treatment start, divided into half year intervals we observed that the treatment association seems most pronounced within the first 12 months and thereafter diminishes with time since treatment start (Supporting Information in Table 1). The potential for immortal-time bias is greater in the subgroup of patients who received at least 6 months of valganciclovir treatment. Calculating the rate ratio can serve as an illustration of potential immortal-time bias.[5,6] Supporting Information in Table 2 gives estimated rate ratios before and after adjustment for potential immortal time of subjects treated for at least 6 months and for those treated for less than 6 months. Both rate ratios are shifted toward a lesser treatment effect. The rate ratio for the 10 patients treated for less than 6 months was greater than unity, indicating a worse outcome than in untreated controls. The shifts in rate ratio for those treated for at least 6 months is less dramatic and remains well below unity, even though immortal time

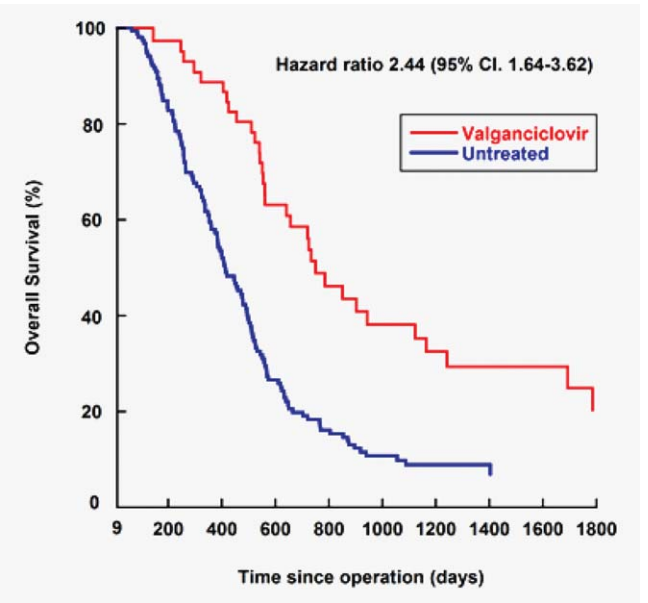

Figure 1. Survival curves with respect to treatment estimated from a treatment-stratified Cox regression model allowing for delayed entry in the treatment group. (Note that the *x*-axis starts at day 9 since the first patient receives treatment on that day and it will therefore constitute the effective origin with respect to the treatment effect.)

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constituted 28.8% of all person-days at risk. The median follow-up time was 406 days (minimum 63 days, maximum 2,075 days) for the controls, 748 days (283, 2,179) for those treated for more than 6 months and 365 days (143, 721) for those treated for less than 6 months.

Further analysis by Cox regression, with treatment status as a time-dependent covariate, showed that the HR ratio comparing patients treated for more than 6 months with untreated controls is 0.441 (95% CI: 0.283–0.687; $p < 0.0003$ by Type 3 Wald chi-square test) and 1.351 (95% CI: 0.659–2.768, $p = 0.4118$) for patients treated less than 6 months. Thus, although the design of our retrospective study potentially could suffer from immortal-time bias when analyzing patients with longer treatment, re-analysis of the data shows that while part of the treatment effect seen in the original analysis for patients treated more than 6 months with valganciclovir may be attributed to this bias, the statistically significant effect remains after adjustment for this effect. The reasons are that the accrued immortal time was small since start of treatment in most cases is relatively close after operation and that the median follow-up time was longer in relation to immortal time. Therefore, we conclude that immortal time bias does not explain the high survival among glioblastoma patients who received valganciclovir treatment as an add-on to standard therapy.

We have so far examined over 300 glioblastomas for CMV; only one was negative.[2] Other groups using different methods to detect the virus also report a similar high prevalence of CMV in glioblastoma.[7–9] Experimental and animal data suggest that treatment for CMV prevents the growth of CMV-positive tumors *in vitro* and *in vivo*,[10] and our retrospective analyses of valganciclovir-treated glioblastoma patients now indicate improved survival, far better than previously reported.[2]

Since valganciclovir prevents CMV replication but cannot eliminate the viral infection, patients who stop therapy would no longer be expected to benefit from the potential suppressive effects of the antiviral treatment. We observed that patients treated with valganciclovir for 6 months had a longer time to recurrence and a lower death rate. Patients who were maintained on continuous valganciclovir treatment further extended their survival. Strikingly, among glioblastoma patients receiving continuous valganciclovir treatment, the first death occurred at 283 days, which is rarely observed in any cohort of glioblastoma patients, who have a very poor prognosis despite optimal aggressive surgical resection, chemotherapy and radiation therapy.

Although this strategy certainly may not cure glioblastoma, it is firmly based on biological evidence that CMV promotes the disease and that decreasing replication of the virus prevents tumor growth. Thus, using CMV-targeted therapies for glioblastoma is a first step toward a therapy that improves survival of patients suffering from this dreadful disease. A randomized trial is the only way to prove or dismiss this hypothesis and should be performed as soon as possible so as to not withhold from patients a potentially effective therapy for glioblastoma.

Yours sincerely,
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References

1. Liu C-J, Hu Y-W. Immortal time bias in retrospective analysis: is there a survival benefit in patients with Glioblastoma receiving prolonged treatment of Valganciclovir. *Int J Cancer* 2013. doi: 10.1002/ijc.28664.
2. Soderberg-Naucler C, Rahbar A, Stragliotto G. Survival in patients with glioblastoma receiving valganciclovir. *N Eng J Med* 2013;369:985–6.
3. Stragliotto G, Rahbar A, Solberg NW, et al. Effects of valganciclovir as an add-on therapy in patients with cytomegalovirus-positive glioblastoma: a randomized, double-blind, hypothesis-generating study. *Int J Cancer* 2013;133:1204–13.
4. Ho AM, Dion PW, Ng CS, et al. Understanding immortal time bias in observational cohort studies. *Anaesthesia* 2013;68:126–30.
5. Suissa S. Immortal time bias in observational studies of drug effects. *Pharmacoepidemiol Drug Saf* 2007;16:241–9.
6. Levesque LE, Hanley JA, Kezouh A, et al. Problem of immortal time bias in cohort studies: example using statins for preventing progression of diabetes. *BMJ* 2010;340:b5087.
7. Cobbs CS, Harkins L, Samanta M, et al. Human cytomegalovirus infection and expression in human malignant glioma. *Cancer Res* 2002;62:3347–50.
8. Bhattacharjee B, Renzette N, Kowalik TF. Genetic analysis of cytomegalovirus in malignant gliomas. *J Virol* 2012;86:6815–24.
9. Ranganathan P, Clark PA, Kuo JS, et al. Significant association of multiple human cytomegalovirus genomic Loci with glioblastoma multiforme samples. *J Virol* 2012;86:854–64.
10. Baryawno N, Rahbar A, Wolmer-Solberg N, et al. Detection of human cytomegalovirus in medulloblastomas reveals a potential therapeutic target. *J Clin Investig* 2011;121:4043–55.

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