Concerns about anti-angiogenic treatment in patients with glioblastoma multiforme

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Abstract

Background: The relevance of angiogenesis inhibition in the treatment of glioblastoma multiforme (GBM) should be considered in the unique context of malignant brain tumours. Although patients benefit greatly from reduced cerebral oedema and intracranial pressure, this important clinical improvement on its own may not be considered as an anti-tumour effect.

Discussion: GBM can be roughly separated into an angiogenic component, and an invasive or migratory component. Although this latter component seems inert to anti-angiogenic therapy, it is of major importance for disease progression and survival. We reviewed all relevant literature. Published data support that clinical symptoms are tempered by anti-angiogenic treatment, but that tumour invasion continues. Unfortunately, current imaging modalities are affected by anti-angiogenic treatment too, making it even harder to define tumour margins. To illustrate this we present MRI, biopsy and autopsy specimens from bevacizumab-treated patients.

Moreover, while treatment of other tumour types may be improved by combining chemotherapy with anti-angiogenic drugs, inhibiting angiogenesis in GBM may antagonise the efficacy of chemotherapeutic drugs by normalising the blood-brain barrier function.

Summary: Although angiogenesis inhibition is of considerable value for symptom reduction in GBM patients, lack of proof of a true anti-tumour effect raises concerns about the place of this type of therapy in the treatment of GBM.
Background

The brain is the highest organised and most complex organ of the body. Some unique features of the brain that have an impact on the biology of brain tumours include extensive three-dimensional structuring with gray and white matter [1], and high density of blood capillaries making it a well-perfused organ; however, a blood-brain barrier (BBB) very selectively regulates the penetration of substances into the brain [2]. With respect to this specific micro-environment, brain cancer, in particular high-grade astrocytoma (malignant glioma, glioblastoma multiforme, GBM) differs from many other cancer types. Whereas this disease usually manifests itself as a focal lesion with central necrosis surrounded by an angiogenic tumour rim (one of the characteristics of GBM), this tumour also invades the surrounding extracellular matrix, using both white matter tracts and blood vessels as substrate [3,4]. Consequently, the presence of migrating glioma cells in brain parenchyma relatively far away from the tumour core is common, complicating curative surgery and radiotherapy. The exact molecular and cellular mechanisms behind cell migration in glioma remain to be elucidated [5].

Despite attempts to improve treatment results over the last 30 years, GBM remains a highly fatal and devastating disease; a cure is extremely rare, even with aggressive treatment [6,7]. The current standard of care is surgical resection to a maximal feasible extent, followed by radiotherapy and systemic temozolomide (TMZ) chemotherapy to a maximal feasible extent, followed by radiotherapy. The exact molecular and cellular mechanisms behind cell migration in glioma remain to be elucidated [5].

However, the question remains whether angiogenesis inhibitors represent a true anti-GBM regimen. Non-randomised phase II trials in recurrent GBM patients demonstrated high response rates ranging from 20% with single agent TMZ [27], to 30% with single agent AZD2171 (Cediranib, a small molecule VEGFR inhibitor) [24], 20% with single agent bevacizumab, and even to 57% when bevacizumab was combined with irinotecan [14-17,28]. These studies also demonstrated longer median PFS (14-24 weeks), and 6-months PFS (17%-46%), compared with historical controls treated with cytotoxic chemotherapy (6-months PFS mostly <30%). Although some have reported relatively long median survival (34-42 weeks) [14,16,28], Norden et al. [29] demonstrated a longer median PFS (8 vs. 22 weeks), but not median survival (39 vs. 37 weeks), of recurrent GBM patients treated with anti-angiogenic therapy. The suggested efficacy of anti-angiogenic drugs in patients with recurrent GBM is based on non-randomised trials with PFS as the main study endpoint.

Determination of the precise time at which GBM progression occurs is challenging. The often robust and sustained changes on MRI by anti-angiogenic drugs may render subsequent MRI scans difficult to interpret [30]. Therefore, the response rate and PFS may not be the optimal endpoints in phase II studies of anti-angiogenic therapy in patients with recurrent GBM. Because of the potential
selection bias in non-randomised studies, and concerns about PFS as study endpoint, we should interpret data from such studies with caution [31]. Randomised trials with survival as primary endpoint are necessary to determine the role of angiogenesis inhibitors in the treatment of patients with recurrent GBM.

A problem with the current enthusiasm for angiogenesis inhibition in GBM is that tumour response evaluations are based on cross sectional contrast enhancing tumour areas [32]. Because anti-angiogenic therapy directly interferes with vessel permeability and gadolinium-DTPA (Gd-DTPA) uptake in the tumours, response evaluations based on contrast enhancing areas are suboptimal for monitoring the effects of anti-angiogenic therapy [30]. In addition, the current methods of imaging are not adequate to identify tumour boundaries, the areas where tumour growth and infiltration takes place. Indeed, also in mouse models of orthotopic glioma, tumour visibility on Gd-DTPA-enhanced MRI scans show an impressive decrease after anti-angiogenic treatment. However, in these models treatment does not affect tumour growth, but merely shifts tumour progression from an expansive to an invasive, angiogenesis-independent phenotype [33,34].
Therefore, also in patients with GBM, the impressive decreases of contrast enhancement in these tumours on anti-angiogenic treatment (Figure 1A-E) are not necessarily synonymous with anti-tumour effects.

In the 1990s, MRI criteria for evaluation of brain tumour size and treatment responses were adjusted after introduction of corticosteroids in GBM treatment [35]. Again, new criteria need to be developed now that angiogenesis inhibitors are being explored for the treatment of GBM and, importantly, for other tumours that metastasise to the brain [36-41]. Promising imaging modalities to evaluate anti-angiogenic treated patients include T2-weighted imaging [42], dynamic contrast enhancement imaging [43], apparent diffusion coefficient imaging [44], diffusion tension imaging [45], diffusion-weighted imaging [46], USPIO-enhanced blood volume imaging [34], (multivoxel) MR spectroscopy [42], positron emitting tomography [47,48], and single-photon emission computed tomography nuclear imaging [49]. However, the clinical value of these modalities for the follow-up of GBM patients treated with angiogenesis inhibitors in trials has yet to be confirmed [50].

Vascularisation and vessel normalisation
The central hypothesis of angiogenesis inhibition as anti-tumour strategy states that tumour growth and expansion are dependent on the ability of the tumour to generate its own vascularisation. In normal vessels, the blood-brain barrier (BBB) remains intact, but due to angiogenesis, this barrier may become ruptured, resulting in leakage of contrast agents and increased signal intensity on T1-weighted imaging. Anti-angiogenic treatments can therefore lead to decreased signal intensity on T1-weighted imaging, which may be interpreted as a decrease in tumour size. However, despite this decreased signal intensity, tumour size may remain unchanged or even increase, as demonstrated by histological examination of recurrent GBM specimens post-bevacizumab treatment (Figure 2A-D).

**Figure 2**
Recurrent GBM: resection and autopsy material, post bevacizumab. (A, B) Recurrent GBM resection material, obtained 6 weeks after last infusion of bevacizumab. Tumour cells co-opt pre-existent vessels with relatively intact BBB (arrows). (A) H&E staining 20×. (B) Glut-1, BBB marker, 20×. (C, D) Recurrent GBM: autopsy was performed 10 weeks after the last infusion of bevacizumab. Near tumour sample shows tumour cells invading along white matter tracks. (C) H&E 20×. (D) Glut-1 BBB marker, 10×.
own supportive vascularisation [51-53]. The actual dependence on angiogenesis is, however, highly dependent on the tumour micro-environment. Indeed, it is an absolute requirement for rapidly proliferating tumours growing in avascular spaces, which encompass most of the subcutaneous murine tumour models (including glioma). Such tumours are indeed very responsive to anti-angiogenic therapy. In clinical tumours (and, in particular, brain cancer), the situation is far more complex. While GBM is considered to be a highly vascular tumour with an extensive angiogenic component driven by VEGF-A (produced by peri-necrotic hypoxic tumour cells), the relative contribution of angiogenic growth aspects is probably low compared to the complete tumour volume. In the angiogenic parts, the quality of the newly formed vessels may be poor [54,55]. Theoretical and preclinical models support the concept that anti-angiogenic treatment leads to vascular normalisation in these areas; immature intratumoural vessels are pruned, vessel hyperpermeability and concomitant tumour interstitial pressure are reduced, and pericyte coverage is restored, resulting in improved tumour vessel perfusion and creating opportunities for combination treatments [56,57]. Vessel normalisation is believed to explain the synergy between bevacizumab and chemotherapy in advanced colorectal cancer patients [9,57]. In confirmation of this concept, it was recently reported that anti-VEGF treatment of subcutaneous melanoma grafts results in vessel normalisation and concomitant better delivery of MRI contrast agents and oxygen to the tumour [58]. In striking contrast, similar treatment of brain tumours (both primary and metastatic) results in reduced contrast-enhanced MRI visibility both in animal models [34,59] and in GBM patients (Figure 1). Thus, functional implications of vessel normalisation may depend on specific features of the tumour micro-environment, enabling an adaptation to circumvent the specific angiogenic blockade [60].

**Invasion and migration**

Next to the angiogenic rim, in GBM large areas can be identified where viable and proliferating tumour cells invade the surrounding highly vascularised normal tissue. In the cerebrum, blood vessels and white matter tracts are typically located next to each other and form corridors for invading GBM cells [4]. Close to the tumour bulk, glioma cells can be found alongside these vessels and white matter tracts (Figure 2A-D) [61]. Utilisation of these blood vessels for metabolic support (known as vessel co-option), offers proliferating tumour cells an efficient escape mechanism for anti-angiogenic therapy. Indeed, co-opted vessels are refractory to anti-angiogenic treatment [33,56,62-65]. Consequently, even during anti-angiogenic therapy, GBM cells continue to migrate through the adjacent cerebrum for considerable distances as is illustrated by the presence of high densities of tumour cells, disperse throughout the brain of a patient who died 10 weeks after the last dose of bevacizumab (Figure 3). Published clinical data appear in line with recently published preclinical data, showing that VEGF inhibition may promote tumour cell invasiveness, also in other tissues than brain [66-69]. Clinical studies have also shown that bevacizumab may alter the recurrence pattern of malignant gliomas by suppressing contrast-enhancing tumours more effectively than non-enhancing, infiltrative tumours [22,70].

**Blood brain barrier and chemotherapy**

Chemotherapeutic drugs are generally not very effective against GBM, partly due to intrinsic resistance [71] but also because of an intact BBB in peripheral parts of the tumour (note the positive staining for BBB-specific endothelial protein glut-1 in Figure 2B,D) that efficiently restricts the distribution of drugs to tumour cells [2]. Even TMZ, which is able to cross the BBB to some extent, probably acts more effectively against tumour cells in more accessible angiogenic parts of the tumour. The synergy of combined anti-angiogenic and chemotherapeutic agents in other malignancies has been partially attributed to normalisation of the tumour vascular bed, as discussed above [53]. Whereas this strategy may work well in tumours outside the brain, in case of GBM normalisation of the tumour vascular bed and reduction of interstitial pressure comes at the expense of restored functionality of the BBB as evidenced by the reduced permeability for Gd-DTPA. The accompanying reduced accessibility of other drugs carries the risk of antagonising the efficacy of such agents.

![Figure 3](http://www.biomedcentral.com/1471-2407/9/444)  
**Recurrent GBM: autopsy material, post bevacizumab.** GBM cells invade almost the whole brain of this recurrent GBM patient (2C, D). H&E stained autopsy specimen of neocortex of the hemisphere opposite to tumour location, 10 weeks after the last infusion of bevacizumab.
Figure 4
Schematic drawing: pre-treatment and during treatment. Schematic drawing of high-grade glioma, pre-treatment (A), and with anti-VEGF treatment (B). (A) Contrast leakage (white) occurs around leaky tumour vessels enhancing the tumour area on MRI. Capillaries in surrounding tissue are not leaky. (B) Contrast-enhanced area is strongly reduced under anti-VEGF treatment. Tumour cells migrate furtively into the surrounding tissue and co-opt existing vasculature.
we have shown this in mice bearing orthotopic intracranial tumours treated with TMZ either with or without concomitant anti-angiogenic treatment with vandetanib (ZD6474) [59]. More recently, it was reported that bevacizumab increases efficacy of TMZ [72] or carboplatin [73]. At this moment the cause of this apparent discrepancy remains unclear but effects of timing and dosing of anti-angiogenic therapy and chemotherapy may play a role here. For example, we previously demonstrated that high doses of vandetanib effectively inhibited angiogenesis and restored vessel permeability, whereas low doses did inhibit angiogenesis but left tumour vasculature hyper-permeable [33]. These features should be carefully investigated for clinical GBM too, to improve control of the different therapeutic modalities on tumour behaviour. Meanwhile, the discussion continues as to whether combining anti-angiogenic treatments with other chemotherapies is beneficial or detrimental (or somewhere in-between).

Conclusions
The relevance of angiogenesis inhibition in the treatment of GBM has been placed in the unique context of malignant brain tumours. Patients may benefit from anti-angiogenic therapy by its reduction of peritumoural oedema and intracranial pressure. Several studies also suggest an anti-tumour effect because of improved response rates and prolonged PFS; however, these data are derived from non-randomised trials with PFS as primary endpoint. Available data on survival prolongation are less robust (phase II) and sometimes even conflicting. High costs and side-effects of angiogenesis inhibitors (e.g. venous and arterial thrombo-embolism and haemorrhage as demonstrated in phase II studies) need to be balanced against advantages related to survival. Therefore, we can only implement angiogenesis inhibitors as standard treatment for patients with GBM when data are available from randomised clinical trials with survival as primary endpoint. Our concerns are not restricted to the limited amount of data on outcome and toxicity, but also arise from preclinical data on the biology of GBM and angiogenesis inhibition.

GBM can be roughly separated into an angiogenic component, and an invasive or migratory component. Although this latter component seems inert to anti-angiogenic therapy, it is of major importance for disease progression and survival (Figure 4). Whereas symptoms are tempered by anti-angiogenic treatment, furtive invasion of the disease continues, unrecognised by standard imaging modalities. Meanwhile, the discussion continues as to whether combining anti-angiogenic treatments with other chemotherapies is beneficial or detrimental (or somewhere in-between).

GBM can be roughly separated into an angiogenic component, and an invasive or migratory component. Although this latter component seems inert to anti-angiogenic therapy, it is of major importance for disease progression and survival (Figure 4). Whereas symptoms are tempered by anti-angiogenic treatment, furtive invasion of the disease continues, unrecognised by standard imaging modalities. Moreover, while treatment of other tumour types is improved by combining chemotherapy with anti-angiogenic drugs, inhibiting angiogenesis in GBM may antagonise the efficacy of chemotherapeutic drugs by normalising the BBB function. Although angiogenesis inhibition is of value for symptom reduction in GBM patients, the possible lack of a true anti-tumour effect raises concerns about the place of this type of therapy in the treatment of patients with GBM.

Summary
1. GBM can be roughly separated into an angiogenic component, and an invasive or migratory component.

2. Although this invasive or migratory component seems inert to anti-angiogenic therapy, it is of major importance for disease progression and survival.

3. Whereas symptoms are tempered by anti-angiogenic treatment, furtive invasion of the disease continues, unrecognised by standard imaging modalities.

4. Moreover, while treatment of other tumour types is improved by combining chemotherapy with anti-angiogenic drugs, inhibiting angiogenesis in GBM may antagonise the efficacy of chemotherapeutic drugs by normalising the BBB function.

5. Although angiogenesis inhibition is of value for symptom reduction in GBM patients, the possible lack of a true anti-tumour effect raises concerns about the place of this type of therapy in the treatment of GBM.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
JJC, OVT, LJAS, DJR, WPJL and WRVF designed the experiments and the study. JJC, OVT, AC, WPJL and WRVF collected data for the study. JJC, OVT, AC, MEVL and WPJL analyzed the data. JJC, OIAS, MEVL, DJR and WRVF enrolled patients. JJC and OVT wrote the first draft of the paper. JJC, OVT, AC, LJAS, MEVL, DJR, WPJL and WRVF contributed to the writing of the paper. All authors read and approved the final manuscript.

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References
1. Mikula S, Trotts I, Stone JM, Jones EG: Internet-enabled high-resolution brain mapping and virtual microscopy. Neuroimage 2007, 35:9-15.
2. Muldoon LL, Soussain C, Jahnke K, Johanson C, Siegal T, Smith QR, Hall WA, Hynynen K, Senter PD, Peereboom DM, et al.: Chemo-therapy delivery issues in central nervous system malignancy: a reality check. J Clin Oncol 2007, 25:2295-2305.
3. Scherer HJ: Structural development in gliomas. Am J Cancer 1938, 34:333-351.
4. Giese A, Westphal M: Glioma invasion in the central nervous system. Neurosurgery 1996, 39:235-250.
21. Pope WB, Demuth T, Berens ME: Autocrine factors that sustain glioma invasion and paracrine biology in the brain microenvironment. J Natl Cancer Inst 2007, 99:1583-1593.

22. Walker MD, Green SB, Byar DP, Alexander E Jr, Zbar B, Brooks J, Frant V, et al.: Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. N Engl J Med 1980, 303:1323-1329.

23. Gonzalez J, Kumar AJ, Conrad CA, Levin VA: Effect of bevacizumab on radiation necrosis of the brain. Int J Radiat Oncol Biol Phys 2007, 67:213-217.

24. Batchelor TT, Sorensen AG, di Tomaso E, Zhang WT, Duda DG, Cohen KS, Kozak KR, Cahill DP, Chen PJ, Zhu M, et al.: AZD 2171: a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. Cancer Cell 2007, 11:83-95.

25. Green R, Wosyshner E, Carvajal G, Nghiemphu P, Lai A, Cloughesy T: Early treatment with bevacizumab in patients with malignant glioma multiforme and gliosarcoma facilitates administration of radiation therapy by rapidly alleviating cerebral edema and improving neurological function. Neuro-Oncology 2007, 9:517-518.

26. Carlson MR, Pope WB, Horvath S, Braunstein JG, Nghiemphu P, Tso CL, Mellinghoff I, Lai A, Liu LM, Mishel PS, et al.: Relationship between survival and edema in malignant gliomas: role of vascular endothelial growth factor and neural perlin-2. Clin Cancer Res 2007, 13:2592-2598.

27. Yung WK, Albright RE, Olson J, Fredericks R, Fink K, Prados MD, Brada M, Spence A, Hohlf RJ, Shapiro VO, et al.: A phase II study of temozolomide vs. procarbazine in patients with glioblastoma multiforme at first recurrence. Br J Cancer 2000, 83:588-593.

28. Cloughesy TF, Prados MD, Wen PY, Mikelsken T, Abrey LE, Schill F, Yung WK, Maxia Z, Dimer Y, Friedman HS: A phase II, randomized, non-comparative clinical trial of the effect of bevacizumab (BV) alone or in combination with irinotecan (CPT) on six-month progression free survival (PF6) in recurrent, treatment-refractory glioblastoma (GBM). J Clin Oncol 2008, 26: May 20 suppl, 2010b.

29. Norden AD, Drappatz J, Muzikansky A, Gerard M, Kimura M, Nishida M, et al.: Regiographic of cerebral edema in patients with brain cancer. Neurology 2007, 69:512-518.

30. Galicich JH, French LA, Melby JC: Use of dexamethasone in treatment of cerebral edema associated with brain tumors. J Cancer 1961, 81:46-53.

31. Kamoun WS, Levy CD, Ferrer CT, Nishida M, et al.: Regression of brain metastases with bevacizumab and erlotinib compared with chemotherapy alone for treatment-refractory glioblastoma (GBM). J Clin Oncol 2008, 26: May 20 suppl, 2010b.

32. Clones A, Gammbarota G, Hamans B, van Tellingen O, Wesseling P, Wesseling P, Heerschap A, Ruiter D, Ryan A, de Waal R: Antiangiogenic therapy of cerebral melanoma metastases results in sustained tumor progression via vessel co-option. Clin Cancer Res 2004, 10:6222-6230.

33. Hoelzinger DB, Demuth T, Berens ME: Autocrine factors that sustain glioma invasion and paracrine biology in the brain microenvironment. J Natl Cancer Inst 2007, 99:1583-1593.

34. Walker MD, Green SB, Byar DP, Alexander E Jr, Zbar B, Brooks J, Frant V, et al.: Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. N Engl J Med 1980, 303:1323-1329.

35. Norden AD, Drappatz J, Muzikansky A, Gerard M, Kimura M, Nishida M, et al.: Regiographic of cerebral edema in patients with brain cancer. Neurology 2007, 69:512-518.

36. Galicich JH, French LA, Melby JC: Use of dexamethasone in treatment of cerebral edema associated with brain tumors. J Cancer 1961, 81:46-53.
43. Hytton N: Dynamic contrast-enhanced magnetic resonance imaging as an imaging biomarker. J Clin Oncol 2006, 24:2329-3298.

44. Bulakbasi N, Guvec I, Onguru O, Erdogan E, Tayfun C, Ucoz T: The added value of the apparent diffusion coefficient calculation to magnetic resonance imaging in the differentiation and grading of malignant brain tumors. J Comput Assist Tomogr 2004, 28:735-746.

45. Price SJ, Jena R, Burnet NG, Carpenter TA, Pickard JD, Gillard JH: Predicting patterns of glioma recurrence using diffusion tensor imaging. Eur Radiol 2007, 17:1675-1684.

46. Chenevert TL, Stegman LD, Taylor JM, Robertson PL, Greenberg HS, Rehemtulla A, Reis BD: Diffusion magnetic resonance imaging: an early surrogate marker of therapeutic efficacy in brain tumors. J Natl Cancer Inst 2000, 92:2029-2036.

47. Chen W, Delaloye S, Silverman DH, Geist C, Czernin J, Sayre J, Satya-murthy N, Pope W, Lai A, Phelps ME, 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, 25:4714-4721.

48. Stadlbauer A, Parante O, Nimsky C, Salomonowicz E, Buchfelder M, Kucher T, Unke R, Ganslandt O: Metabolic imaging of cerebral gliomas: spatial correlation of changes in O-(2-18F-fluoroethyl)-L-tyrosine PET and proton magnetic resonance spectroscopic imaging. J Nucl Med 2008, 49:721-729.

49. Vos MJ, Hoekstra OS, Barkhof F, Berkhof J, van Groenin-gen CJ, Vandenbos WP, Slotman BJ, Postma TJ: Thallium-201 single-photon emission computed tomography as an early predictor of outcome in recurrent glioma. J Clin Oncol 2003, 21:3559-3565.

50. Miller JC, Pien HH, Sahani D, Sorensen AG, Thrall JH: Imaging angiogenesis: applications and potential for drug development. J Nat Cancer Inst 2005, 97:172-187.

51. Folkman J: Seminars in Medicine of the Beth Israel Hospital, Boston. Clinical applications of research on angiogenesis. N Engl J Med 1997, 337:1757-1763.

52. Fisher BJ, Ellis LM: Neoplastic angiogenesis—not all blood vessels are created equal. N Engl J Med 2004, 351:215-216.

53. Jain RK: Oncology, basic science and management. Vol. 1: Angiogenesis in brain tumours. Nat Rev Neurosci 2007, 8:1002-1012.

54. Holsh J, Maisonpierre PC, Compton D, Boland P, Alexander CR, Zagzag D, Yancopoulos GD, Wiegand SJ: Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science 1999, 284:1994-1998.

55. Jain RK: Molecular regulation of vessel maturation. Nat Med 2003, 9:685-693.

56. Hicklin DJ, Ellis LM: Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. J Clin Oncol 2005, 23:1011-1027.

57. Jain RK: Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science 2005, 307:58-62.

58. Eichhorn ME, Striet S, Luedemann S, Kleespies A, Noth U, Lamszus K, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Menon U, Menon P, Lyomana A, Morice A, Chauvel P, Chauvel P, et al.: Tumor microcirculation in highly vascularized melanomas: added value of the apparent diffusion coefficient calculation and intravital fluorescence microscopy. Eur Radiol 2008, 18:169-177.

59. van Kempen LC, Leenders WP: Tumors can adapt to anti-angiogenic therapy depending on the stromal context: lessons from endothelial cell biology. Eur J Cell Biol 2006, 85:61-68.

60. Bergers G, Hanahan D: Modes of resistance to anti-angiogenic therapy. Nat Rev Cancer 2008, 8:592-603.

61. Lefranc F, Brochot J, Kiss R: Possible future issues in the treatment of glioblastomas: special emphasis on cell migration and the resistance of migrating glioblastoma cells to apoptosis. J Clin Oncol 2005, 23:2411-2422.

62. Lamszus K, Kunkei P, Westphal M: Invasion as limitation to antiangiogenic glioma therapy. Acta Neurochir Suppl 2003, 88:169-177.

63. van Kempen LC, Leenders WP: Tumors can adapt to anti-angiogenic therapy depending on the stromal context: lessons from endothelial cell biology. Eur J Cell Biol 2006, 85:61-68.

64. Martens T, Laabs Y, Gunther HS, Kemming D, Zhu Z, Witte L, Hagem C, Westphal M, Lamszus K: Inhibition of glioblastoma growth in a highly invasive nude mouse model can be achieved by targeting epidermal growth factor receptor but not vascular endothelial growth factor receptor-2. Clin Cancer Res 2008, 14:5447-5458.

65. Rubenstein J, Kim J, Ozawa T, Zhang M, Westphal M, Dene DF, Shuman MA: Anti-VEGF antibody treatment of glioblastoma prolongs survival but results in increased vascular cooption. Neoplasia 2000, 2:306-314.

66. Loges S, Mazzone M, Hohenwarter P, Carmeliet P: Silencing or fueling metastasis with VEGF inhibitors: antiangiogenesis revisited. Cancer Cell 2009, 15:167-170.

67. Ebos JM, Lee CR, Cruz-Munoz W, Bjarnason GA, Christensen JG, Kerbel RS: Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. Cancer Cell 2009, 15:233-239.

68. Paez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Vinals F, Inoue M, Bergers G, Hanahan D, Casanovas O: Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer Cell 2009, 15:220-231.

69. Du R, Lu KY, Petritsch C, Liu P, Ganss R, Passeygue E, Song H, Vandenberg S, Johnson RS, Werb Z, et al.: HIF-1 alpha induces the Recruitment of Bone Marrow-Derived Vascular Modulatory Cells to Regulate Tumor Angiogenesis and Invasion. Cancer Cell 2008, 13:206-220.

70. Narayana A, Kelly P, Golfinos J, Parker E, Johnson G, Knopp E, Zagzag D, Fischer I, Raza S, Medabalmi P, et al.: Antiangiogenic therapy using bevacizumab in recurrent high-grade glioma: impact on local control and patient survival. J Neurosurg 2009, 110:173-180.

71. Hegi ME, Dieras V, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros J, Hainfellner JA, Mason W, Mariani L, et al.: MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 2005, 352:997-1003.

72. Mathieu V, De NN, Le MM, Deville J, Gasson JF, Dehoux M, Kiss R, Lefranc F: Combining bevacizumab with temozolomide increases the antitumor efficacy of temozolomide in a human glioblastoma orthotopic xenograft model. Neurosurgery 2008, 10:1383-1392.

73. Jahnke K, Muldoon LL, Varallyay CG, Lewin SJ, Kraemer DF, Neuwelt EA: Bevacizumab and carbolipin increase survival and asymptomatic tumor volume in a glioma model. Neuro Oncol 2009, 11:142-150.

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