Antiangiogenic Therapy for Glioblastoma: Current Status and Future Prospects

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Abstract

Glioblastoma is characterized by high expression levels of proangiogenic cytokines and microvascular proliferation, highlighting the potential value of treatments targeting angiogenesis. Antiangiogenic treatment likely achieves a beneficial impact through multiple mechanisms of action. Ultimately, however, alternative proangiogenic signal transduction pathways are activated, leading to the development of resistance, even in tumors that initially respond. The identification of biomarkers or imaging parameters to predict response and to herald resistance is of high priority. Despite promising phase II clinical trial results and patient benefit in terms of clinical improvement and longer progression-free survival, an overall survival benefit has not been demonstrated in four randomized phase III trials of bevacizumab or cilengitide in newly diagnosed glioblastoma or cediranib or enzastaurin in recurrent glioblastoma. However, future studies are warranted. Predictive markers may allow appropriate patient enrichment, combination with chemotherapy may ultimately prove successful in improving overall survival, and novel agents targeting multiple proangiogenic pathways may prove effective.

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Introduction

Glioblastoma, the most common primary malignant brain tumor, affects more than 3 per 100,000 individuals each year. Median survival is less than 1 year in population-based studies. Older age and lower performance status are associated with less aggressive care and shorter survival. The current standard of care includes maximal safe resection followed by radiotherapy plus concomitant and adjuvant chemotherapy with temozolomide. Elderly patients, i.e., those aged 65 to 70 years, who are not considered candidates for combined chemotherapy and radiation mainly on the basis of comorbidities or impaired performance status, may be treated with radiation or temozolomide alone based on the promoter methylaton status of O^6-methylguanine DNA methyltransferase (*MGMT*), a mediator of resistance to alklyating chemotherapy drugs. In patients with tumors lacking *MGMT* promoter methylation radiotherapy alone is acceptable, whereas in patients with *MGMT* promoter methylation temozolomide with or without radiation is acceptable (1).

Angiogenesis has emerged as a primary target of drug development for glioblastoma over the past decade. This development was triggered by the disappointing outcomes with cytotoxic drugs and the recognition that the extensive pathologic vascularization should make this disease potentially susceptible to antiangiogenic therapy. Bevacizumab, a humanized antibody to vascular endothelial growth factor (VEGF), received accelerated approval for recurrent glioblastoma in the United States and many other countries based on radiographic response rates (2, 3). In contrast, because of the lack of a controlled trial, bevacizumab did not receive approval in the European Union (EU), resulting in different standards of care between the United States and the EU. Although randomized trials in newly diagnosed glioblastoma patients have not demonstrated an overall survival benefit, the final status of bevacizumab in this setting has yet to be fully determined, as well be discussed subsequently. Other VEGF-targeting agents either have been or will continue to be explored in glioblastoma (4).

Mechanisms of Action and Resistance

The mechanisms of action of antiangiogenic therapies for solid tumors are multiple and may act in concert to delay tumor progression and ultimately prolong survival in several cancers. Folkman originally hypothesized that antiangiogenic

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agents confer an antitumor effect through induction of endothelial cell apoptosis, inhibition of new blood vessel growth, obliteration of small vessels, and decreased tumor perfusion, culminating in decreased delivery of oxygen and nutrients ("tumor starvation"; ref. 5). However, during the initial stages of treatment, antiangiogenic agents may transiently "normalize" abnormal tumor vasculature by reducing blood vessel diameter and permeability, which paradoxically improves tumor perfusion, reduces interstitial pressure, and improves tumor oxygenation (6–8), potentially sensitizing for radiotherapy and increasing tumor exposure to cytotoxic chemotherapy (ref. 7; Fig. 1). Antiangiogenic therapy may also prevent VEGF-mediated vascular regrowth following endothelial cell injury after genotoxic therapies (9–11). Antiangiogenic agents may exhibit intrinsic antitumor activity, for example, against glioblastoma stem-like cells (GSC) residing in the perivascular niche (12, 13). Antiangiogenic agents may interfere with VEGF-mediated recruitment of tumor-infiltrating VEGFR1 expressing monocytes (14). A potential role exists for antiangiogenic therapy in augmenting host immunity by reducing VEGF-mediated immune suppression (15) and thereby improving the efficacy of immunotherapy (16). The relative importance of these multiple mechanisms of action to the therapeutic benefit of antiangiogenic therapy is unknown, and different mechanisms may be operative in distinct subsets of patients as well as at different stages of the disease.

The realization that antiangiogenic therapies provide transient clinical benefit and delay tumor progression has prompted an effort to better understand mechanisms of resistance to this class of therapeutic agent, as discussed subsequently. High rates of radiographic response rates and decreased cerebral edema indicate a reduction in vascular permeability due to interruption of VEGF-A (originally termed vascular permeability factor) signaling (3, 6, 17). However, a lack of antitumor effect observed in some orthotopic rodent xenograft models of glioblastoma (18) suggests that angiogenesis inhibitors, such as cediranib, have limited intrinsic antitumor activity and that their main benefit may be limited to reductions in permeability and vasogenic cerebral edema (3, 6, 17). Notwithstanding a better understanding of the potential benefits of using an optimal dose, schedule, and drug combination, data from phase III clinical trials (19, 20) of bevacizumab suggest that some glioblastomas may be intrinsically resistant to antiangiogenic therapy. Inherent vessel insensitivity to the effect of VEGF inhibition could partially mediate this intrinsic resistance (21). Several adaptive resistance mechanisms may counteract any potential initial benefit afforded by antiangiogenic therapy. In the setting of VEGF signaling inhibition the tumor and its microenvironment release alternative proangiogenic growth factors to promote VEGF-independent angiogenesis (22–24), which may be further augmented by the recruitment of proangiogenic myeloid cells such as monocytes, M2-skewed macrophages, granulocytes, and myeloid-derived suppressor cells (14, 25, 26). In addition, functional vessels are characteristically covered with pericytes that may protect endothelial cells from apoptosis in the

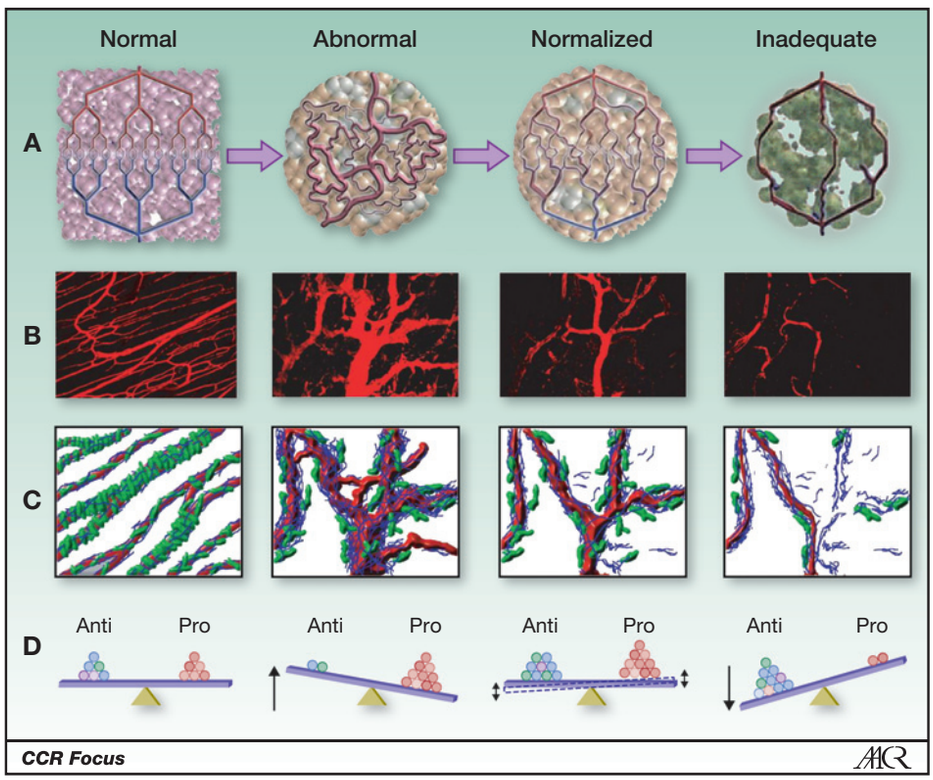

Figure 1. Normalization of tumor vasculature. A, tumor vasculature is structurally and functionally abnormal. One potential mechanism of action for antiangiogenic therapies is transient improvement in both the structure and the function of tumor vessels. However, sustained antiangiogenic treatment may eventually result in a vasculature that is both resistant to further treatment and inadequate for the delivery of drugs or oxygen. B, vessel structural patterns before, during, and with sustained VEGFR2 blockade. C, diagram depicting the concomitant changes in pericyte coverage (green) and basement membrane thickness (blue) before, during, and with sustained VEGFR2 blockage. D, changes in the balance of pro- and antiangiogenic factors leading to the phenotypic changes noted above. Reprinted from Jain (98).

face of VEGF blockade. Finally, adaptive resistance has been characterized by a transition to a mesenchymal and more invasive tumor phenotype (27–29). In the setting of antiangiogenic therapy, glioblastoma cells co-opt normal blood vessels (30) as a route of invasion into the surrounding brain. Although initial reports implied that anti-VEGF therapies were associated with nonenhancing radiographic tumor progression (31) originally interpreted as an increase in tumor invasion, subsequent reports did not support this observation (19, 20, 32, 33).

Clinical Trials

Clinical trials evaluating antiangiogenic agents for glioblastoma initially lagged behind other cancer indications due to concern about potentially serious adverse events in brain tumor patients, notably intracranial hemorrhage or stroke. However, early clinical experience confirmed the rarity of such events and that the toxicity profile of antiangiogenic agents for glioblastoma was not significantly different from that in other cancer indications; thereafter, clinical study of antiangiogenic agents for glioblastoma accelerated. A multitude of antiangiogenic agents have been evaluated for glioblastoma, including tyrosine kinase inhibitors (17, 34–48), monoclonal antibodies against VEGFR, and a soluble decoy receptor (ref. 49; Table 1). Because clinical development is most advanced for bevacizumab, we focus herein on the design, results, and conclusions of the major bevacizumab trials for glioblastoma.

Bevacizumab for recurrent glioblastoma

Dramatic overall radiographic response (ORR) rates and reassuring safety data led to two phase II studies that subsequently became the basis of the FDA accelerated approval of bevacizumab as monotherapy for recurrent glioblastoma in 2009 (Table 2; ref. 50). Of note, both studies compared outcome with historical benchmarks and included independent radiologic review. The BRAIN study randomized patients to bevacizumab ($n = 85$) or bevacizumab plus irinotecan ($n = 82$) but was not designed to detect differences between the two treatment arms (3). Outcomes for the bevacizumab and bevacizumab plus irinotecan arms included ORR rates of 28.2% and 37.8%, 6-month progression-free survival rates (PFS-6) of 42.6% and 50.3%, and median overall survival (OS) of 9.2 months and 8.7 months, respectively. A single-arm study of bevacizumab among 48 patients treated at the NCI noted ORR and PFS-6 rates of 35% and 29%, respectively, and a median OS of 7.75 months (2). Although the BRAIN and NCI trials generated unprecedented ORR and PFS-6 rates, the European Medicines Agency declined to approve bevacizumab for recurrent glioblastoma due to the absence of a non-bevacizumab control arm, a modest OS increment versus historic controls, inadequate elucidation of true antitumor effect, and challenges with radiographic response assessment (51).

Thereafter, attempts to augment the benefit of single-agent bevacizumab included studies evaluating bevacizumab combined with chemotherapeutics (31, 52–61), targeted therapies (62–64), and reirradiation (65–67). Unfortunately, all of these combinatorial regimens failed to improve outcome beyond that of bevacizumab monotherapy, possibly due to a decrease of drug delivery to the tumor (8). A single exception is a phase II study in which 148 patients with recurrent glioblastoma were randomly assigned to lomustine, bevacizumab, or lomustine plus bevacizumab (Table 2; ref. 68). The outcome was notably improved for the combination arm including PFS-6 of 41%, compared with 11% and 18% for lomustine and bevacizumab alone, respectively. The combination arm also showed improved OS at 9 months (OS9), the primary endpoint of this trial. The OS9 rates were 59% for the combination arm and 43% and 38% for lomustine and bevacizumab alone, respectively. Two aspects of this study warrant special comment. First, this is the only study to date that incorporates a comparative, randomized statistical design with a non-bevacizumab control arm with minimal crossover to bevacizumab. Second, it is the first study to report a bevacizumab combination with

Table 1. Representative clinical trials of VEGF/VEGFR targeting therapeutics among recurrent glioblastoma patients

Agent	Mechanism	Dose	Patients (*n*)	ORR (%)	PFS-6 (%)	OS (median, months)	Reference
Aflibercept	Soluble decoy VEGFR	4 mg/kg biweekly	42	18	7.7	9.8	(49)
Cediranib	VEGFR TKI	30 mg daily	118	15.3	16	8.0	(97)
Nintedanib	VEGFR TKI	200 mg twice a day	13	0	4	8.1	(48)
Pazopanib	VEGFR TKI	800 mg daily	35	5.7	3	8.8	(34)
Pazopanib (+ lapatinib)	VEGFR TKI	400 mg daily	41	5	7.5	NR	(47)
Sorafenib (+ daily TMZ)	VEGFR TKI	400 mg daily	32	3	9.4	10.4	(35)
Sunitinib	VEGFR TKI	37.5 mg daily	32	10	10.4	9.4	(42)
Vandetanib	VEGFR TKI	300 mg daily	32	12.5	6.5	6.3	(41)
Bevacizumab	Humanized anti-VEGF mAb	10 mg/kg biweekly	85	28	43	9.3	(3)

Abbreviations: TKI, tyrosine kinase inhibitor; TMZ, temozolomide.

Table 2. Landmark clinical trials of bevacizumab for glioblastoma

Trial	Regimen	Patients (*n*)	Median PFS (mo)	PFS-6 (%)	Median OS (mo)	Reference
Recurrent glioblastoma						
Brain	BEV	85	4.2	42.6	9.2	(3)
Brain	BEV + irinotecan	82	5.6	50.3	8.7	(3)
NCI	BEV	48	4.0	29	7.8	(2)
BELOB	BEV	50	3	18	8	(68)
BELOB	Lomustine	46	2	11	8	(68)
BELOB	BEV + lomustine	44	11	41	11	(68)
Newly diagnosed glioblastoma						
RTOG 0825	BEV + TMZ/XRT	312	10.7 (HR, 0.79; $P = 0.007$)	NR	15.7	(78)
RTOG 0825	TMZ/XRT	309	7.3	NR	16.1	(78)
AVAGlio	BEV + TMZ/XRT	458	10.6 (HR, 0.64; $P < 0.0001$)	NR	16.9	(77)
AVAGlio	TMZ/XRT	463	6.2	NR	16.8	(77)

Abbreviations: BEV, bevacizumab; NR, not reported; TMZ, temozolomide; XRT, radiation therapy.

improved outcome compared with bevacizumab monotherapy. Yet, the bevacizumab-alone arm underperformed in this trial, and the differences between PFS and OS in all arms suggest that further interventions had a great impact on outcome in this trial. An ongoing phase III study to further evaluate these findings (EORTC 26101, NCT01290939) randomizes recurrent glioblastoma patients to lomustine or lomustine plus bevacizumab with a primary endpoint of OS.

Resistance to bevacizumab inevitably develops, and such patients typically die rapidly due to ineffective therapies (2, 31, 53, 69–72). Retrospective data suggest that bevacizumab continuation beyond initial progression may modestly improve outcome (73). Prospective evaluation of this approach is forthcoming via an ongoing trial (TAMIGA). Nonetheless, effective therapies for bevacizumab-refractory glioblastomas are desperately needed.

Bevacizumab for newly diagnosed glioblastoma

Initial single-arm, phase II studies of bevacizumab in combination with temozolomide and radiation for newly diagnosed glioblastoma patients noted a near doubling of median PFS to 13 to 14 months compared with historic benchmarks and a nominal median OS increment to 20 months (74–76). Two randomized, placebo-controlled phase III studies, RTOG 0825 and AVAglio, reported extension of PFS but no difference in OS (77, 78; Table 2). Specifically, the median PFS rate was 47% to 71% longer for bevacizumab recipients compared with controls, but OS was not significantly different in the two treatment arms. Because 30% to 40% of controls on each study received bevacizumab at progression, crossover is a potential confounder in terms of the impact on OS, although this remains a matter of speculation. Importantly, both studies assessed predefined clinical and molecular prognostic factors for association with outcome but failed to identify any of these patient subgroups more or less likely to benefit from bevacizumab; however, there is ongoing investigation in both trials to determine whether more complex genetic signatures may define subgroups more likely to benefit from bevacizumab in combination with chemoradiation, as discussed below and elsewhere.

Both RTOG 0825 and AVAglio assessed other measures of clinical benefit. The investigators from the AVAglio trial noted preserved Karnofsky performance status and lower corticosteroid requirement among bevacizumab recipients. Unexpectedly, results from validated measures of quality of life (QOL), including the EORTC QLQ-C30 and BN20 questionnaires that were incorporated by both studies, were conflicting. Among bevacizumab recipients, consistently improved QOL scores were reported across five prospectively defined domains on AVAglio, whereas lower scores were noted for several domains on RTOG 0825. Moreover, in RTOG 0825, symptom burden was increased in the bevacizumab arm versus the control arm using the MDASI-BT. The explanation for these discordant results remains unclear. Investigators in RTOG 0825 assessed radiographic response solely by enhancing tumor (Macdonald criteria; ref. 79) and may have failed to identify early progression among bevacizumab recipients. In contrast, AVAglio assessed both enhancing and nonenhancing tumor (RANO criteria; ref. 80). RTOG 0825, but not AVAglio, incorporated formal neurocognitive testing, and these investigators noted diminished processing speed and executive function among bevacizumab recipients compared with controls. These notable findings warrant follow-up investigation. In summary, clinical trial data to date support improved PFS but lack of significant OS benefit with bevacizumab among recurrent and newly diagnosed glioblastoma patients.

Biologic and Imaging Markers

The increased understanding of the molecular profile of glioblastoma suggests that subgroups of these patients may respond differentially to distinct classes of antiangiogenic agents. There are a number of tumor tissue and circulating

candidate biomarkers for predicting the efficacy of antiangiogenic agents. Tumor tissue biomarkers that have been assessed, but not confirmed (78, 81) include a nine-gene signature representative of the mesenchymal subtype of glioblastoma (82, 83), VEGF expression (84, 85), *O6-methylguanine methyltransferase* promoter methylation status (78), epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor a (PDGFR-a), and c-KIT for VEGFR2 inhibition (6, 86). Negative predictive markers for the use of bevacizumab in newly diagnosed glioblastoma include an expanded set of mesenchymal genes (81), whereas the proneural molecular (tumors with *IDH* mutations excluded) subtype of glioblastoma specifically benefitted from bevacizumab (85) versus the other three Cancer Genome Atlas glioblastoma molecular subtypes. Independent cross-trial confirmation of these putative predictive markers is needed, and their use in current clinical practice should be discouraged.

Circulating cytokines are attractive candidate biomarkers. However, in the AVAglio trial, pretreatment plasma VEGF and sVEGFR2 levels were not associated with PFS or OS (20, 85, 87). Matrix metalloproteinase (MMP)-2 is a plasma candidate biomarker for efficacy of bevacizumab (88). Elevated soluble VEGFR1, a negative regulator of the VEGF signaling cascade, has been proposed as a resistance biomarker in other solid tumor types (89).

Radiographic response as defined by a reduction in tumor contrast-enhancement on brain CT or MRI scans may not reflect intrinsic antitumor activity since antiangiogenic treatment notably targeting VEGF signaling may rapidly reduce vessel permeability and contrast extravasation. This rapid and usually transient radiographic change is sometimes termed "pseudoresponse." Consequently, antiangiogenic treatments have compelled a focus on brain tumor imaging leading to the introduction of novel, candidate techniques to accurately define tumor response and tumor progression. Some of these MRI methods include apparent diffusion coefficient (ADC; ref. 90), dynamic contrast-enhanced (DCE) and dynamic susceptibility-contrast (DSC) techniques to assess baseline and dynamic features of glioblastoma vasculature (91, 92), as well as vessel architectural imaging (VAI), which exploits a temporal shift in the magnetic resonance signal, forming the basis for vessel caliber estimation (93). VAI techniques demonstrate vessel-normalizing microcirculation during VEGF inhibition with cediranib, a pan-VEGF receptor tyrosine kinase inhibitor (93). The T_1-derived parameter K^{Trans} may reflect not only vessel normalization but also efficacy with VEGF inhibition. Cerebral blood flow may increase early after initiation of anti-VEGF therapy and identify responders, and it is associated with improved tumor oxygenation status (86). Dopamine and amino acid positron emission tomography has been evaluated as an early imaging parameter of response to anti-VEGF therapy (94, 95). Further assessment of these imaging techniques and implementation of uniform imaging protocols in prospective randomized trials is essential to determine their ultimate predictive value.

Future Directions

Significant effort and investment have been dedicated to the development of antiangiogenic therapies for glioblastoma. Consequently, new criteria for the assessment of disease by neuroimaging have been defined (80), new concepts of clinical trial design have been developed (96), and the quality of clinical trial design, conduct, and analysis has been improved (20, 78, 97). Nevertheless, an overall survival benefit has yet to be identified after five randomized phase III trials in the newly diagnosed and recurrent glioblastoma setting. Where do we go from here?

First, future pivotal phase III trials of antiangiogenic agents should be conducted on the basis of data from well-designed, placebo-controlled, randomized phase II trials when feasible (96). Second, it is highly likely that a future survival advantage is likely to come from the combination of antiangiogenic and cytotoxic treatments, similar to other solid tumor types. As noted, the only positive OS data from a randomized (phase II) trial were for recurrent glioblastoma with chemotherapy plus bevacizumab. Third, although striking differences in patient response to and duration of benefit from VEGF inhibitors among patients with glioblastoma have been observed, none of the promising neuroimaging, histologic, and circulating markers associated with radiographic or clinical benefit have yet been validated. This, until now, missed opportunity for drug development is equally unfortunate for the field of neuro-oncology and for the pharmaceutical industry and even more for patients who may derive benefit from this treatment approach. Thus, intensive effort should focus on the identification and validation of such predictive markers. Fourth, with improved cellular and rodent glioma models, including patient-derived and stem-like cell models and feasible animal imaging techniques available, more preclinical studies focusing on predictive biomarkers and mechanisms of escape are feasible and should supplement the ongoing efforts of moving antiangiogenic agents forward.

Disclosure of Potential Conflicts of Interest

T.T. Batchelor reports receiving commercial research grants from AstraZeneca, Millenium, and Pfizer; speakers bureau honoraria from Research To Practice; and is a consultant/advisory board member for Agenus, Kirin, Merck, Oakstone, Proximagen, and UpToDate. D.A. Reardon reports receiving speakers bureau honoraria from Merck/Schering and Roche/Genentech. J.F. de Groot reports receiving a commercial research grant from AstraZeneca and is a consultant/advisory board member for Roche/Genentech. W. Wick is a consultant/advisory board member for Apogenix and Roche. M. Weller reports receiving commercial research support from Merck Serono and Roche. No other potential conflicts of interest were disclosed.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.F. de Groot

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.F. de Groot, W. Wick, M. Weller

Writing, review, and/or revision of the manuscript: T.T. Batchelor, D.A. Reardon, J.F. de Groot, W. Wick, M. Weller

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T.T. Batchelor

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References

1. Weller M, van den Bent M, Hopkins K, Tonn JC, Stupp R, Falini A, et al. EANO guideline on the diagnosis and treatment of anaplastic gliomas and malignant glioblastoma. Lancet Oncol 2014;15:e395–403.
2. Kreisl TN, Kim L, Moore K, Duic P, Royce C, Stroud I, et al. Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. J Clin Oncol 2009;27:740–5.
3. Friedman HS, Prados MD, Wen PY, Mikkelsen T, Schiff D, Abrey LE, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol 2009;27:4733–40.
4. Wick W, Puduvalli VK, Chamberlain MC, van den Bent MJ, Carpentier AF, Cher LM, et al. Phase III study of enzastaurin compared with lomustine in the treatment of recurrent intracranial glioblastoma. J Clin Oncol 2010;28:1168–74.
5. Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med 1971;285:1182–6.
6. Batchelor TT, Sorensen AG, diTomaso E, Zhang WT, Duda DG, Cohen KS, et al. AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. Cancer Cell 2007;11:83–95.
7. Winkler F, Kozin SV, Tong RT, Chae SS, Booth MF, Garkavtsev I, et al. Kinetics of vascular normalization by VEGFR2 blockade governs brain tumor response to radiation: role of oxygenation, angiopoietin-1, and matrix metalloproteinases. Cancer Cell 2004;6:553–63.
8. Van der Veldt AA, Lubberink M, Bahce I, Walraven M, de Boer MP, Greuter HN, et al. Rapid decrease in delivery of chemotherapy to tumors after anti-VEGF therapy: implications for scheduling of anti-angiogenic drugs. Cancer Cell 2012;21:82–91.
9. Duda DG, Jain RK, Willett CG. Antiangiogenics: the potential role of integrating this novel treatment modality with chemoradiation for solid cancers. J Clin Oncol 2007;25:4033–42.
10. Shaked Y, Kerbel RS. Antiangiogenic strategies on defense: on the possibility of blocking rebounds by the tumor vasculature after chemotherapy. Cancer Res 2007;67:7055–8.
11. Gorski DH, Beckett MA, Jaskowiak NT, Calvin DP, Mauceri HJ, Salloum RM, et al. Blockage of the vascular endothelial growth factor stress response increases the antitumor effects of ionizing radiation. Cancer Res 1999;59:3374–8.
12. Calabrese C, Poppleton H, Kocak M, Hogg TL, Fuller C, Hamner B, et al. A perivascular niche for brain tumor stem cells. Cancer Cell 2007;11:69–82.
13. Folkins C, Man S, Xu P, Shaked Y, Hicklin DJ, Kerbel RS. Anticancer therapies combining antiangiogenic and tumor cell cytotoxic effects reduce the tumor stem-like cell fraction in glioma xenograft tumors. Cancer Res 2007;67:3560–4.
14. de Groot JF, Piao Y, Tran H, Gilbert M, Wu HK, Liu J, et al. Myeloid biomarkers associated with glioblastoma response to anti-VEGF therapy with aflibercept. Clin Cancer Res 2011;17:4872–81.
15. Terme M, Pernot S, Marcheteau E, Sandoval F, Benhamouda N, Colussi O, et al. VEGFA-VEGFR pathway blockade inhibits tumor-induced regulatory T-cell proliferation in colorectal cancer. Cancer Res 2013;73:539–49.
16. Shrimali RK, Yu Z, Theoret MR, Chinnasamy D, Restifo NP, Rosenberg SA. Antiangiogenic agents can increase lymphocyte infiltration into tumor and enhance the effectiveness of adoptive immunotherapy of cancer. Cancer Res 2010;70:6171–80.
17. Batchelor TT, Duda DG, di Tomaso E, Ancukiewicz M, Plotkin SR, Gerstner E, et al. Phase II study of cediranib, an oral pan-vascular endothelial growth factor receptor tyrosine kinase inhibitor, in patients with recurrent glioblastoma. J Clin Oncol 2010;28:2817–23.
18. Kamoun WS, Ley CD, Farrar CT, Duyverman AM, Lahdenranta J, Lacorre DA, et al. Edema control by cediranib, a vascular endothelial growth factor receptor-targeted kinase inhibitor, prolongs survival despite persistent brain tumor growth in mice. J Clin Oncol 2009;27:2542–52.
19. Gilbert MR, Sulman EP, Mehta MP. Bevacizumab for newly diagnosed glioblastoma. N Engl J Med 2014;370:2048–9.
20. Chinot OL, Wick W, Cloughesy T. Bevacizumab for newly diagnosed glioblastoma. N Engl J Med 2014;370:2049.
21. Sitohy B, Nagy JA, Jaminet SC, Dvorak HF. Tumor-surrogate blood vessel subtypes exhibit differential susceptibility to anti-VEGF therapy. Cancer Res 2011;71:7021–8.
22. DeLay M, Jahangiri A, Carbonell WS, Hu YL, Tsao S, Tom MW, et al. Microarray analysis verifies two distinct phenotypes of glioblastomas resistant to antiangiogenic therapy. Clin Cancer Res 2012;18:2930–42.
23. Casanovas O, Hicklin DJ, Bergers G, Hanahan D. Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. Cancer Cell 2005;8:299–309.
24. Lu KV, Bergers G. Mechanisms of evasive resistance to anti-VEGF therapy in glioblastoma. CNS Oncol 2013;2:49–65.
25. Shojaei F, Wu X, Qu X, Kowanetz M, Yu L, Tan M, et al. G-CSF-initiated myeloid cell mobilization and angiogenesis mediate tumor refractoriness to anti-VEGF therapy in mouse models. Proc Natl Acad Sci U S A 2009;106:6742–7.
26. de Groot J, Liang J, Kong LY, Wei J, Piao Y, Fuller G, et al. Modulating antiangiogenic resistance by inhibiting the signal transducer and activator of transcription 3 pathway in glioblastoma. Oncotarget 2012;3:1036–48.
27. Piao Y, Liang J, Holmes L, Henry V, Sulman E, de Groot JF. Acquired resistance to anti-VEGF therapy in glioblastoma is associated with a mesenchymal transition. Clin Cancer Res 2013;19:4392–403.
28. Lu KV, Chang JP, Parachoniak CA, Pandika MM, Aghi MK, Meyronet D, et al. VEGF inhibits tumor cell invasion and mesenchymal transition through a MET/VEGFR2 complex. Cancer Cell 2012;22:21–35.
29. Du R, Lu KV, Petritsch C, Liu P, Ganss R, Passegue E, et al. HIF1alpha induces the recruitment of bone marrow-derived vascular modulatory cells to regulate tumor angiogenesis and invasion. Cancer Cell 2008;13:206–20.
30. de Groot JF, Fuller G, Kumar AJ, Piao Y, Eterovic K, Ji Y, et al. Tumor invasion after treatment of glioblastoma with bevacizumab: radiographic and pathologic correlation in humans and mice. Neuro Oncol 2010;12:233–42.
31. Norden AD, Young GS, Setayesh K, Muzikansky A, Klufas R, Ross GL, et al. Bevacizumab for recurrent malignant gliomas: efficacy, toxicity, and patterns of recurrence. Neurology 2008;70:779–87.
32. Pope WB, Xia Q, Paton VE, Das A, Hambleton J, Kim HJ, et al. Patterns of progression in patients with recurrent glioblastoma treated with bevacizumab. Neurology 2011;76:432–7.
33. Wick A, Dorner N, Schafer N, Hofer S, Heiland S, Schemmer D, et al. Bevacizumab does not increase the risk of remote relapse in malignant glioma. Ann Neurol 2011;69:586–92.
34. Iwamoto FM, Lamborn KR, Robins HI, Mehta MP, Chang SM, Butowski NA, et al. Phase II trial of pazopanib (GW786034), an oral multi-targeted angiogenesis inhibitor, for adults with recurrent glioblastoma (North American Brain Tumor Consortium Study 06–02). Neuro Oncol 2010;12:855–61.
35. Reardon DA, Vredenburgh JJ, Desjardins A, Peters K, Gururangan S, Sampson JH, et al. Effect of CYP3A-inducing anti-epileptics on sorafenib exposure: results of a phase II study of sorafenib plus daily temozolomide in adults with recurrent glioblastoma. J Neurooncol 2011;101:57–66.
36. Pan E, Yu D, Yue B, Potthast L, Chowdhary S, Smith P, et al. A prospective phase II single-institution trial of sunitinib for recurrent malignant glioma. J Neurooncol 2012;110:111–8.

37. Gerstner ER, Eichler AF, Plotkin SR, Drappatz J, Doyle CL, Xu L, et al. Phase I trial with biomarker studies of vatalanib (PTK787) in patients with newly diagnosed glioblastoma treated with enzyme inducing antiepileptic drugs and standard radiation and temozolomide. J Neurooncol 2011;103:325–32.
38. Brandes AA, Stupp R, Hau P, Lacombe D, Gorlia T, Tosoni A, et al. EORTC study 26041–22041: phase I/II study on concomitant and adjuvant temozolomide (TMZ) and radiotherapy (RT) with PTK787/ZK222584 (PTK/ZK) in newly diagnosed glioblastoma. Eur J Cancer 2010;46:348–54.
39. Hainsworth JD, Ervin T, Friedman E, Priego V, Murphy PB, Clark BL, et al. Concurrent radiotherapy and temozolomide followed by temozolomide and sorafenib in the first-line treatment of patients with glioblastoma multiforme. Cancer 2010;116:3663–9.
40. Lee EQ, Kuhn J, Lamborn KR, Abrey L, DeAngelis LM, Lieberman F, et al. Phase I/II study of sorafenib in combination with temsirolimus for recurrent glioblastoma or gliosarcoma: North American Brain Tumor Consortium Study 05–02. Neuro Oncol 2012;14:1511–8.
41. Kreisl TN, McNeill KA, Sul J, Iwamoto FM, Shih J, Fine HA. A phase I/II trial of vandetanib for patients with recurrent malignant glioma. Neuro Oncol 2012;14:1519–26.
42. Kreisl TN, Smith P, Sul J, Salgado C, Iwamoto FM, Shih JH, et al. Continuous daily sunitinib for recurrent glioblastoma. J Neurooncol 2013;111:41–8.
43. Drappatz J, Norden AD, Wong ET, Doherty LM, Lafrankie DC, Ciampa A, et al. Phase I study of vandetanib with radiotherapy and temozolomide for newly diagnosed glioblastoma. Int J Radiat Oncol Biol Phys 2010;78:85–90.
44. Reardon DA, Egorin MJ, Desjardins A, Vredenburgh JJ, Beumer JH, Lagattuta TF, et al. Phase I pharmacokinetic study of the vascular endothelial growth factor receptor tyrosine kinase inhibitor vatalanib (PTK787) plus imatinib and hydroxyurea for malignant glioma. Cancer 2009;115:2188–98.
45. Reardon DA, Conrad CA, Cloughesy T, Prados MD, Friedman HS, Aldape KD, et al. Phase I study of AEE788, a novel multitarget inhibitor of ErbB- and VEGF-receptor-family tyrosine kinases, in recurrent glioblastoma patients. Cancer Chemother Pharmacol 2012;69:1507–18.
46. Reardon DA, Vredenburgh JJ, Coan A, Desjardins A, Peters KB, Gururangan S, et al. Phase I study of sunitinib and irinotecan for patients with recurrent malignant glioma. J Neurooncol 2011;105:621–7.
47. Reardon DA, Groves MD, Wen PY, Nabors L, Mikkelsen T, Rosenfeld S, et al. A phase I/II trial of pazopanib in combination with lapatinib in adult patients with relapsed malignant glioma. Clin Cancer Res 2013;19:900–8.
48. Muhic A, Poulsen HS, Sorensen M, Grunnet K, Lassen U. Phase II open-label study of nintedanib in patients with recurrent glioblastoma multiforme. J Neurooncol 2013;111:205–12.
49. de Groot JF, Lamborn KR, Chang SM, Gilbert MR, Cloughesy TF, Aldape K, et al. Phase II study of aflibercept in recurrent malignant glioma: a North American Brain Tumor Consortium study. J Clin Oncol 2011;29:2689–95.
50. Cohen MH, Shen YL, Keegan P, Pazdur R. FDA drug approval summary: Bevacizumab (avastin) as treatment of recurrent glioblastoma multiforme. Oncologist 2009;14:1131–8.
51. Wick W, Weller M, van den Bent M, Stupp R. Bevacizumab and recurrent malignant gliomas: a European perspective. J Clin Oncol 2010;28:e188–9; author reply e190–2.
52. Francesconi AB, Dupre S, Matos M, Martin D, Hughes BG, Wyld DK, et al. Carboplatin and etoposide combined with bevacizumab for the treatment of recurrent glioblastoma multiforme. J Clin Neurosci 2010;17:970–4.
53. Reardon DA, Desjardins A, Peters KB, Gururangan S, Sampson JH, McLendon RE, et al. Phase II study of carboplatin, irinotecan, and bevacizumab for bevacizumab naive, recurrent glioblastoma. J Neurooncol 2012;107:155–64.
54. Reardon DA, Desjardins A, Vredenburgh JJ, Gururangan S, Sampson JH, Sathornsumetee S, et al. Metronomic chemotherapy with daily, oral etoposide plus bevacizumab for recurrent malignant glioma: a phase II study. Br J Cancer 2009;101:1986–94.
55. Ali SA, McHayleh WM, Ahmad A, Sehgal R, Braffet M, Rahman M, et al. Bevacizumab and irinotecan therapy in glioblastoma multiforme: a series of 13 cases. J Neurosurg 2008;109:268–72.
56. Bokstein F, Shpigel S, Blumenthal DT. Treatment with bevacizumab and irinotecan for recurrent high-grade glial tumors. Cancer 2008;112:2267–73.
57. Kang TY, Jin T, Elinzano H, Peereboom D. Irinotecan and bevacizumab in progressive primary brain tumors, an evaluation of efficacy and safety. J Neurooncol 2008;89:113–8.
58. Zuniga RM, Torcuator R, Jain R, Anderson J, Doyle T, Ellika S, et al. Efficacy, safety and patterns of response and recurrence in patients with recurrent high-grade gliomas treated with bevacizumab plus irinotecan. J Neurooncol 2009;91:329–36.
59. Hasselbalch B, Eriksen JG, Broholm H, Christensen IJ, Grunnet K, Horsman MR, et al. Prospective evaluation of angiogenic, hypoxic and EGFR-related biomarkers in recurrent glioblastoma multiforme treated with cetuximab, bevacizumab and irinotecan. APMIS 2010;118:585–94.
60. Nghiemphu PL, Liu W, Lee Y, Than T, Graham C, Lai A, et al. Bevacizumab and chemotherapy for recurrent glioblastoma: a single-institution experience. Neurology 2009;72:1217–22.
61. Desjardins A, Reardon DA, Coan A, Marcello J, Herndon JE 2nd, Bailey L, et al. Bevacizumab and daily temozolomide for recurrent glioblastoma. Cancer 2012;118:1302–12.
62. Sathornsumetee S, Desjardins A, Vredenburgh JJ, McLendon RE, Marcello J, Herndon JE, et al. Phase II trial of bevacizumab and erlotinib in patients with recurrent malignant glioma. Neuro Oncol 2010;12:1300–10.
63. Galanis E, Anderson SK, Lafky JM, Uhm JH, Giannini C, Kumar SK, et al. Phase II study of bevacizumab in combination with sorafenib in recurrent glioblastoma (N0776): a north central cancer treatment group trial. Clin Cancer Res 2013;19:4816–23.
64. Drappatz J, Lee EQ, Hammond S, Grimm SA, Norden AD, Beroukhim R, et al. Phase I study of panobinostat in combination with bevacizumab for recurrent high-grade glioma. J Neurooncol 2012;107:133–8.
65. Cuneo KC, Vredenburgh JJ, Sampson JH, Reardon DA, Desjardins A, Peters KB, et al. Safety and efficacy of stereotactic radiosurgery and adjuvant bevacizumab in patients with recurrent malignant gliomas. Int J Radiat Oncol Biol Phys 2012;82:2018–24.
66. Gutin PH, Iwamoto FM, Beal K, Mohile NA, Karimi S, Hou BL, et al. Safety and efficacy of bevacizumab with hypofractionated stereotactic irradiation for recurrent malignant gliomas. Int J Radiat Oncol Biol Phys 2009;75:156–63.
67. Cabrera AR, Cuneo KC, Vredenburgh JJ, Sampson JH, Kirkpatrick JP. Stereotactic radiosurgery and bevacizumab for recurrent glioblastoma multiforme. J Natl Compr Canc Netw 2012;10:695–9.
68. Taal W, Oosterkamp HM, Walenkamp AM, Dubbink HJ, Beerepoot LV, Hanse MC, et al. Single-agent bevacizumab or lomustine versus a combination of bevacizumab plus lomustine in patients with recurrent glioblastoma (BELOB trial): a randomised controlled phase 2 trial. Lancet Oncol 2014;15:943–53.
69. Reardon DA, Desjardins A, Peters K, Gururangan S, Sampson J, Rich JN, et al. Phase II study of metronomic chemotherapy with bevacizumab for recurrent glioblastoma after progression on bevacizumab therapy. J Neurooncol 2011;103:371–9.
70. Lu-Emerson C, Norden AD, Drappatz J, Quant EC, Beroukhim R, Ciampa AS, et al. Retrospective study of dasatinib for recurrent glioblastoma after bevacizumab failure. J Neurooncol 2011;104:287–91.
71. Iwamoto FM, Abrey LE, Beal K, Gutin PH, Rosenblum MK, Reuter VE, et al. Patterns of relapse and prognosis after bevacizumab failure in recurrent glioblastoma. Neurology 2009;73:1200–6.
72. Quant EC, Norden AD, Drappatz J, Muzikansky A, Doherty L, Lafrankie D, et al. Role of a second chemotherapy in recurrent malignant glioma patients who progress on bevacizumab. Neuro Oncol 2009;11:550–5.
73. Reardon DA, Herndon JE II, Peters KB, Desjardins A, Coan A, Lou E, et al. Bevacizumab continuation beyond initial bevacizumab progression among recurrent glioblastoma patients. Br J Cancer 2012;107:1481–7.
74. Lai A, Tran A, Nghiemphu PL, Pope WB, Solis OE, Selch M, et al. Phase II study of bevacizumab plus temozolomide during and after radiation

therapy for patients with newly diagnosed glioblastoma multiforme. J Clin Oncol 2011;29:142–8.
75. Vredenburgh JJ, Desjardins A, Kirkpatrick JP, Reardon DA, Peters KB, Herndon JE 2nd, et al. Addition of bevacizumab to standard radiation therapy and daily temozolomide is associated with minimal toxicity in newly diagnosed glioblastoma multiforme. Int J Radiat Oncol Biol Phys 2012;82:58–66.
76. Vredenburgh JJ, Desjardins A, Reardon DA, Peters KB, Herndon JE 2nd, Marcello J, et al. The addition of bevacizumab to standard radiation therapy and temozolomide followed by bevacizumab, temozolomide, and irinotecan for newly diagnosed glioblastoma. Clin Cancer Res 2011;17:4119–24.
77. Chinot OL, Wick W, Mason W, Henriksson R, Saran F, Nishikawa R, et al. Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. N Engl J Med 2014;370:709–22.
78. Gilbert MR, Dignam JJ, Armstrong TS, Wefel JS, Blumenthal DT, Vogelbaum MA, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. N Engl J Med 2014;370:699–708.
79. Macdonald DR, Cascino TL, Schold SC Jr, Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. J Clin Oncol 1990;8:1277–80.
80. Wen PY, Macdonald DR, Reardon DA, Cloughesy TF, Sorensen AG, Galanis E, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. J Clin Oncol 2010;28:1963–72.
81. Sulman EP, Won M, Blumenthal DT, Vogelbaum MA, Colman H, Jenkins RB, et al. Molecular predictors of outcome and response to bevacizumab (BEV) based on analysis of RTOG 0825, a phase III trial comparing chemoradiation (CRT) with and without BEV in patients with newly diagnosed glioblastoma (GBM). J Clin Oncol 2013;31:(suppl; abstr LBA2010).
82. Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, Wu TD, et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell 2006;9:157–73.
83. Gilbert MR, Wang M, Aldape KD, Stupp R, Hegi ME, Jaeckle KA, et al. Dose-dense temozolomide for newly diagnosed glioblastoma: a randomized phase III clinical trial. J Clin Oncol 2013;31:4085–91.
84. Sathornsumetee S, Cao Y, Marcello JE, Herndon JE 2nd,McLendon RE, Desjardins A, et al. Tumor angiogenic and hypoxic profiles predict radiographic response and survival in malignant astrocytoma patients treated with bevacizumab and irinotecan. J Clin Oncol 2008;26:271–8.
85. Phillips H, Sandmann T, Li C, Cloughesy T, Chinot O, Wick W, et al. Correlations of molecular subtypes with survival in AVAglio (bevacizumab [BV] plus radiotherapy [RT] and temozolomide [T] for newly diagnosed glioblastoma [GBM]). J Clin Oncol 2014;32:5s.(suppl; abstr 2001).
86. Batchelor TT, Gerstner ER, Emblem KE, Duda DG, Kalpathy-Cramer J, Snuderl M, et al. Improved tumor oxygenation and survival in glioblastoma patients who show increased blood perfusion after cediranib and chemoradiation. Proc Natl Acad Sci U S A 2013;110:19059–64.
87. Nishikawa R, Saran F, Mason W, Wick W, Cloughesy TF, Henriksson R, et al. Biomarker (BM) evaluations in the phase III AVAglio study of bevacizumab (Bv) plus standard radiotherapy (RT) and temozolomide (T) for newly diagnosed glioblastoma (GBM). J Clin Oncol 2013;31:(suppl; abstr 2023).
88. Tabouret E, Boudouresque F, Barrie M, Matta M, Boucard C, Loundou A, et al. Association of matrix metalloproteinase 2 plasma level with response and survival in patients treated with bevacizumab for recurrent high-grade glioma. Neuro Oncol 2014;16:392–9.
89. Duda DG, Willett CG, Ancukiewicz M, di Tomaso E, Shah M, Czito BG, et al. Plasma soluble VEGFR-1 is a potential dual biomarker of response and toxicity for bevacizumab with chemoradiation in locally advanced rectal cancer. Oncologist 2010;15:577–83.
90. Pope WB, Kim HJ, Huo J, Alger J, Brown MS, Gjertson D, et al. Recurrent glioblastoma multiforme: ADC histogram analysis predicts response to bevacizumab treatment. Radiology 2009;252:182–9.
91. Waldman AD, Jackson A, Price SJ, Clark CA, Booth TC, Auer DP, et al. Quantitative imaging biomarkers in neuro-oncology. Nat Rev Clin Oncol 2009;6:445–54.
92. O'Connor JP, Jackson A, Parker GJ, Roberts C, Jayson GC. Dynamic contrast-enhanced MRI in clinical trials of antivascular therapies. Nat Rev Clin Oncol 2012;9:167–77.
93. Emblem KE, Mouridsen K, Bjornerud A, Farrar CT, Jennings D, Borra RJ, et al. Vessel architectural imaging identifies cancer patient responders to anti-angiogenic therapy. Nat Med 2013;19:1178–83.
94. Chen W, Delaloye S, Silverman DH, Geist C, Czernin J, Sayre J, et al. Predicting treatment response of malignant gliomas to bevacizumab and irinotecan by imaging proliferation with [18F] fluorothymidine positron emission tomography: a pilot study. J Clin Oncol 2007;25:4714–21.
95. Harris RJ, Cloughesy TF, Pope WB, Nghiemphu PL, Lai A, Zaw T, et al. 18F-FDOPA and 18F-FLT positron emission tomography parametric response maps predict response in recurrent malignant gliomas treated with bevacizumab. Neuro Oncol 2012;14:1079–89.
96. Galanis E, Wu W, Cloughesy T, Lamborn K, Mann B, Wen PY, et al. Phase 2 trial design in neuro-oncology revisited: a report from the RANO group. Lancet Oncol 2012;13:e196–204.
97. Batchelor TT, Mulholland P, Neyns B, Nabors LB, Campone M, Wick A, et al. Phase III randomized trial comparing the efficacy of cediranib as monotherapy, and in combination with lomustine, versus lomustine alone in patients with recurrent glioblastoma. J Clin Oncol 2013;31:3212–8.
98. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science 2005;307:58–62.