2. Wong ET, Hess KR, Gleason MJ, et al. Outcomes and prognostic factors in recurrent glioma patients enrolled onto phase II clinical trials. *J Clin Oncol* 1999;17:2572–2578.
3. Lamborn KR, Yung WK, Chang SM, et al. Progression-free survival: an important end point in evaluating therapy for recurrent high-grade gliomas. *Neuro Oncol* 2008;10:162–170.
4. Wick WW, Weller M; Neuro-oncology Working Group of the German Cancer Society et al. NOA-04 randomized Phase III study of sequential radiochemotherapy of oligoastrocytic tumors of WHO-grade III with PCV or temozolomide. *J Clin Oncol* 2010;28(15S), 5874–80.

doi:10.1093/neuonc/nor048

Trabedersen to target transforming growth factor-β: when the journey is not the reward, in reference to Bogdahn et al. (Neuro-Oncology 2011;13:132–142)

Dear Editor:

Neuro-Oncology recently published the first safety and efficacy data on a transforming growth factor (TGF)-β-inhibiting approach in a brain tumor trial.[1] TGF-β is a potent cytokine with multiple biological activities which has become an attractive target in glioblastoma[2] because of its immunosuppressive properties[3] and its role in angiogenesis, migration, and invasion.[4,5] Recently, TGF-β was also implicated in the maintenance of the glioma-initiating cell pool.[6] Consequently, a TGF-β inhibitory compound such as the antisense oligonucleotide trabedersen (AP 12009)[7] may be expected to reduce angiogenesis, migration, and invasion and promote the activity of natural killer and cytotoxic T cells, resulting in benefits for the treated patient cohort. However, the AP 12009-G004 trial, which compared standard chemotherapy (temozolomide or procarbazine-lomustine-vincristine [PCV]) with 10 or 80 μM trabedersen in a cohort of 145 patients with recurrent or refractory anaplastic astrocytoma or glioblastoma, was negative for the prespecified primary endpoint. That endpoint (documented at www.clinicaltrials.gov, accessed January 10, 2011) was defined as the cumulative rate of patients experiencing complete remission (CR), partial remission (PR), or stable disease (SD).[8] Despite this overall negative result, the authors state in the ABSTRACT that trabedersen results in 3-fold survival at 2 and 3 years compared with chemotherapy in a small subgroup of 9 versus 15 glioblastoma patients. We are concerned that a breakdown of a study population into such small subpopulations creates a risk of overinterpretating apparent differences that may arisen by chance and are not supported by adequate biometrical analyses.

First, the prestudy characteristics of the study groups differed. Five of the 28 glioblastoma patients (17%) had not been treated with radiotherapy prior to study entry in the low-dose trabedersen arm. Given that this was a study with of "recurrent and /refractory" glioblastoma, one wonders what standard of care these patients had been refractory to? Although radiotherapy-naïve patients were also present in the other groups, at rates of 8%-10% the differences between the groups in terms of previous treatment may have influenced the findings.

Second, the reference chemotherapy arm is remarkable in that only 6 months of chemotherapy, likely corresponding to 6 cycles of temozolomide or 3 cycles of PCV, were planned. Was this considered "standard chemotherapy?" The median treatment duration for PCV was 29 days, which corresponds to 1 cycle. The contemporary British trial ISRCTN83176944, which evaluated temozolomide and PCV for recurrent high-grade glioma that progressed after radiotherapy, allowed a PCV treatment duration of 166 days,[9] similar to the German NOA-04 trial with PCV at recurrence after radiotherapy in anaplastic glioma (152 days),[10] strongly suggesting that the reference arm patients received inadequate treatment.

Third, the analysis of the trial is flawed in that outcome data are presented in a *per protocol* way declared here as the "primary efficacy population," that is, the population of patients treated, not those intended to be treated. However, the rates of loss varied between the groups: 8 patients randomly assigned to trabedersen were excluded during the trial, compared with only 2 patients in the chemotherapy arm. The loss of these probably poor-prognosis patients likely had disproportionate effects in the low-dose trabedersen arm because this arm had the lowest total number of patients in its primary efficacy population. We worry that further clinical development of trabedersen might be built on the basis of the "superiority" of low- *versus* high-dose trabedersen in this comparison. Yet, an optimal dose cannot be derived from an inadequately powered comparison of 2 choices.

Fourth, as noted above, the trial's primary outcome measure was to be the overall response rate, or the percentage of patients with CR, PR, or SD according to the Macdonald criteria.[8] However, the primary endpoint reported was the "tumor control rate" at 6 months, which resembles the more commonly used concept of progression-free survival at 6 months. The reason for changing the original primary endpoint remains unclear, but should have been justified in the publication.

Fifth, almost all statements of significance or nonsignificance are related to comparisons of low numbers of cases; this is especially troubling given that anaplastic astrocytoma patients, who represented only 39 patients distributed among 3 study arms, are the basis for most of the reported conclusions. The profound prognostic heterogeneity of this patient population, on the basis of molecular markers including 1p/19q status, *MGMT* promoter methylation status, and *IDH* mutation status, is firmly established.[10] Any imbalance in these factors could skew study results in various directions, but none of these factors was mentioned in the article. It was also not stated whether all patient specimens had undergone central pathologic analysis or whether the data were reported by local or central pathologists. Moreover, radiological assessment of response is now

known to be a challenge,[11] and there is no information about who assessed response in this trial.

Sixth, the *ad hoc* and nonstandard definition of the concept PFS14 as an endpoint seems to suggest that other retrospectively crafted endpoints were also tested. The adoption of this measure is explained as reflecting an outcome for cases with "sufficient MRI data available for interpretable analysis," but no real justification for needing such an analysis is provided. Furthermore, Table 2 in the article shows that in the small subgroup of anaplastic astrocytoma patients at 14 months, MRI scans were missing for 25% in the low-dose arm, 40% in the high-dose arm, and 42% in the chemotherapy arm; this challenges the view that the MRI data were sufficient for analysis.

Seventh, no information about salvage treatment by study arm was provided, preventing readers from being able to determine if the interpretation of the overall survival data was appropriate.

Despite the disappointing outcome of this trial, we remain confident that TGF-β is a relevant target in glioblastoma. Going forward, it will be important to subject data relating to trabedersen to central review and to investigate the extent to which the oligonucleotide inhibits its target *in vivo*. It will be important to incorporate biological endpoints into future immunotherapy trials in glioblastoma.

We concur with the notion that trabedersen did not produce unexpected or harmful toxicity.[1] Trabedersen neutralizes only TGF-β_2, whereas TGF-β_1 or TGF-β_3 released by glioma or glioma-infiltrating (e.g., microglial) cells will at least not directly be affected. Despite persistent safety concerns,[12] the future of anti-TGF-β agents may be brighter for small molecule antagonists of the TGF-β receptor, which have shown truly promising activity in relevant rodent glioma models.[13,14]

Conflict of interest statement. Wolfgang Wick, MD: Advisory boards for Antisense Pharma, Astra Zeneca, BMS, Eli Lilly, MSD, Merck, Pfizer, Roche/Genentech, and Schering-Plough. Speakers honoraria from Merck, Roche, Schering-Plough, and Wyeth/Pfizer. Unrestricted research funding from Eli Lilly and Schering-Plough for projects unrelated to the current manuscript.

Michael Weller, MD: Investigator at Tübingen, Germany, in the AP 12009-G004 trial.

Advisory boards for Astra Zeneca, Bayer Schering, BMS, MSD, Merck, Miltenyi Biotech, Roche/Genentech, and Schering-Plough. Speakers honoraria from Merck Serono, Roche, and Schering-Plough. Unrestricted research funding from Merck Serono, MSD, and Roche for projects unrelated to the current manuscript.

Wolfgang Wick[1] and Michael Weller[2]
[1]*Department of Neurooncology, University Clinic Heidelberg, and Clinical Cooperation Unit Neurooncology, German Cancer Research Center, Heidelberg, Germany*
[2]*Department of Neurology, University Hospital Zurich, Zurich, Switzerland*

References

1. Bogdahn U, Hau P, Stockhammer G, et al. Targeted therapy for high-grade glioma with the TGF-β2 inhibitor trabedersen: results of a randomized and controlled phase IIb study. *Neuro Oncol*. 2011; 13:132–142.
2. Wick W, Naumann U, Weller M. Transforming growth factor-β: a molecular target for the future therapy of glioblastoma. *Curr Pharm Design*. 2006;12:341–349.
3. Weller M, Fontana A. The failure of current immunotherapy for malignant glioma. Tumor-derived TGF-β, T cell apoptosis, and the immune privilege of the brain. *Brain Res Rev*. 1995;21:128–151.
4. Wick W, Grimmel C, Wild-Bode C, et al. Ezrin-dependent promotion of glioma cell clonogenicity, motility and invasion mediated by BCL-2 and TGF-β_2. *J Neurosci*. 2001;21:3360–3368.
5. Friese MA, Wischhusen J, Wick W, et al. RNA interference targeting TGF-$\beta_{1,2}$ enhances NKG2D-mediated anti-glioma immune response, inhibits glioma cell migration and invasiveness and abrogates tumorigenicity *in vivo*. *Cancer Res*. 2004;64:7596–7603.
6. Penuelas S, Anido J, Prieto-Sánchez RM, et al. TGF-beta increases glioma-initiating cell self-renewal through the induction of LIF in human glioblastoma. *Cancer Cell*. 2009;15:315–327.
7. Jachimczak P, Bogdahn U, Scheider J, et al. The effect of transforming growth factor-beta 2-specific phosphothioate-anti-sense oligodeoxynucleotides in reversing cellular immunosuppression in malignant glioma. *J Neurosurg*. 1993;78:944–951.
8. Macdonald DR, Cascino TL, Schold SC, et al. Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol*. 1990;8:1277–1280.
9. Brada M, Stenning S, Gabe R, et al. Temozolomide versus procarbazine, lomustine, and vincristine in recurrent high-grade glioma. *J Clin Oncol*. 2010;28:4601–4608.
10. Wick W, Hartmann C, Engel C, et al. for the Neurooncology Working Group (NOA) of the German Cancer Society. NOA-04 randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with PCV or temozolomide. *J Clin Oncol*. 2009;27:5874–5880.
11. Wen PY, Macdonald DA, Reardon DA, et al. Updated Response Assessment Criteria For High-Grade Gliomas: Response Assessment in Neuro-Oncology (RANO) Working Group. *J Clin Oncol*. 2010;28:1963–1972.
12. Nicholas J, Laping NJ, 1Jeffrey I, Everitt JI, Kendall S, Frazier KS, et al. Tumor-specific efficacy of transforming growth factor-βRI inhibition in Eker rats. *Clin Cancer Res*. 2007;13:3087–3099.
13. Uhl M, Aulwurm S, Wischhusen J, et al. SD-208, a novel TGF-β receptor I kinase inhibitor, inhibits growth and invasiveness and enhances immunogenicity of murine and human glioma cells *in vitro* and *in vivo*. *Cancer Res*. 2004;64:7954–7961.
14. Tran TT, Uhl M, Ma JY, et al. Inhibiting TGF-ß signaling restores immune surveillance in the SMA-560 glioma model. *Neuro Oncol*. 2007;9:259–270.

Address correspondence to Wolfgang Wick, Department of Neurooncology, Neurology Clinic and National Center for Tumor Diseases, University of Heidelberg, Im Neuenheimer Feld 400, D-69120 Heidelberg, Germany; tel: +49-6221-56-7075; fax: +49-6221-56-7554; e-mail: wolfgang.wick@med.uni-heidelberg.de.

doi:10.1093/neuonc/nor046