

7

Reversibility of capillary density after discontinuation of bevacizumab treatment

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Abstract

Background

VEGF inhibition is known to decrease capillary density. Decreased capillary density may be the basis for VEGF inhibitor related side effects. We investigated whether the effects of bevacizumab on capillary density are reversible.

Patients and methods

Capillary density, assessed by sidestream dark field imaging of the mucosal surface of the lip, was measured at baseline, after 6 weeks of bevacizumab treatment and >3 months after discontinuation. Additional measurements included blood pressure (BP) measurements, flow-mediated dilation (FMD), nitroglycerin-mediated dilation (NMD), and aortic pulse wave velocity (PWV).

Results

Fourteen patients were included. Seven patients completed measurements at all 3 pre-defined timepoints. Capillary density significantly decreased after 6 weeks of bevacizumab treatment and was reversible after discontinuation of the bevacizumab ($p=0.00001$ using a general linear model repeated measures test). Blood pressure, FMD and NMD remained unchanged. Mean PWV increased after 6 weeks treatment ($p=0.027$) and decreased after bevacizumab discontinuation. Amongst the 6 patients with the best response were the 3 patients showing the clearest decrease in capillary density after 6 weeks of bevacizumab treatment.

Conclusions

Bevacizumab induced decrease in capillary density is reversible. Non-invasive assessment of capillary density during treatment with anti-angiogenic drugs may be useful as a marker of treatment efficacy.

Introduction

Bevacizumab, a monoclonal antibody against the vascular endothelial growth factor (VEGF), is currently being used in the treatment of various types of cancer, including colorectal, breast, renal cell and non-small-cell lung cancer.¹⁻⁶

A clear decrease in capillary density has been reported in the treatment with two VEGF inhibitors, telatinib and bevacizumab.^{7,8} Decreased vascular density, or rarefaction, may be a possible mechanism for various side effects seen in the treatment with VEGF inhibitors. One of these side effects is hypertension, with grade 3-4 hypertension, using the common toxicity criteria, developing in 9-16% of bevacizumab-treated patients develops.^{9,10} Research in rats also showed that vascular rarefaction may be the cause of sunitinib-induced hypothyroidism and bevacizumab-induced enteric perforations.¹¹ Since bevacizumab-treated patients may have a prognosis of several years in palliative setting and many years when adjuvant schedules will prove to be beneficial, the patient group is relatively large. Increased knowledge on bevacizumab-induced vascular changes may help to identify patients at risk for complications, stimulate the sense of urgency to monitor patients and to start early treatment in case of hypertension, hypothyroidism, encephalopathy or other vascular-related complications.¹²

The aim of this study is to investigate whether the bevacizumab-associated vascular and blood pressure effects are reversible after discontinuation of bevacizumab treatment. An additional goal is to provide more information on the mechanisms in the development of bevacizumab-associated side effects.

Patients and Methods

Eligibility Criteria and study outline

Patients receiving bevacizumab monotherapy or combination therapy for breast or colorectal cancer were eligible for participation. Patients with previous bevacizumab treatment, major surgery within the last 4 weeks, or clinical significant cardiovascular disease in the previous year were excluded. Written informed consent was obtained from all patients before any study related procedure was performed, and approval from the institutional medical ethical review boards was obtained before study initiation.

Blood pressure measurements and vascular assessments were performed at baseline (<7 days before first bevacizumab administration), after 6 weeks (before third 3-weekly bevacizumab administration) and >3 months after last bevacizumab admin-

istration. All measurements were performed by the same experienced investigator, in a quiet, temperature-controlled room.

Assessments

Capillary density measurements with SDF imaging

Patients were situated in a supine position with the investigator at the head side of the bed. An SDF hand-held device (MicroScan Video Microscope System, MicroVision Medical) was introduced into the open mouth and gently pushed to the mucosal surface of the inner lip. SDF imaging consists of a light guide surrounded by light-emitting diodes that emit green light (540 F 50 nm) which penetrates the tissue and directly illuminates the tissue microcirculation. The SDF technique is described in detail in previous publications.^{7,13,14} Images of the mucosal microcirculation were projected on a computer screen. The final on-screen magnification of the images obtained with the SDF imaging device was 325 times original. When images of satisfying quality were seen, video images of at least 30 s were obtained. Images were obtained from four different lip quadrants (mucosal readings of the left and right upper inner lip quadrant and the left and right lower inner lip quadrant) using the SDF probe. From every quadrant, at least three 30-s video images were obtained. Video images were stored on digital videotape in avi format. Off line, at least five still frames of each quadrant were captured from these video images. The number of capillary loops per frame was counted. Capillary density for each frame was expressed as the mean number of capillary loops per mm². The mean capillary density per lip quadrant and total lip was calculated. Using offline computer analysis, capillary diameter was measured. The mean diameter of at least 80 capillaries per visit of each patient was calculated. All measurements were done by one technician, not blinded to the time point in treatment of the patients. Off-line analysis (counting of the number of capillary loops and measurement of capillary diameter) was done by two observers (JR and NS), who were blinded to the time point in treatment of the patients. The reproducibility of the SDF technique to determine capillary density in healthy volunteers was moderate to high, showing a coefficient of variation of 4.6%.⁷

Peripheral blood pressure measurements

Brachial blood pressure was measured at home, 4 times a day for at least three consecutive days, after 15 min rest, measuring thrice in a supine position with 5-min intervals, using an automatic device (Datex-Ohmeda S/5 Light Monitor, Datex-Ohmeda, Inc.) with appropriate cuff size. For statistical analysis, we used the mean of the measurements.

Central blood pressure measurements

Application tonometry of the radial and external carotid artery (SphygmoCor SCOR-PVx device, AtCor) was done. The mean of the three peripheral blood pressure measurements was used to calculate central aortic pressure.¹⁵

Flow mediated dilation

The FMD measurements were done in a quiet, temperature-controlled room. Postischemic vasodilator responses in the brachial artery were measured using a Wall Track System (WTS 2, Pie Medical). This system consists of a standard 7.5-MHz linear array ultrasound transducer connected to a PC equipped with a data acquisition board and software. Subjects were investigated in a supine position, and three ECG leads were attached. Ischemia was induced in the forearm by inflation of a blood pressure cuff just below the elbow of the right arm for 5 min. After deflation of the cuff, changes in brachial artery wall diameter were measured every 20 s for 4 min. WTS measurements were stored and analyzed off line using WTS software. FMD was expressed as percentage change in brachial artery diameter after ischemia.

Nitroglycerin-mediated dilation

NMD was assessed in the same way as FMD, with the exception that 0.4 mg of nitroglycerin was given sublingually, instead of cuff inflation and deflation, before measurements were started.

Aortic pulse wave velocity

Measurements were done at the right carotid and femoral arteries using standard blood pressure transducers (SphygmoCor SCOR-PVx device, AtCor) with simultaneous electrographic gating. This enabled the base of the pressure wave to be recorded and the time delay between the carotid and femoral waves to be calculated. The distance between the two sites was measured. PWV was defined as the distance traveled by the pressure waves divided by the time delay.

Statistical analysis

Continuous variables are presented as mean values and categorical variables as frequencies (percentages), unless otherwise stated. Comparisons between variables at baseline, after 6 weeks, and after discontinuation of bevacizumab treatment, were made with a general linear model repeated measures test or with a paired Student's t-test were appropriate, and were two-sided, with a level of significance of $\alpha = 0.05$. All calculations were made by computer-assisted analyses using SPSS 16.0 (Chicago, IL) statistical package for Windows.

Results

Between March 2007 and July 2008, a total of 14 patients were enrolled in the study. In 8 patients measurements could be performed after discontinuation of bevacizumab treatment. Baseline patient and treatment characteristics of both groups are listed in Table 1.

Patients received bevacizumab for colorectal cancer (12 patients) or breast cancer (2 patients). All patients had metastasized disease. Bevacizumab dose was 7.5 mg/kg/3weeks for the patients with colorectal cancer and 10 mg/kg/2weeks for the breast cancer patients. Bevacizumab was combined with various chemotherapy schedules; capecitabine plus oxaliplatin (7 patients), capecitabine (3 patients), irinotecan (2 patients), paclitaxel and capecitabine (1 patient), paclitaxel (1 patient).

Reversibility of vascular changes

Measurements after discontinuation of bevacizumab treatment could be performed in 8 patients. In one of these patients SDF data were not available due to technical reasons.

Capillary density measurements with SDF imaging

Using a general linear model repeated measures test on the 7 patients with 3 evaluable SDF measurements, before, during and after bevacizumab treatment, the change in capillary density due to bevacizumab treatment was significant, with a p-value of 0.00001 (Fig 1). More importantly, after discontinuation of the bevacizumab treatment the changes in capillary density showed to be reversible, with a p-value of 0.001 (Fig 1).

Figure 2 shows the SDF images of a representative patient, demonstrating visible capillary loops at baseline, decreased capillary density after 6 weeks of bevacizumab treatment, and normalization of capillary density >3 months after bevacizumab discontinuation.

Of the 7 patients, 6 patients were without any systemic anticancer therapy in the three months before the third visit. One patient was still treated with capecitabine plus oxaliplatin, however without bevacizumab.

At baseline, 2 patients received a single blood pressure lowering agent (thiazide diuretic or angiotensin-II receptor antagonist), 3 patients received two agents (β -blocker plus thiazide diuretic, β -blocker plus angiotensin converting enzyme (ACE) inhibitor, and β -blocker plus angiotensin-II receptor antagonist, all in 1 patient respectively), and 1 patient received 3 blood pressure lowering agents (β -blocker plus loop diuretic plus angiotensin-II receptor antagonist). These blood pressure lowering medication schedules remained unchanged for all 6 of the above described patients for the duration of the

Table 1. Baseline demographics and patient characteristics

| Patient characteristics | Pts with data at 3 visits, including after bevacizumab discontinuation | Pts with data before and during bevacizumab, without data after discontinuation |
|---|--|---|
| <i>N</i> | 8 | 14 |
| Male gender | 4 (50) | 6 (43) |
| Age (years, range) | 59 (49-71) | 61 (45-74) |
| <i>Center</i> | | |
| Leiden University Medical Center | 6 (75) | 8 (57) |
| Rijnland Hospital Leiderdorp | 1 (13) | 4 (29) |
| Deaconess Hospital Leiden | 1 (13) | 2 (14) |
| <i>Additional cardiovascular risk factors</i> | | |
| BMI (kg/m ² , range) | 27.3 (22.8-33.7) | 26.2 (20.7-33.7) |
| Nicotine abuse; in past or present | 5 (63) | 6 (43) |
| History of CVD | 1 (13) | 2 (14) |
| History of hypertension | 2 (25) | 4 (29) |
| Renal impairment (creatinine > ULN) | 0 (0) | 0 (0) |
| <i>WHO performance scale</i> | | |
| 0 | 5 (63) | 7 (50) |
| 1 | 3 (38) | 6 (43) |
| unknown | 0 (0) | 1 (7) |
| <i>Prior treatment</i> | | |
| Surgery | 5 (63) | 7 (50) |
| Chemotherapy | 1 (13) | 5 (36) |
| Radiotherapy | 4 (50) | 5 (36) |
| Blood pressure lowering drugs at entry | 3 (38) | 6 (43) |
| <i>Tumor type</i> | | |
| Colorectal cancer | 7 (88) | 12 (86) |
| Breast cancer | 1 (13) | 2 (14) |

BMI: body mass index; CVD: cardiovascular disease; ULN: upper limit of normal; WHO: world health organization

Data are presented as n (%) unless otherwise specified

entire study. One additional patient, patient 14, was started on antihypertensive treatment with a calcium antagonist during bevacizumab treatment.

Blood pressure, vascular function and vascular structure assessments

With a general linear model repeated measures test there were no significant changes in blood pressure or vascular function and vascular structure parameters, besides FMD (Table 2). Comparing results of visit 3 with visit 2, FMD remained unchanged after discontinuation of bevacizumab treatment.

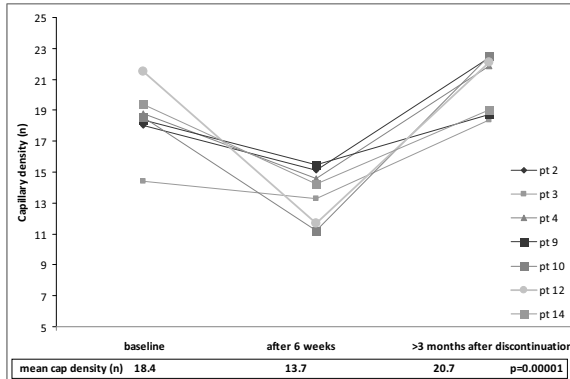


Fig. 1. Capillary density of the 7 patients with 3 evaluable SDF measurements. Visit 1: baseline; visit 2: after 6 weeks of bevacizumab treatment; visit 3: >3 months after discontinuation of bevacizumab treatment. P-value generated by general linear model repeated measures test

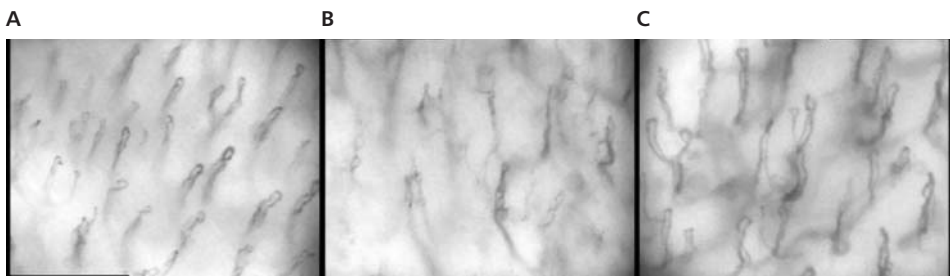


Fig. 2. SDF images demonstrating visible capillary loops of a representative patient. A: at baseline; B: after 6 weeks of bevacizumab treatment; C: >3 months after bevacizumab discontinuation.

Vascular changes during bevacizumab treatment

In a total of 14 patients measurements were performed before and during treatment with bevacizumab.

In 6 patients additional measurements after discontinuation of bevacizumab treatment could not be performed due to various reasons; 2 patients died, 2 patients were in poor clinical condition due to tumor progression, 1 moved out of the region and 1 was still receiving bevacizumab treatment.

Capillary density measurements with SDF imaging

In all 14 patients, the number of capillary loops markedly decreased from 18.2 at baseline to 13.3 ($p=0.00002$) after 6 weeks treatment with bevacizumab (Table 2).

Table 2. Hemodynamic and vascular function/structure variables; mean values of all patients.

2A: 8 patients with measurements available at all 3 predefined timepoints; baseline, after 6 weeks of bevacizumab treatment and >3 months after discontinuation. 2B: 14 patients with data available at baseline and after 6 weeks of bevacizumab treatment, but not after bevacizumab discontinuation.

2A

| n=8 | Visit 1 baseline values | Visit 2 after 6 weeks treatment | Visit 3 >3 months after discontinuation | Repeated measures overall p-value ‡ | Visit 1 vs. 2 p-value † | Visit 2 vs. 3 p-value † |
|--------------------------|-------------------------------|---------------------------------------|---|---|-------------------------------|-------------------------------|
| Capillary density (n) ** | 18.4 | 13.7 | 20.7 | 0.00001 * | 0.002 * | 0.001 * |
| Capillary diameter (mm) | 6.9 | 5.6 | 5.7 | 0.094 | 0.051 | 0.816 |
| pSBP (mm Hg) | 128.4 | 134.1 | 132.6 | 0.536 | 0.286 | 0.768 |
| pDBP (mm Hg) | 83.9 | 90.0 | 85.3 | 0.203 | 0.042 * | 0.166 |
| cSBP (mm Hg) | 134.4 | 135.5 | 132.3 | 0.877 | 0.891 | 0.570 |
| cDBP (mm Hg) | 83.8 | 86.4 | 86.3 | 0.634 | 0.484 | 0.963 |
| FMD (%) | 7.0 | 3.5 | 3.8 | 0.031 * | 0.039 * | 0.699 |
| NMD (%) | 15.5 | 16.1 | 9.7 | 0.186 | 0.302 | 0.060 |
| PWV (m/s) | 8.9 | 9.7 | 9.4 | 0.169 | 0.064 | 0.558 |

* p < 0.05

** patient 6 no SDF data available at visit 3

‡ general linear model repeated measures test

† paired Student's t-test

n: number; pSBP: peripheral systolic blood pressure; pDBP: peripheral diastolic blood pressure; cSBP: central systolic blood pressure; cDBP: central diastolic blood pressure; FMD: flow mediated dilatation; NMD: nitroglycerin mediated dilatation; PWV: aortic pulse wave velocity.

2B

| n=14 | Visit 1 baseline values | Visit 2 after 6 weeks treatment | Visit 1 vs. 2 p-value † |
|--------------------------|----------------------------|---------------------------------------|----------------------------|
| Capillary density (n) ** | 18.2 | 13.3 | 0.00002* |
| Capillary diameter (mm) | 6.9 | 5.6 | 0.002 * |
| pSBP (mm Hg) | 129.3 | 133.1 | 0.368 |
| pDBP (mm Hg) | 82.2 | 87.7 | 0.033 * |
| cSBP (mm Hg) | 134.8 | 139.6 | 0.322 |
| cDBP (mm Hg) | 83.4 | 87.9 | 0.081 |
| FMD (%) | 6.3 | 3.2 | 0.006 * |
| NMD (%) | 12.5 | 13.7 | 0.521 |
| PWV (m/s) | 8.9 | 9.6 | 0.027 * |

* p < 0.05

† paired Student's t-test

n: number; pSBP: peripheral systolic blood pressure; pDBP: peripheral diastolic blood pressure; cSBP: central systolic blood pressure; cDBP: central diastolic blood pressure; FMD: flow mediated dilatation; NMD: nitroglycerin mediated dilatation; PWV: aortic pulse wave velocity.

Blood pressure, vascular function and vascular structure assessments

There was no significant change in most of the blood pressure parameters after 6 weeks treatment with bevacizumab (Table 2). There was a significant increase in peripheral diastolic blood pressure. The decrease in FMD from 6.3% to 3.2% after 6 weeks treatment was statistically significant ($p=0.006$) when compared with baseline (Table 2). There was no change in NMD. Mean PWV significantly increased from 8.9 m/s at baseline to 9.6 m/s after 6 weeks treatment ($p= 0.027$; Table 2).

Association of capillary density with bevacizumab efficacy

Figure 3 shows the relationship between capillary density changes and bevacizumab efficacy. Amongst the 6 patients that were alive and showed no tumor progression within 6 months of start of bevacizumab treatment were the 3 patients showing the clearest decrease in capillary density after 6 weeks of bevacizumab treatment.

Discussion

We conducted this study to investigate whether bevacizumab-associated vascular and blood pressure effects are reversible after discontinuation of bevacizumab treatment. We additionally aimed at providing more information on the mechanisms in the development of bevacizumab-associated side effects.

To our knowledge, this is the first study describing effects on blood pressure and the vascular system after discontinuation of VEGF inhibitory treatment. Until now, the reversibility of vascular changes was never documented in humans. We now report that the bevacizumab induced decrease in capillary density is reversible. Although this study was performed in a limited number of patients, our results are consistent and in line with previous preclinical data. Preclinical data show rapid reversibility of capillary regression after cessation of VEGF inhibition in normal organs and tumors in mice.¹⁶⁻¹⁸ Strikingly, most capillaries grew back within 2 weeks after cessation of treatment.

In this journal, Mourad et al reported endothelial dysfunction and capillary rarefaction in 18 bevacizumab treated patients.⁸ We previously reported a reduction in capillary density (rarefaction) and microvascular flow, associated with a reduced vasodilatory capacity induced by telatinib, an orally active, small molecule inhibitor of VEGFR-2, VEGFR-3, platelet-derived growth factor receptor- α , and c-Kit.⁷ With the current study, we confirm that treatment with bevacizumab results in a clear decrease in capillary density. And, after studying both a small molecule tyrosine kinase inhibitor of the VEGF receptor and a monoclonal antibody against VEGF, we conclude that the induced decrease in

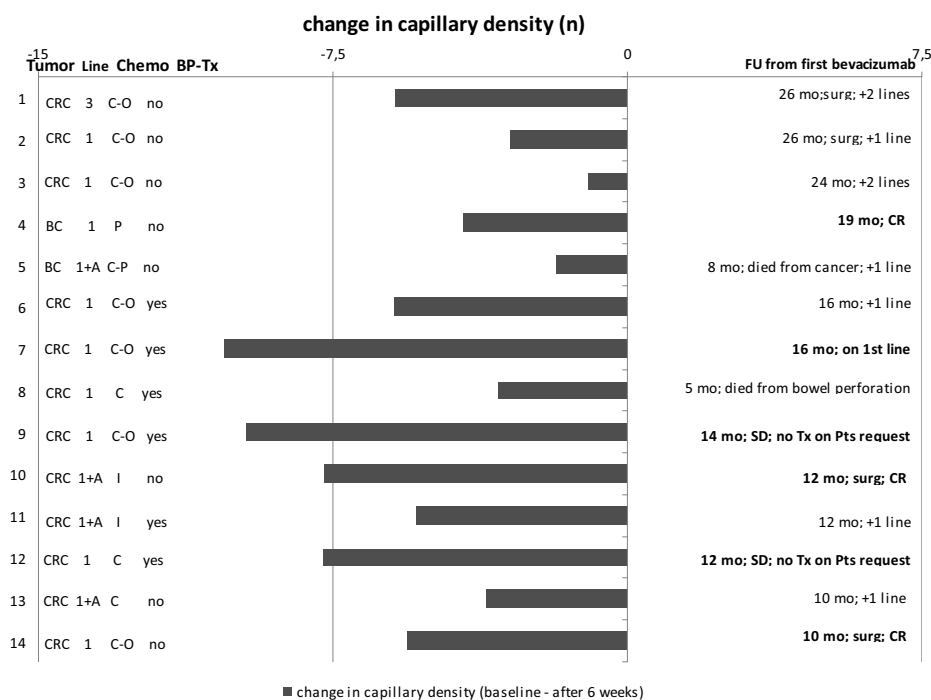


Fig. 3. Change in capillary density after 6 weeks of bevacizumab treatment, compared to baseline. Individuals clinical and efficacy parameters are added. In bold patients that were alive and showed no tumor progression within 6 months of start of bevacizumab treatment. BP-Tx: blood pressure lowering treatment before start of bevacizumab treatment; CRC: colorectal cancer; BC: breast cancer; A: adjuvant chemotherapy; C: capecitabine; O: oxalipatin; P: paclitaxel; I: irinotecan; mo: months; surg: surgery; CR: complete response; Tx: therapy; Pt: patient

In this study there was a significant decrease in capillary density after 6 weeks of bevacizumab treatment, however, there was no significant increase in blood pressure. This might be explained by the timing of the measurements, i.e. after six weeks or 2 half-lives of bevacizumab. The decrease in capillary density may be induced before an increase in blood pressure develops. Other explanations may be the limited number of patients, or the use of blood pressure lowering agents in almost half of the patients.

We designed our study, such that our data can be extrapolated to the general patient population treated with bevacizumab containing anti cancer treatment. To maximally imitate daily life, we decided not to exclude patients using blood pressure lowering agents at baseline. While multiple measurements were performed in the same patients and patients therefore were their own internal control, this resulted in accurate statisti-

cal data. We also decided to include patients receiving bevacizumab in combination with various cytotoxic agents. Since vascular changes were seen in all patients, we can conclude that the changes in vascular parameters are linked to the bevacizumab treatment, and are irrespective of the cytotoxic agents used in the bevacizumab combination.

The sidestream dark field (SDF) method is a very elegant method for measuring capillary density. It is a non-invasive, not painful method, with no side effects for the test subject. Measurements can be performed in almost all subjects while the mucosal surface of the inner lip is intact and easy to reach in most patients. Moreover, the measurement time for the patient is limited, while large parts of the data analysis can be performed off line. The technique is fairly easy to learn, and reproducibility of data is generally high.^{7,13,14}

Our results may have several implications in the treatment of cancer patients with VEGF inhibitors. Recently, increased blood pressure during treatment with VEGF inhibitors has been associated with a longer time to tumor progression.¹⁹⁻²² Possibly, capillary density measured by the noninvasive SDF method can be used as an even earlier marker for response. In our study we could show that decreased capillary density during bevacizumab treatment may be associated with a better prognosis. However, this is exploratory and confirmation with adequate sample size is needed. Also, theoretically, combining capillary density measurements with blood pressure changes can result in a better predictive marker than blood pressure alone. Moreover, we would like to stimulate the sense of urgency to monitor patients, since the reversibility of vascular changes after discontinuation of VEGF-inhibiting therapy might suggest that timely intervention may reduce or prevent certain side effects with sometimes high morbidity and even mortality. This is the first study reporting that the bevacizumab-induced capillary density decrease is reversible after discontinuation of bevacizumab treatment. In combination with earlier results in VEGF tyrosine kinase inhibitor treatment, we also report that VEGF-associated rarefaction is a class-effect generated by all VEGF-inhibitors.

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