The Oncologist®

Clinical Trial Results

Phase II Trial of Bevacizumab in Combination With Temozolomide as First-Line Treatment in Patients With Metastatic Uveal Melanoma

Sophie Piperno-Neumann,[a] Alhassane Diallo,[b] Marie-Christine Etienne-Grimaldi,[f] François-Clément Bidard,[a] Manuel Rodrigues,[a] Corine Plancher,[b] Pascale Mariani,[c] Nathalie Cassoux,[c] Didier Decaudin,[e] Bernard Asselain,[b] Vincent Servois[d]

Departments of [a]Medical Oncology, [b]Biostatistics, [c]Surgical Oncology, and [d]Radiology and Nuclear Medicine, and [e]Preclinical Investigation Laboratory, Institut Curie, Paris, France; [f]Onco-Pharmacology Laboratory, Centre Antoine Lacassagne, Nice, France

Trial Information

- **European Clinical Trials Identifier:** EudraCT 2009-011751-46
- **Sponsor:** Institut Curie
- **Principal Investigator:** Sophie Piperno-Neumann
- **IRB Approved:** Yes

Lessons Learned

- Trials dedicated to metastatic uveal melanoma are needed because of the poor prognosis of this rare cancer and because its biology is distinct from that of cutaneous melanoma.
- Agents targeting the MEK/ERK/MAP kinase pathways are being tested.

Abstract

Background. In experimental models, bevacizumab suppressed in vitro growth and in vivo hepatic metastasis of ocular melanoma cells. Additional preclinical data suggested a potential benefit when combining bevacizumab with dacarbazine.

Methods. This noncomparative phase II study evaluated a combination of bevacizumab (10 mg/kg on days 8 and 22) with temozolomide (150 mg/m^2 on days 1–7 and 15–21) in 36 patients with metastatic uveal melanoma (MUM). The primary endpoint was the progression-free rate (PFR) at 6 months. Using a modified 2-step Fleming plan, at least 10 of 35 patients were required to support a predefined PFR at 6 months of 40%. Secondary objectives were progression-free survival (PFS), overall survival (OS), and safety; liver perfusion computed tomography (CT) for response imaging; and impact of *VEGF-A* gene polymorphisms on bevacizumab pharmacodynamics.

Results. First- and second-step analyses revealed nonprogression at 6 months in 3 of 17 and 8 of 35 patients, respectively. Finally, the 6-month PFR was 23% (95% confidence interval [CI]: 10–39), with long-lasting stable disease in 5 patients (14%). Median PFS and OS were 12 weeks and 10 months, respectively. No unexpected toxicity occurred. Liver perfusion CT imaging was not useful in assessing tumor response, and *VEGF-A* gene polymorphisms were not correlated with toxicity or survival.

Conclusion. In patients with MUM, a combination of bevacizumab plus temozolomide achieved a 6-month PFR of 23%. ***The Oncologist*** 2016;21:281–282f

Discussion

Up to 50% of patients with uveal melanoma (UM) develop metastases mainly to the liver [1]. Metastatic uveal melanoma (MUM) has a poor prognosis; survival rates have remained unchanged for decades [2]. Historically, treatments for metastatic cutaneous melanoma have been applied to patients with MUM, despite the diseases' distinct biologies [3]. Various chemotherapy agents have been tested; the response rates ranged from 0% to 15%, with median OS and PFS of 6–12 months and 3 months, respectively [4]. Because systemic treatments, so far, have had so little impact on survival, the current standard of care for patients with MUM is, thus, clinical trial participation.

Low-dose temozolomide (TMZ) exhibits antiangiogenic activity in several tumor models, including UM xenografts [5]. A phase II study in 14 patients with MUM reported stable disease in 2 patients and a median PFS of 1.8 months [6].

In an orthotopic UM mouse model, bevacizumab (BEV) by intraperitoneal injection suppressed primary tumor growth and the formation of hepatic micrometastasis [7]. Malignant melanocytes exposed to dacarbazine dramatically upregulate vascular endothelial growth factor (VEGF) production [8], suggesting a potential antitumor benefit might be achieved by adding an anti-VEGF agent to dacarbazine. The SAKK 50/07 trial combining TMZ and BEV in 62 patients with metastatic

Correspondence: Sophie Piperno-Neumann, M.D., Department of Medical Oncology, Institut Curie, 26 rue d'Ulm, 75248 Paris cedex 05, France. Telephone: 33(0)-1-44-32-40-68; E-Mail: sophie.piperno-neumann@curie.fr Received December 9, 2015; accepted for publication January 11, 2016; published Online First on February 24, 2016. http://dx.doi.org/10.1634/theoncologist.2015-0501

Table 1. Patient characteristics at baseline

Characteristic	Patient data ($n = 35$)[a]
Age, years, median (range)	55 (29–72)
Male/female (%)	19/16 (54/46)
Primary tumor, mm, median (range)	
LTD	15 (13–18)
Thickness	7.7 (5.5–10)
Primary tumor, treatment (%)	
Proton beam therapy	22 (63)
Enucleation	10 (28)
Brachytherapy	3 (9)
Time to metastasis[b], months, median (range)	38 (17–62)
ECOG performance status (%)	
0	28 (80)
1	7 (20)
Metastatic sites (%)	
Liver only	29 (83)
Liver + other site	5 (14)
Lung only	1 (3)
Elevated LDH (%), >UNL	10 (29)
Size of the largest metastasis, cm, median (range)	3 (3–15)
Prior metastasis treatment (%)	
Liver surgery	2 (6)
Liver RFA	2 (6)
Extrahepatic surgery	2 (6)

[a]Data given as no. (%) unless otherwise indicated.
[b]Time elapsed between diagnosis of primary ocular tumor and metastasis.
Abbreviations: ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; LTD, largest tumor diameter; RFA, radiofrequency ablation; UNL, upper normal limit.

melanoma reported response and survival rates significantly higher in patients with wild-type BRAF melanoma [9].

In this phase II, single-arm, single-institution study (approved by both an ethics committee and health authorities; European Clinical Trials Identifier: EudraCT 2009-011751-46), we evaluated the 6-month progression-free rate (PFR) with first-line treatment in patients with MUM. From May 2010 to May 2012, 36 patients with MUM were enrolled. The treatment plan included six 28-day cycles of BEV 10 mg/kg (on days 8 and 22) and TMZ 150 mg/m^2 (on days 1–7 and 15–21), followed by BEV maintenance in patients whose disease had not progressed.

Disease imaging (CT or magnetic resonance imaging) was performed every three cycles according to RECIST criteria version 1.0 [10]. Adverse events were assessed according to the National Cancer Institute's Common Toxicity Criteria version 3.0.

We studied prospectively the influence of *VEGF-A* gene polymorphisms on BEV pharmacodynamics in patients with MUM, as well as the role of liver perfusion CT imaging for response prediction. Liver perfusion CT imaging was scheduled at baseline, and after 1 and 3 months of treatment; target lesion analysis comprised RECIST evaluation and measurement of perfusion parameters. *VEGF-A* polymorphisms were analyzed by polymerase chain reaction restriction fragment length polymorphism on DNA extracted from a 9-mL blood sample [11].

All 35 evaluable patients (Table 1) received a median number of 4 treatment cycles (range: 2–6 cycles). With a median follow-up of 26 months (range: 19–40 months), stable disease ≥6 months was the best response in 8 patients. The 6-month PFR was 23% (95% CI: 10%–39%). Median PFS and OS were 12 weeks (95% CI: 11–24 weeks) and 10 months (95% CI: 8–15 months), respectively (Figs. 1, 2). This combination was tolerable, but did not reach the planned 6-month PFR in patients with MUM.

Trial Information

Disease	Uveal melanoma
Stage of disease / treatment	Metastatic / Advanced
Prior Therapy	None
Type of study - 1	Phase II
Type of study - 2	Single Arm
Primary Endpoint	6-month PFR
Secondary Endpoint	Progression-Free Survival
Secondary Endpoint	Overall Survival
Secondary Endpoint	Overall Response Rate
Secondary Endpoint	Safety
Secondary Endpoint	Tolerability
Secondary Endpoint	Influence of VEGF-A gene polymorphisms on bevacizumab pharmacodynamics
Secondary Endpoint	Liver perfusion computed tomography for response prediction
Additional Details of Endpoints or Study Design	We hypothesized that bevacizumab could not provide an objective response except for long-lasting stable disease. The 6-month PFR was chosen as a reasonable endpoint, and the number of patients was calculated, based on the following assumptions: a 6-month PFR of 15% with conventional chemotherapy [4] and an expected 6-month PFR of 40% with the BEV-TMZ combination. A 2-step Fleming design was used to allow for early discontinuation in the event of insufficient efficacy (type I error 3%; type II error 6%). Initially, 17 patients were to be recruited in the first step. If fewer than 3 of the 17 patients were progression-free at 6 months, the trial

	would be discontinued owing to lack of clinical efficacy. Otherwise, an additional 18 patients would be enrolled, for a total of 35 evaluable patients. At the end of the second step, if no more than 9 of the 35 patients were progression-free at 6 months, the combination would be considered as poorly effective; if 10 or more patients were progression-free at 6 months, the BEV-TMZ combination would be considered worthy of further testing.
Investigator's Analysis	No sufficient activity for further development

Drug Information

Drug 1	
Generic/Working name	Bevacizumab
Trade name	Avastin
Company name	Genentech
Drug type	Antibody
Drug class	Angiogenesis - VEGF
Dose	10 mg/kg
Route	IV
Schedule of Administration	Days 8 and 22 in 28-day cycle × 6 cycles; maintenance in nonprogressive patients
Drug 2	
Generic/Working name	Temozolomide
Trade name	Temodal
Company name	Merck
Drug type	Chemotherapy
Drug class	Alkylating agent
Dose	150 mg/m^2
Route	Oral
Schedule of Administration	Days 1–7 and 15–21 in 28-day cycle × 6 cycles.

Patient Characteristics

Number of patients, male	19
Number of patients, female	16
Stage	Stage IV / metastatic
Age	Median (range): 55 years (29–72 years)
Number of prior systemic therapies	Median (range): 0
Performance Status: ECOG	0 — 28 1 — 7 2 — 0 3 — 0 unknown — 0
Other	Eastern Cooperative Oncology Group 4 = 0
Cancer Types or Histologic Subtypes	Uveal Melanoma 35

Primary Assessment Method

Control Arm: Total Patient Population	
Number of patients screened	37
Number of patients enrolled	36
Number of patients evaluable for toxicity	35
Number of patients evaluated for efficacy	35
Response assessment CR	*n* = 0 (0%)
Response assessment PR	*n* = 0 (0%)
Response assessment SD	*n* = 8 (23%)
Response assessment PD	*n* = 27 (77%)
Six-month progression-free rate	23

(Median) duration assessments PFS	12 weeks
(Median) duration assessments OS	10 months
(Median) duration assessments duration of treatment	4 months

Adverse Events

Adverse Events At All Dose Levels, Cycle 1

Name	*NC/NA	1	2	3	4	5	All Grades
Hemoglobin	83%	14%	3%	0%	0%	0%	17%
Leukocytes (total WBC)	71%	14%	6%	6%	3%	0%	29%
Neutrophils/granulocytes (ANC/AGC)	79%	3%	6%	3%	9%	0%	21%
Platelets	48%	31%	9%	6%	6%	0%	52%
Febrile neutropenia (fever of unknown origin without clinically or microbiologically documented infection) (ANC $<1.0 \times 10^9$/L, fever $\geq$38.5°C)	100%	0%	0%	0%	0%	0%	0%
Fever (in the absence of neutropenia, where neutropenia is defined as ANC $<1.0 \times 10^9$/L)	94%	6%	0%	0%	0%	0%	6%
Fatigue (asthenia, lethargy, malaise)	48%	43%	9%	0%	0%	0%	52%
Pruritus/itching	91%	6%	3%	0%	0%	0%	9%
Rash/desquamation	97%	3%	0%	0%	0%	0%	3%
Nausea	71%	20%	9%	0%	0%	0%	29%
Gastrointestinal - abdominal pain	77%	23%	0%	0%	0%	0%	23%
Diarrhea	94%	6%	0%	0%	0%	0%	6%
Constipation	60%	26%	11%	3%	0%	0%	40%
Pain - myalgia	91%	9%	0%	0%	0%	0%	9%
Hemorrhage/bleeding	91%	9%	0%	0%	0%	0%	9%
Coagulation - thromboembolic event	100%	0%	0%	0%	0%	0%	0%
Hypertension	88%	6%	6%	0%	0%	0%	12%
Proteinuria	67%	21%	12%	0%	0%	0%	33%
Creatinine	91%	9%	0%	0%	0%	0%	9%
Bilirubin (hyperbilirubinemia)	91%	9%	0%	0%	0%	0%	9%
AST, SGOT (serum glutamic oxaloacetic transaminase)	54%	46%	0%	0%	0%	0%	46%
ALT, SGPT (serum glutamic pyruvic transaminase)	46%	51%	3%	0%	0%	0%	54%

Adverse Events Legend
*No Change from Baseline/No Adverse Event
The administered-dose intensity closely matched the planned schedule in the 35 treated patients. The most commonly reported treatment-related adverse events were grade 1 or 2 nausea, constipation, and abdominal pain. Seven patients experienced grade 3 toxicity: four patients had neutropenia, and three had either thrombocytopenia, constipation, or pruritus. Nine patients were affected by grade 4 toxicity, consisting of neutropenia in 3, thrombocytopenia in 4, and venous thromboembolism in 1. All adverse events related to bevacizumab, such as proteinuria or hypertension, were grades 1 to 2, and did not require temporarily suspending or discontinuing bevacizumab.

Serious Adverse Events

Name	Grade	Attribution
Febrile neutropenia	4	TMZ
Pneumonitis	4	TMZ
Vomiting	4	Disease progression

Serious Adverse Events Legend
Serious adverse events were reported in 2 patients, namely, grade 4 febrile neutropenia and pneumonitis in 1, and grade 4 vomiting in the other.

Assessment, Analysis, and Discussion

Completion	Study completed
Pharmacokinetics / Pharmacodynamics	Not Collected
Investigator's Assessment	No sufficient activity for further development

Uveal melanoma preferentially spreads to the liver hematogenously. Vascular density and expression of angiogenic factors in the primary tumor are associated with poor prognosis [12]. A combination of low-dose TMZ and BEV has been shown to be synergistic in reducing tumor angiogenesis and increasing survival in glioblastoma-bearing mice. Three

mechanisms have been implicated: (a) decreased nutrient supply for tumor repopulation, (b) vascular network normalization facilitating cytotoxic drug diffusion into the tumor, and (c) enhancement of chemotherapy-induced antiangiogenic effects [13]. Preclinical experiments with BEV were conducted in five UM patient-derived xenografts (PDXs) obtained from primary tumors or liver metastasis, as already described [7]. Tumor growth inhibition ranged from 33% to 89% in all 5 UM PDXs tested, and these models also displayed a high sensitivity to TMZ (supplemental online Figure 1).

The study's enrollment has been completed in 2 years, reflecting the lack of standard of care in this rare tumor with a very poor prognosis when it metastasizes. The tested combination had an acceptable safety profile, consistent with published data: 2 patients experienced serious adverse events, and 45% of patients had reversible grade 3–4 toxicities.

Our primary endpoint was not met. The hypothesis might have been too optimistic, with a targeted 6-month PFR of 40% in a small sample of 35 evaluable patients. In a randomized phase II trial comparing selumetinib versus dacarbazine or TMZ in 120 patients receiving first-line treatment for MUM, Carvajal et al. reported a 6-month PFR of 23%, and a median PFS of 15.9 weeks in the selumetinib arm versus 5.7% and 7 weeks in the conventional chemotherapy arm, respectively [14].

Five patients displayed long-lasting stable disease (11–35 months) during BEV maintenance therapy. Of these, 4 were still alive at 27–47 months from the date of inclusion. All five patients had liver metastases, and two of them also had lung lesions. The disease-free interval from the primary tumor diagnosis was short for 2 patients (14 and 22 months), but longer than expected for the others (4, 12, and 14 years). Furthermore, three patients received a second line of treatment and experienced some subsequent slow metastatic progression.

Bevacizumab's mechanism of action in intraocular tumors is far from understood. A recent study revealed that an intraocular BEV injection stimulated the growth of B16 melanoma cells placed into the anterior chamber of murine eyes [15]. Interestingly, in vitro exposure of B16 and human uveal melanoma cells to BEV resulted in paradoxical VEGF-A upregulation involving the HIF-1α pathway. In another experiment, BEV did not dramatically impact VEGF-A inhibition of cytokine expression in three different UM cell lines, suggesting compensatory mechanisms might reduce the drug's effects following BEV administration [16]. Ischemic conditions caused by anti-VEGF treatment may lead to the recruitment of proangiogenic bone marrow-derived cells, as demonstrated in glioblastoma [17]. UM tumors in patients whose survival is poor contain M2 macrophages, rendering this hypothesis plausible [18]. Another hypothesis might be that VEGF expression is modulated by UM cells themselves, either by the tumor microenvironment or via VEGF inhibitors. Further research appears warranted in this area.

Our prospective analysis of an association of *VEGF-A* gene polymorphisms and toxicity and patient outcome with bevacizumab-based therapy in MUM did not find an association with any of the five functional analyzed *VEGF-A* polymorphisms in this small cohort (supplemental online Table 1), as previously reported in a larger study with BEV in metastatic breast cancer [19].

CT perfusion imaging is a useful tool for assessing the vascularization of liver metastasis, with improved quantification of tumor neoangiogenesis [20]. The feasibility of CT perfusion was clearly demonstrated by our study, and the hypervascularity of UM liver metastases was confirmed by significantly increased blood flow and blood volume values compared with normal liver (Table 2), as previously shown in liver metastases from carcinoid tumors [21]. To minimize the variations in perfusion parameter measurements related to patient characteristics (i.e., cardiovascular condition, extent of liver metastases, or underlying liver disease), the analysis was conducted on paired samples, each patient acting as his or her own control. Moreover, our acquisition parameters complied with the current international guidelines [22]. In contrast with most studies on primary and secondary liver tumors, we showed that baseline permeability surface-area product (PS) measured at the most vascularized metastatic area was lower than that of normal liver parenchyma. No significant difference in perfusion parameters was seen before and after 1 or 3 months of treatment (Table 3). To date, only one study reported PS to be lower in liver metastases from neuroendocrine tumors than in normal liver [23].

Tumor vessels generally exhibit larger pores than normal liver capillaries; exchanges between compartments are increased, allowing small molecules like iodinated contrast agents to diffuse more rapidly. PS values, which reflect the abundance and permeability of tumor vessels, are thus usually higher. According to recent data, the vascularization of UM is partly due to a mechanism, "vasculogenic mimicry," that is distinct from the tumor angiogenic switch, and this may provide UM with an alternative microcirculation [24]. Thereby, tumor lesions are vascularized by channels directly lined with tumor cells but devoid of endothelial cells, and independently of angiogenesis. These connecting loops of circulating channels directly join normal vessels involved in tumor growth. We thus assume that the iodinated contrast agents used in CT diffuse more rapidly in the interstitial compartment. Given this scenario, the bicompartmental (i.e., intravascular and interstitial) model usually relied on in CT perfusion imaging may not be appropriate in this particular cancer. Further studies are needed to better understand blood supply patterns in UM and develop new imaging techniques.

In conclusion, this combination of BEV with TMZ for first-line therapy of MUM demonstrated an acceptable safety profile and a low 6-month PFR of 23% despite long-lasting stable disease in 14% of patients. *VEGF-A* gene polymorphisms were not able to discriminate patients without significant toxicity or clinical activity with the combination. We were unable to document the usefulness of hepatic CT perfusion imaging in assessing response compared with RECIST criteria, but we observed lower PS values in UM liver metastases than in normal liver parenchyma.

Acknowledgments

We thank the patients who participated in the study and their families, the investigators and Unité de Gestion des Essais Cliniques at Institut Curie for their helpful assistance in trial coordination and administrative issues management, as well as Dr. N. Ady-Vago from Roche France who made the study possible, and Dr. G. Cremer for medical writing assistance

(copyediting and editorial assistance). The study was funded by the French Ministry of Health (French national program for clinical research PHRC 2010-02-59) and Roche France. Results were presented in part at the American Society of Clinical Oncology Annual Meetings (Melanoma Poster Session) held in 2012 and 2013 in Chicago, Illinois.

Disclosures

Sophie Piperno-Neumann: Roche France (RF); **Manuel Rodrigues**: Hoffman-La Roche (Other). The other authors indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/ inventor/patent holder; (SAB) Scientific advisory board

References

1. Diener-West M, Reynolds SM, Agugliaro DJ et al. Development of metastatic disease after enrollment in the COMS trials for treatment of choroidal melanoma: Collaborative Ocular Melanoma Study Group Report No. 26. Arch Ophthalmol 2005;123: 1639–1643.

2. Augsburger JJ, Corrêa ZM, Shaikh AH. Effectiveness of treatments for metastatic uveal melanoma. Am J Ophthalmol 2009;148:119–127.

3. Van Raamsdonk CD, Bezrookove V, Green G et al. Frequent somatic mutations of *GNAQ* in uveal melanoma and blue naevi. Nature 2009;457:599–602.

4. Leyvraz S, Keilholz U. Ocular melanoma: What's new? Curr Opin Oncol 2012;24:162–169.

5. Némati F, Sastre-Garau X, Laurent C et al. Establishment and characterization of a panel of human uveal melanoma xenografts derived from primary and/or metastatic tumors. Clin Cancer Res 2010;16:2352–2362.

6. Bedikian AY, Papadopoulos N, Plager C et al. Phase II evaluation of temozolomide in metastatic choroidal melanoma. Melanoma Res 2003;13: 303–306.

7. Yang H, Jager MJ, Grossniklaus HE. Bevacizumab suppression of establishment of micrometastases in experimental ocular melanoma. Invest Ophthalmol Vis Sci 2010;51:2835–2842.

8. Lev DC, Ruiz M, Mills L et al. Dacarbazine causes transcriptional up-regulation of interleukin 8 and vascular endothelial growth factor in melanoma cells: A possible escape mechanism from chemotherapy. Mol Cancer Ther 2003;2:753–763.

9. von Moos R, Seifert B, Simcock M et al. First-line temozolomide combined with bevacizumab in metastatic melanoma: A multicentre phase II trial (SAKK 50/07). Ann Oncol 2012;23:531–536.

10. Therasse P, Arbuck SG, Eisenhauer EA et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000; 92:205–216.

11. Formento JL, Etienne-Grimaldi MC, Francoual M et al. Influence of the VEGF-A 936C>T germinal polymorphism on tumoral VEGF expression in head and neck cancer. Pharmacogenomics 2009;10: 1277–1283.

12. Notting IC, Missotten GS, Sijmons B et al. Angiogenic profile of uveal melanoma. Curr Eye Res 2006;31:775–785.

13. Mathieu V, De Nève N, Le Mercier M et al. Combining bevacizumab with temozolomide increases the antitumor efficacy of temozolomide in a human glioblastoma orthotopic xenograft model. Neoplasia 2008;10:1383–1392.

14. Carvajal RD, Sosman JA, Quevedo JF et al. Effect of selumetinib vs chemotherapy on progression-free survival in uveal melanoma: A randomized clinical trial. JAMA 2014;311:2397–2405.

15. el Filali M, Ly LV, Luyten GP et al. Bevacizumab and intraocular tumors: An intriguing paradox. Mol Vis 2012;18:2454–2467.

16. Logan P, Burnier J, Burnier MN Jr. Vascular endothelial growth factor expression and inhibition in uveal melanoma cell lines. Ecancermedicalscience 2013;7:336.

17. Du R, Lu KV, Petritsch C et al. HIF1alpha induces the recruitment of bone marrow-derived vascular modulatory cells to regulate tumor angiogenesis and invasion. Cancer Cell 2008;13:206–220.

18. Bronkhorst IH, Ly LV, Jordanova ES et al. Detection of M2-macrophages in uveal melanoma and relation with survival. Invest Ophthalmol Vis Sci 2011;52:643–650.

19. Etienne-Grimaldi MC, Formento P, Degeorges A et al. Prospective analysis of the impact of VEGF-A gene polymorphisms on the pharmacodynamics of bevacizumab-based therapy in metastatic breast cancer patients. Br J Clin Pharmacol 2011;71: 921–928.

20. García-Figueiras R, Goh VJ, Padhani AR et al. CT perfusion in oncologic imaging: A useful tool? AJR Am J Roentgenol 2013;200:8–19.

21. Ng CS, Charnsangavej C, Wei W et al. Perfusion CT findings in patients with metastatic carcinoid tumors undergoing bevacizumab and interferon therapy. AJR Am J Roentgenol 2011;196:569–576.

22. Miles KA, Lee TY, Goh V et al. Current status and guidelines for the assessment of tumour vascular support with dynamic contrast-enhanced computed tomography. Eur Radiol 2012;22:1430–1441.

23. Ng CS, Hobbs BP, Chandler AG et al. Metastases to the liver from neuroendocrine tumors: Effect of duration of scan acquisition on CT perfusion values. Radiology 2013;269:758–767.

24. Folberg R, Hendrix MJ, Maniotis AJ. Vasculogenic mimicry and tumor angiogenesis. Am J Pathol 2000;156:361–381.

Figures and Tables

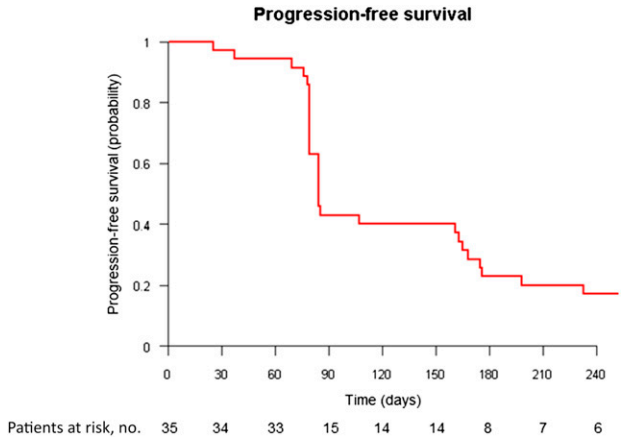


Figure 1. Kaplan-Meier curve of progression-free survival.

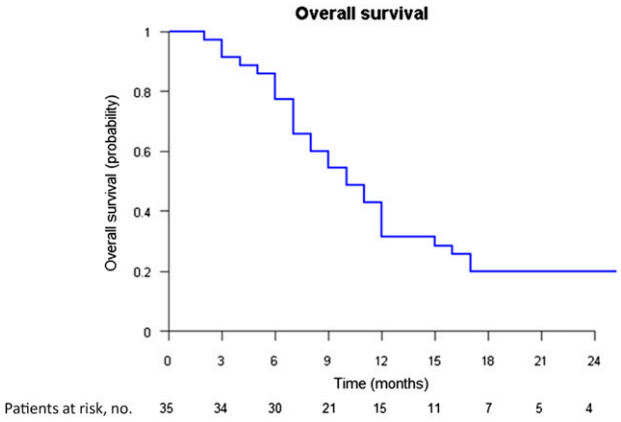
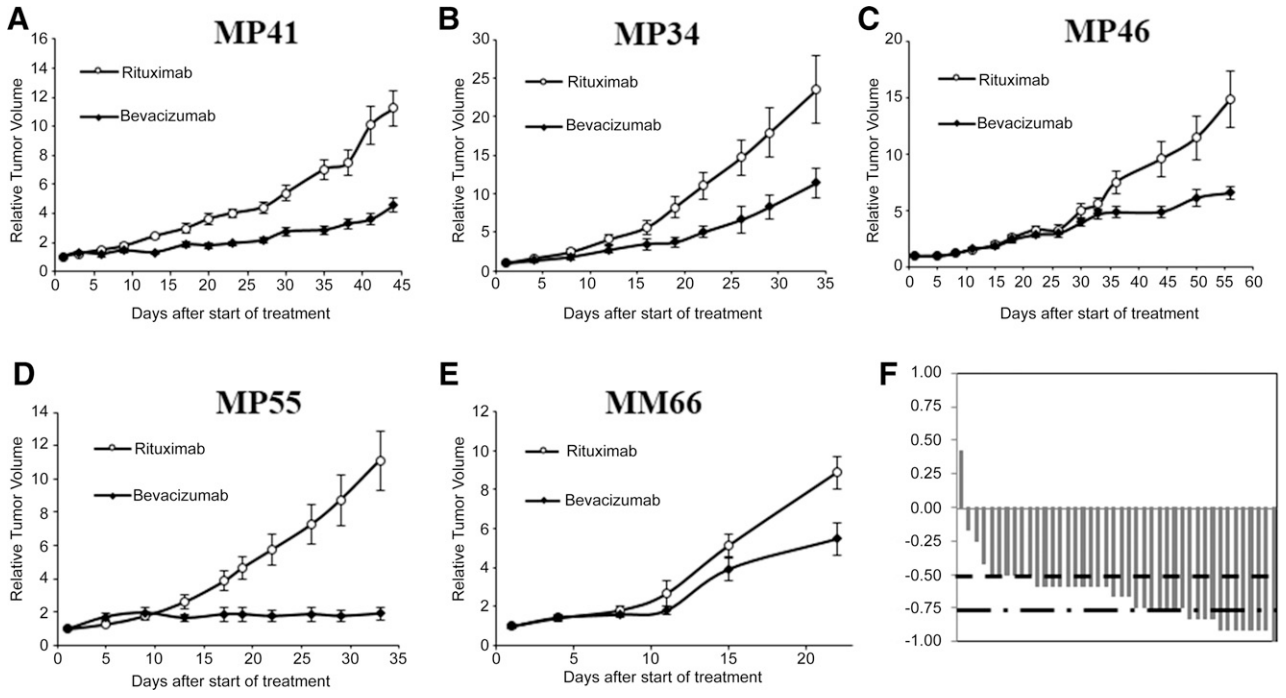


Figure 2. Kaplan-Meier curve of overall survival.



Supplemental Figure 1. In vivo responses of UM PDXs to bevacizumab. Bevacizumab (♦) was administered intraperitoneally at a dose of 10 mg/kg twice a week in MP34 **(A)**, MP41 **(B)**, MP46 **(C)**, MP55 **(D)**, and MM26 **(E)** UM PDXs. Mice in the control group (O) received rituximab with the same schedule as the treated animals. Tumor growth was evaluated by plotting the mean of the relative tumor volume ± SD per group. Between 8 to 10 mice per group were included in in vivo experiments. Overall response rate in all bevacizumab-treated mice **(F)**.

Table 2. Perfusion CT parameters of liver metastasis and normal liver parenchyma at baseline ($n = 32$)[a]

Parameter	Metastasis (median)	Normal liver (median)	*p* value
BF (mL/100 g/min)	372.5	225.5	.0018
BV (mL/100 g)	36.5	20.5	.0007
MTT (sec)	5	6	.4984
PS (mL/100 g/min)	57	66	.0311

[a]CT perfusion images were obtained after injecting 50 mL of nonionic contrast agent (Ultravist 370 mg/mL; Bayer Schering Pharma; Berlin, Germany, http://pharma.bayer.com) using a 64-row multidetector CT scanner (VCT; GE Healthcare, Waukesha, WI, http://www3.gehealthcare.com) and analyzed with the CT perfusion software 4 (version 4.3.1, Advantage Windows 4.5; GE Healthcare).
Abbreviations: BF, blood flow; BV, blood volume; MTT, mean transit time; PS, permeability surface-area product.

Table 3. Perfusion CT parameters in liver metastasis at baseline vs. 1 month and 3 months after treatment[a]

Parameter	Baseline (n =32) Median	1 month after treatment (n = 29) Median	1 month after treatment (n = 29) p value	3 months after treatment (n = 24) Median	3 months after treatment (n = 24) p value
BF (mL/100 g/min)	372.5	316	.61	297	.53
BV (mL/100 g)	36.5	37	.24	32	.18
MTT (sec)	5	7	.77	6	.88
PS (mL/100 g/min)	57	44	.27	46	.07

[a]Continuous variables were expressed as mean ± SD. Perfusion parameters between were compared using Wilcoxon signed-rang test. The statistical analysis of median values was conducted on paired samples, each patient acting as his own control.
Abbreviations: BF, blood flow; BV, blood volume; MTT, mean transit time; PS, permeability surface area product.

Supplemental Table 1. Linkage disequilibria between *VEGFA* gene polymorphisms[a]

		−2578[b] C>A			−1498[c] T>C			−1154[d] G>A			−634[e] G>C		
		CC	CA	AA	TT	TC	CC	GG	GA	AA	GG	GC	CC
−1498 T>C	**TT**	8	0	0									
	TC	0	18	0									
	CC	0	1	5									
			$p < .001$[f]										
−1154G>A	**GG**	8	3	0	8	3	0						
	GA	0	15	2	0	15	2						
	AA	0	1	3	0	0	4						
			$p < .001$			$p < .001$							
−634G>C	**GG**	2	9	5	2	8	6	3	9	4			
	GC	2	10	0	2	10	0	4	8	0			
	CC	4	0	0	4	0	0	4	0	0			
			$p = .001$			$p < .001$			$p = .013$				
−936C>T	**CC**	6	11	5	6	10	6	7	11	4	11	8	3
	CT	2	8	0	2	8	0	4	6	0	5	4	1
	TT	0	0	0	0	0	0	0	0	0	0	0	0
			ns			ns			ns			ns	

[a]Polymerase chain reaction–restriction fragment length polymorphism on DNA from a baseline 9-mL blood sample (Paxgene Blood DNA kit; Qiagen) in 32 patients. The influence of the different *VEGF-A* gene polymorphisms, considered as binary variables (−2578 CC vs. CA+AA, −1498 CC+CT vs. TT, −1154 AA+AG vs. GG, −634 GG vs. GC+CC, 936 CC vs. CT+TT), was tested using the Fisher's exact test for toxicity and using the log-rank test for progression-free survival and overall survival.
[b]−2578 C>A (rs 699947): The literature is inconsistent regarding the minor allele. In this study, A was found to be the minor allele; AA (n = 5) and CA (n = 19) patients were thus regrouped and compared with CC (n = 8).
[c]−1498 C>T (rs 833061): C and T allele frequencies are similar in white people. In this study, CT (n = 18) and CC (n = 6) were regrouped and compared with TT (n = 8).
[d]−1154 G>A (rs 1570360): G is the most common allele; AG (n = 17) and AA (n = 4) patients were thus regrouped and compared with GG (n = 11).
[e]−634 G>C (rs 2010963): G is the most common allele; GC (n = 12) and CC (n = 4) patients were thus regrouped and compared to GG (n = 16).
[f]p values of Fischer's exact test are given.
[g]−936 C>T (rs 3025039): C is the most common allele, and there was no homozygous patient for the minor allele; CC (n = 22) patients were thus compared with CT (n = 10).
Abbreviations: NS, not statistically significant.