Clinical Trial Results

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

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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/m² 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%.

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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
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/m² (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 bevacizumab 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.

### 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)      | 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 metastasisb, 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)                  |

aData given as no. (%) unless otherwise indicated.

bTime 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.

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Bevacizumab Plus Temozolomide for Metastatic UM

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**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/m²
- **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
**Adverse Events**

| 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 × 10^9/L, fever ≥38.5°C) | 100%   | 0%  | 0%  | 0%  | 0%  | 0%  | 0%         |
| Fever (in the absence of neutropenia, where neutropenia is defined as ANC <1.0 × 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 bicompartamental (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.

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DISCLOSURES

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

(CA) 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.

FIGURES AND TABLES

Figure 1. Kaplan-Meier curve of progression-free 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 (○) 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)§

| 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   |

§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

| Parameter | Baseline (n = 32) | 1 month after treatment (n = 29) | 3 months after treatment (n = 24) |
|-----------|------------------|---------------------------------|----------------------------------|
|           | Median           | Median p value                   | Median 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                           |

*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

|          | −2578<sup>b</sup> C>A | −1498<sup>c</sup> T>C | −1154<sup>d</sup> G>A | −634<sup>e</sup> G>C |
|----------|-----------------------|-----------------------|-----------------------|---------------------|
|          | CC        | CA       | AA       | TT        | TC       | CC       | GG       | GA       | AA       | GG       | GC       | CC       |
| −1498 T>C | TT       | 0        | 0        | 8        | 3        | 0        | 8        | 3        | 0        | 8        | 3        | 0        |
|          | TC       | 0        | 18       | 0        | 15       | 2        | 0        | 15       | 2        | 0        | 15       | 2        |
|          | CC       | 0        | 1        | 5        | 4        | 0        | 4        | 0        | 4        | 0        | 4        | 0        |
| p        | <.001     | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    |
| −1154G>A | GG       | 8        | 3        | 0        | 8        | 3        | 0        | 8        | 3        | 0        | 8        | 3        |
|          | GA       | 0        | 15       | 2        | 0        | 15       | 2        | 0        | 15       | 2        | 0        | 15       |
|          | AA       | 0        | 1        | 3        | 0        | 0        | 4        | 0        | 4        | 0        | 4        | 0        |
| p        | <.001     | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    |
| −634G>C  | GG       | 2        | 9        | 5        | 2        | 8        | 6        | 3        | 9        | 4        | 8        | 0        |
|          | GC       | 2        | 10       | 0        | 2        | 10       | 0        | 4        | 8        | 0        | 8        | 0        |
|          | CC       | 4        | 0        | 0        | 4        | 0        | 0        | 4        | 0        | 0        | 4        | 0        |
| p        | <.001     | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    |
| −936C>T  | CC       | 6        | 11       | 5        | 6        | 10       | 6        | 7        | 11       | 4        | 11       | 8        |
|          | CT       | 2        | 8        | 0        | 2        | 8        | 0        | 4        | 8        | 0        | 5        | 4        |
|          | TT       | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| p        | <.001     | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    | <.001    |
| ns       | ns       | ns       | ns       | ns       | ns       | ns       | ns       | ns       | ns       | ns       | ns       | ns       |

<sup>a</sup>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 TT vs. TT, −1154 AA + AG vs. GG, −634 GG vs. GC + CC, 936 CC vs. TT), was tested using the Fisher’s exact test for toxicity and using the log-rank test for progression-free survival and overall survival.

<sup>b</sup>−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).

<sup>c</sup>−1498 T>C (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).

<sup>d</sup>−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).

<sup>e</sup>−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).

<sup>f</sup>p values of Fisher’s exact test are given.

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