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A Randomized Trial of Bevacizumab, an Anti-Vascular Endothelial Growth Factor Antibody, for Metastatic Renal Cancer

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ABSTRACT

BACKGROUND

Mutations in the tumor-suppressor gene *VHL* cause oversecretion of vascular endothelial growth factor by clear-cell renal carcinomas. We conducted a clinical trial to evaluate bevacizumab, a neutralizing antibody against vascular endothelial growth factor, in patients with metastatic renal-cell carcinoma.

METHODS

A randomized, double-blind, phase 2 trial was conducted comparing placebo with bevacizumab at doses of 3 and 10 mg per kilogram of body weight, given every two weeks; the time to progression of disease and the response rate were primary end points. Crossover from placebo to antibody treatment was allowed, and survival was a secondary end point.

RESULTS

Minimal toxic effects were seen, with hypertension and asymptomatic proteinuria predominating. The trial was stopped after the interim analysis met the criteria for early stopping. With 116 patients randomly assigned to treatment groups (40 to placebo, 37 to low-dose antibody, and 39 to high-dose antibody), there was a significant prolongation of the time to progression of disease in the high-dose-antibody group as compared with the placebo group (hazard ratio, 2.55; $P < 0.001$). There was a small difference, of borderline significance, between the time to progression of disease in the low-dose-antibody group and that in the placebo group (hazard ratio, 1.26; $P = 0.053$). The probability of being progression-free for patients given high-dose antibody, low-dose-antibody, and placebo was 64 percent, 39 percent, and 20 percent, respectively, at four months and 30 percent, 14 percent, and 5 percent at eight months. At the last analysis, there were no significant differences in overall survival between groups ($P > 0.20$ for all comparisons).

CONCLUSIONS

Bevacizumab can significantly prolong the time to progression of disease in patients with metastatic renal-cell cancer.

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STUDIES OF THE HEREDITARY FORM OF clear-cell renal carcinoma, which occurs in the von Hippel–Lindau syndrome, led to the identification of the von Hippel–Lindau tumor suppressor gene (*VHL*). The gene is mutated both in hereditary renal-cell carcinoma (where one mutation is a germ-line mutation) and in most cases of sporadic clear-cell renal carcinoma (where both alleles have acquired mutations or deletions).^{1,2} One consequence of these mutations is the overproduction of vascular endothelial growth factor through a mechanism involving hypoxia-inducible factor α .³⁻⁷ In addition, both *VHL*-deficient mice and vascular endothelial growth factor–knockout mice die in utero from defective vasculogenesis.^{8,9} Thus, by its regulation of vascular endothelial growth factor, the von Hippel–Lindau protein is tightly linked to angiogenesis. Vascular endothelial growth factor stimulates the growth of endothelial cells and appears to be a central factor in angiogenesis, particularly during embryogenesis, ovulation, wound healing, and tumor growth.¹⁰

Studies of human tumor xenografts in immunodeficient mice showed that neutralization of vascular endothelial growth factor inhibited the growth of a variety of model tumors.^{11,12} Presta and colleagues “humanized” the murine antibody used in these studies, A.4.6.1, by placing its complementarity-determining (antigen-binding) regions into a human IgG1 constant-region framework and modifying further amino acid residues to optimize antigen binding.¹³ In the resulting product, bevacizumab (or rhMAB-VEGF), 7 percent of the amino acids are from the murine antibody. In phase 1 testing, bevacizumab had a low toxicity profile in most patients, had a terminal elimination half-life of approximately 21 days, and did not induce antibodies to bevacizumab.¹⁴ The severe toxic effects that occurred in the phase 1 trial were infrequent intratumoral bleeding (including fatal hemoptysis), pulmonary emboli, and peripheral venous thrombosis. We conducted a randomized, placebo-controlled phase 2 trial of bevacizumab in patients with advanced renal-cell carcinoma.

METHODS

PATIENTS

Patients with histologically confirmed renal cancer of the clear-cell type, measurable metastatic disease, and documented progression of disease were eligi-

ble for this study. Other requirements included an Eastern Cooperative Oncology Group (ECOG) performance status of 2 or lower and previous therapy with interleukin-2 (or contraindications to standard interleukin-2 therapy). The exclusion criteria were a history of central nervous system involvement, any other therapy or major surgery within the previous four weeks, a history of intratumoral bleeding, a serum creatinine level of more than 2 mg per deciliter (17 μ mol per liter), a serum bilirubin level of more than 2 mg per deciliter (34 μ mol per liter), and ischemic vascular disease.

All patients gave written informed consent. This protocol was approved by the institutional review board of the National Cancer Institute (NCI). The study was sponsored by the Cancer Therapy Evaluation program of the NCI, and bevacizumab was supplied by Genentech under a cooperative research and development agreement with the NCI. Trial design, data accrual (with the exception of assays for vascular endothelial growth factor and bevacizumab performed by Genentech on coded patient specimens), data analysis, and manuscript preparation were performed entirely by the authors.

The patients were evaluated by physical examination, magnetic resonance imaging of the brain, and complete computed tomographic scanning no more than one month before randomization, five weeks after the beginning of therapy, and then every two months for the first year of therapy and every three months for the second year of therapy.

A complete response was defined as the absence of all evidence of disease for at least a month. A partial response was defined as a decrease of at least 50 percent in the sum of the products of the maximal perpendicular diameters of measured lesions, lasting for a minimum of one month, with no progression of any lesion or appearance of new lesions. Minor and mixed responses were not included as responses.

Annual interim evaluations were performed by an independent data safety and monitoring board, and the method of O’Brien and Fleming was used to determine the threshold for statistical significance at each interim evaluation that would constitute grounds to recommend termination of the trial.¹⁵ For the first year of the trial, this threshold was a P value of 0.0006 or less; for the second year, it was a P value of 0.015 or less; and for the third year, it was a P value of 0.047 or less. The estimated and actual accrual rates were similar enough that these proposed intervals did not require revision.

RANDOMIZATION AND TREATMENT

In this phase 2 study, the patients were stratified according to whether or not they had received interleukin-2 therapy and were then randomly assigned to receive either a vehicle-only placebo, 3 mg of bevacizumab per kilogram of body weight, or 10 mg of bevacizumab per kilogram. During all treatment and evaluations, neither the patients nor any participating health care personnel were aware of the treatment assignment. Based on pharmacokinetic modeling, treatment with bevacizumab began with one loading dose, in which 150 percent of the assigned dose was administered by intravenous infusion over a 2-hour period, and then, beginning one week later, the standard assigned dose was administered (by progressively shorter infusions that reached a minimum of 30 minutes) every two weeks. Plasma levels of vascular endothelial growth factor and serum levels of bevacizumab were measured. The plasma vascular endothelial growth factor assay used the 3.5.F.8 murine antibody for both capture and detection. This assay detects both free and bevacizumab-bound vascular endothelial growth factor equally, with a lower limit of detection of 40 pg per milliliter.

EVALUATION

For the purposes of end-point evaluation, the criteria for declaring tumor progression were the unequivocal appearance of new lesions; an increase of more than 25 percent in the product of the maximal perpendicular diameters of any measured lesion, as compared with base-line evaluation (or the smallest size subsequent to base line); or a tumor-related deterioration in ECOG performance status to 3 or more. For a declaration of progressive disease to be made, the lesions had to attain a minimal diameter of 1.5 cm (to ensure accurate measurement).

The indications for removing patients from the study and unblinding their treatment assignments were as follows. To permit adequate time for the initial assessment of the therapy while protecting patients with rapid disease progression who were assigned to placebo, the evaluation conducted five weeks after enrollment differed from subsequent evaluations. At five weeks, patients with increases of more than 2 cm in any lesion, a clinically significant deterioration in performance status, or new, severe symptoms (e.g., bone pain or nerve compression) were removed from the study. At all other evaluations, the indication for removal from the study was progressive disease. These different indications for

removal from the study did not affect the end-point analyses, which were always based on tumor progression, as defined above.

STATISTICAL ANALYSIS

Using NCI Surgery Branch historical data from patients with no response to interleukin-2 therapy, we used the following criteria to estimate the sample size necessary to detect a doubling of the time to progression in patients receiving either dose of bevacizumab as compared with those receiving placebo: a 24-month accrual period, a 12-month evaluation period after the completion of accrual, a power of 80 percent, and an overall alpha of 0.05 to detect a doubling of the hazard ratio for each of the two primary comparisons (high-dose antibody vs. placebo and low-dose antibody vs. placebo). The calculation indicated that 40 patients per group would be required (50 were permitted, to allow for some patients who could not be evaluated).

The primary evaluation was based on the time from enrollment to disease progression; a secondary analysis examined the time to disease progres-

Table 1. Characteristics of Patients before Treatment.*

| Characteristic | High-Dose Bevacizumab (N=39) | Low-Dose Bevacizumab (N=37) | Placebo (N=40) |
|--|------------------------------|-----------------------------|----------------|
| Median age (yr) | 53 | 54 | 53 |
| Male sex (%) | 74 | 84 | 68 |
| ECOG performance status (no.)† | | | |
| 0 | 30 | 30 | 31 |
| 1 or 2 | 9 | 7 | 9 |
| Prior interleukin-2 therapy (no.) | 37 | 34 | 37 |
| Prior chemotherapy (no.) | 10 | 7 | 8 |
| Prior radiation therapy (no.) | 8 | 6 | 12 |
| Prior nephrectomy (no.) | 35 | 33 | 38 |
| Anemia (no.) | 14 | 15 | 16 |
| Hypercalcemia (no.) | 12 | 18 | 14 |
| Interval from diagnosis to randomization (no.) | | | |
| <1 yr | 14 | 13 | 12 |
| 1–2 yr | 8 | 6 | 9 |
| >2 yr | 17 | 18 | 19 |
| Liver involvement (no.) | 10 | 10 | 10 |
| Bone involvement (no.) | 2 | 3 | 6 |

* P>0.05 for all comparisons.

† ECOG denotes Eastern Cooperative Oncology Group. Higher performance-status numbers indicate greater impairment.

sion from the five-week assessment, in order to determine whether the effect of treatment was delayed and to ensure that small variations in the interval from the pretreatment evaluation to the time of randomization did not affect the uniform determination of the time to progression. Each P value was adjusted for the performance of two primary comparisons on the basis of treatment groups.

The time to progression and the overall response rate were the primary end points, and the analyses were performed on an intention-to-treat basis. Survival was declared a secondary end point, because patients whose disease progressed while they were receiving placebo were offered crossover either to 3 mg of bevacizumab per kilogram alone or to a combination of 3 mg of bevacizumab per kilogram and thalidomide. The time to progression of disease and survival were assessed with use of Kaplan–Meier curves and tested for significance by the log-rank test. Hazard ratios were determined with the Cox proportional-hazards model. All P values are two-tailed.

RESULTS

Between October 1998 and September 2001, 116 patients were enrolled, of whom 108 had progressive disease during the course of the study. The median follow-up time from study entry was 27 months. Forty patients were randomly assigned to placebo, 37 to low-dose bevacizumab, and 39 to high-dose bevacizumab. All planned doses of the study drug were given unless grade 3 toxic effects occurred, in which case doses were withheld as specified by the study protocol. Only one patient (who was assigned to low-dose bevacizumab) was lost to follow-up after therapy. The three groups had similar demographic and clinical characteristics and laboratory results (Table 1). All patients received at least one dose of the assigned drug, and 114 of the 116 patients underwent at least one planned follow-up evaluation (evidence concerning disease progression was available for the remaining 2 patients).

There were no life-threatening toxic effects (grade 4, major organ) or deaths possibly related to bevacizumab (Table 2). Hypertension and asymptomatic proteinuria were associated with bevacizumab therapy (Table 2). Of 13 patients with grade 2 or 3 hypertension, 7 (54 percent) had grade 2 or 3 proteinuria; of 63 patients with grade 0 or 1 hypertension, 10 (16 percent) had grade 2 or 3 proteinuria ($P=0.007$ by Fisher's exact test). None of these patients, or any other patient, had renal insufficiency. Hypertension and proteinuria uniformly decreased after the cessation of therapy, but death from renal cancer, the slow rate of correction of hypertension and proteinuria, and the commencement of other therapies prevented the documentation of complete resolution of these toxic effects in all but one patient.

There were no episodes of grade 4 hypertension during randomized therapy, but in one patient who was initially assigned to placebo, hypertension with coma developed after the patient crossed over to low-dose bevacizumab plus thalidomide. These complications resolved completely after therapy was stopped. Typically, hypertension during the study was treated by the patients' private physicians with standard regimens for essential hypertension. Among all bevacizumab-treated patients who required therapy for newly diagnosed hypertension (for whom the dates of onset could be most accurately determined), the median interval from the first dose of bevacizumab to the onset of hypertension was 131 days (range, 7 to 316). Grade 1 or 2 hemoptysis developed in four patients (one receiving

Table 2. Toxic Effects of Treatment.*

| Effect | High-Dose Bevacizumab (N=39) | Low-Dose Bevacizumab (N=37) | Placebo (N=40) |
|---|------------------------------------|-----------------------------------|-------------------|
| | number | | |
| Epistaxis | 8† | 5 | 1 |
| Hypertension | 14† (8†) | 1 | 2 |
| Fever without infection | 4 | 1 | 0 |
| Malaise | 13 | 6 | 6 |
| Hematuria | 5† | 1 | 0 |
| Hyponatremia | 3 | 4† | 0 |
| Proteinuria ($\geq 1+$ or ≥ 150 mg/24 hr) | 25† (3) | 15 (2) | 15 |
| Elevated alanine aminotransferase | 4 | 2 | 0 |
| Chest pain | 2 (2) | 0 | 0 |

* The table lists all toxic effects of any grade that occurred in at least 10 percent of patients receiving either dose of antibody and that were more frequent than in patients receiving placebo. The number of patients with grade 3 toxic effects is shown in parentheses (there were no grade 4 or 5 events; every bevacizumab-associated grade 3 toxic effect occurring in more than one patient is shown). Grade 3 hypertension was defined as hypertension not completely controlled by one standard medication. Grade 3 proteinuria was defined as urinary excretion of more than 3.5 g of protein per 24 hours. Other toxic effects were graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0).

† Unadjusted $P \leq 0.05$ for the comparison with placebo (by chi-square test, or by Fisher's exact test if the expected frequency was less than 5).

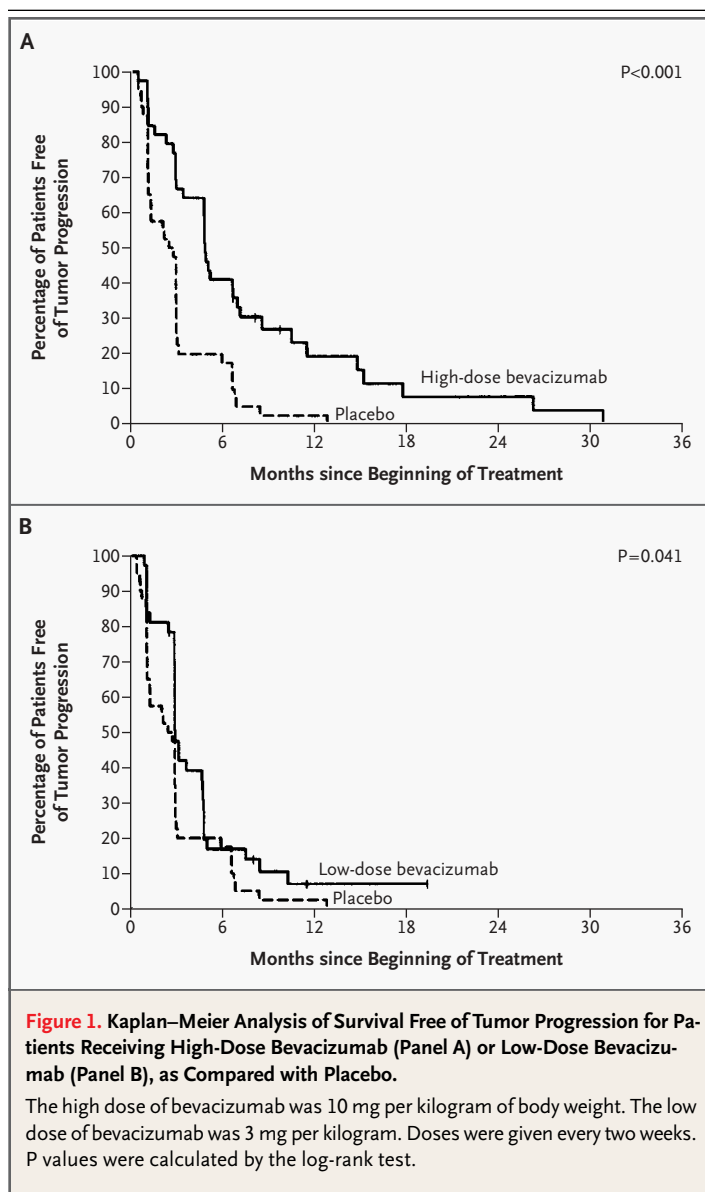
high-dose bevacizumab, one receiving low-dose bevacizumab, and two receiving placebo), and one patient receiving placebo had a pulmonary embolus.

At the second interim evaluation (which analyzed the data on 110 patients), the NCI data safety and monitoring board recommended closure of accrual on the basis of the difference between the placebo and high-dose bevacizumab groups in the time to progression of disease. According to intention-to-treat analysis, progression-free survival in the group receiving 10 mg of bevacizumab per kilogram (with a median time to progression of 4.8 months) was significantly longer than that in the placebo group (with a median time to progression of 2.5 months, $P < 0.001$ by the log-rank test) (Fig. 1A). The difference between the time to progression of disease in the group receiving 3 mg of bevacizumab per kilogram (median time, 3.0 months) and that in the placebo group was of borderline significance ($P = 0.041$ by the log-rank test) (Fig. 1B).

The planned analysis of progression from the five-week assessment yielded the same results. The percentages of patients assigned to high-dose bevacizumab, low-dose bevacizumab, and placebo who had no tumor progression were 64 percent, 39 percent, and 20 percent, respectively, four months after randomization and 30 percent, 14 percent, and 5 percent eight months after randomization. A Cox proportional-hazards model yielded hazard ratios for the time to progression of disease of 2.55 among patients given high-dose bevacizumab ($P < 0.001$) and 1.26 among those given low-dose bevacizumab ($P = 0.053$), as compared with those given placebo.

Only four patients had objective responses (all of which were partial responses), and all of these had received high-dose bevacizumab; thus, the response rate for high-dose bevacizumab was 10 percent (95 percent confidence interval, 2.9 to 24.2 percent). One patient had a partial response for the maximal treatment period of two years. This patient then stopped therapy, had a relapse six months later, and is currently having a second partial response after retreatment under a compassionate exemption (Fig. 2). Another patient treated for two years had a sustained minor response, had a relapse after stopping therapy, and had another minor response after being retreated.

Measurements of plasma vascular endothelial growth factor were available for 113 patients. Of these, 76 had a base-line level below the lower limit of detection (40 pg per milliliter). There were no significant associations between a detectable pretreat-



ment level of vascular endothelial growth factor and the clinical response or the time to progression in either bevacizumab group (all P values were greater than 0.20). However, the limited sensitivity of the assay does not permit the definitive conclusion that there is no correlation between the base-line plasma level of vascular endothelial growth factor and the clinical response or the time to progression. After antibody therapy was started, the plasma levels of vascular endothelial growth factor rose steadily (the assay measures both free and antibody-bound vascular endothelial growth factor). After 5 weeks

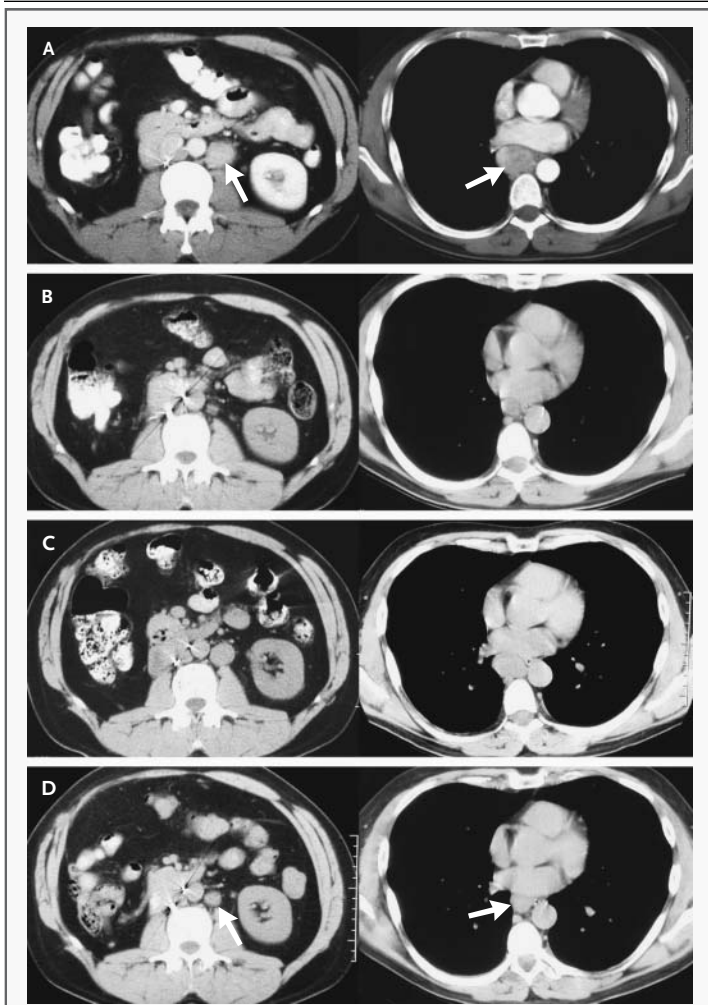


Figure 2. Serial Radiographs of a Patient Treated with High-Dose Bevacizumab.

Panel A shows the pretreatment assessment (arrows indicate lymph-node metastases). Panel B shows a radiograph obtained two years later, when treatment was stopped during a partial response. Panel C shows relapse of tumor six months thereafter. Panel D shows a second partial response 3 months after therapy was restarted, which is ongoing at more than 18 months as of this writing.

and 13 weeks of therapy, all bevacizumab-treated patients had detectable plasma levels of vascular endothelial growth factor. The median levels were 196 and 246 pg per milliliter, respectively, for patients receiving high-dose bevacizumab and 155 and 170 pg per milliliter for patients receiving low-dose bevacizumab. The percentages of patients assigned to placebo who had undetectable plasma levels of vascular endothelial growth factor at baseline, 5 weeks, and 13 weeks were 66 percent, 67 per-

cent, and 75 percent, respectively. Patients receiving low-dose bevacizumab had mean (\pm SE) peak and trough serum levels of bevacizumab of 101 ± 9 and 39 ± 3 μ g per milliliter, respectively; patients receiving high-dose bevacizumab had mean peak and trough levels of 392 ± 24 and 157 ± 13 μ g per milliliter, respectively. In both groups, the trough levels were above that needed to abolish detectable free vascular endothelial growth factor in the plasma of patients in previous phase 1 studies.¹⁴

At the most recent analysis, in February 2003, 19 of 116 patients (16 percent) were alive, and there were no significant differences in survival between the treatment groups (all P values were greater than 0.20) (Fig. 3). The complete radiographic records of 113 patients (3 were no longer complete at the time of audit) were blindly audited by a team of extramural radiologists under the supervision of the Cancer Therapy and Evaluation Program of the NCI. The prolongation of time to progression of disease was confirmed radiologically.

DISCUSSION

We selected vascular endothelial growth factor as a target for treatment of clear-cell kidney cancer because mutations in the von Hippel–Lindau tumor-suppressor gene, which probably cause most sporadic clear-cell kidney cancers, result in overproduction of this growth factor by the tumors. In our study, the aim was to neutralize vascular endothelial growth factor with a humanized monoclonal antibody (bevacizumab) in patients with metastatic clear-cell renal cancer. Using a randomized, double-blind, placebo-controlled design, we found that the time to tumor progression was prolonged by a factor of 2.55 in patients given 10 mg of bevacizumab per kilogram every two weeks, as compared with patients in the placebo group. Survival was not a primary end point in this trial, which allowed patients to cross over from placebo to bevacizumab therapy at the time of disease progression. Indeed, the survival of bevacizumab-treated patients was not significantly different from that of the patients receiving placebo.

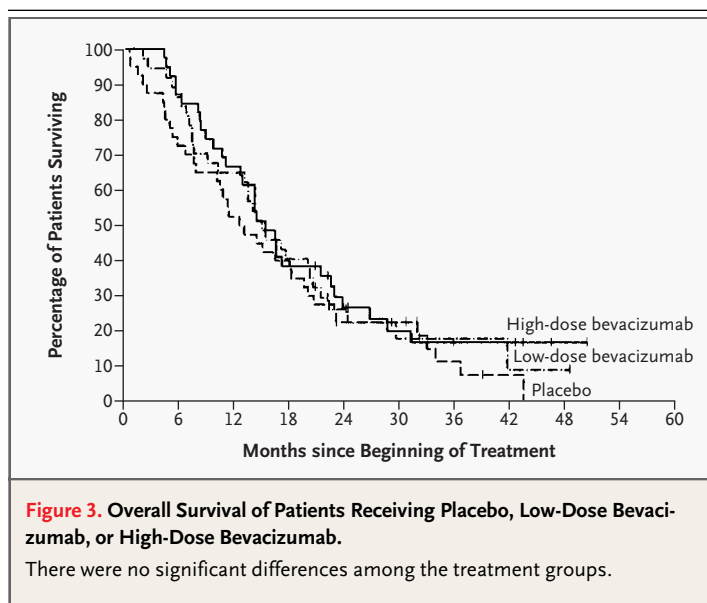
During bevacizumab therapy, the plasma level of vascular endothelial growth factor rose. It is important to note that the assay we used measured both free and antibody-bound vascular endothelial growth factor. The explanation for this increase and its clinical significance are unknown, but it might have been due to diminished clearance of bevaciz-

zumab-bound, inactive vascular endothelial growth factor or to an antibody-mediated blockade of the binding of vascular endothelial growth factor to its receptors.

Hypotheses about the mechanism responsible for the delay we observed in tumor progression are based on *in vitro* data, the results of treatment of human tumor xenografts in immunodeficient mice, and studies of human renal cancer. These data suggest that the antitumor effects of the antibody against vascular endothelial growth factor are due to inhibition of angiogenesis. Both *in vitro* and *in tumor xenografts*, vascular endothelial growth factor has potent angiogenic activity, which is inhibited by neutralizing antibodies to vascular endothelial growth factor; the result is a decrease in tumor blood flow and microvessel densities.¹¹ Human clear-cell renal cancers have significantly higher microvessel counts than non-clear-cell renal cancers, and these counts are correlated with the expression of vascular endothelial growth factor.¹⁶ Endothelial cells and hematopoietic cells (but not renal cancer cells) are the predominant cells that express receptors for vascular endothelial growth factor, but the inhibition of the growth of human tumor xenografts in immunodeficient mice argues against contributions from an immunologic mechanism. For all these reasons, the inhibitory effect of bevacizumab on the growth of clear-cell renal cancer is likely to be due to its antiangiogenic action.

Antiangiogenic strategies for the treatment of cancer have generated widespread enthusiasm based on promising *in vitro* and preclinical studies. The concepts that growing tumors require the manufacture of new blood vessels and that very little of the rest of the normal adult body has such a requirement have led to the belief that there is valuable therapeutic potential in this area. Early clinical studies of antiangiogenic compounds such as endostatin, TNP-470, and thalidomide were not designed to assess their clinical efficacy.^{17,18} In retrospect, only a randomized assessment of a time-to-progression end point could have demonstrated the activity of bevacizumab in renal cancer. Reliance on major response rates would have resulted in the conclusion that this drug was ineffective. Nevertheless, without a demonstration of improved overall survival, this single-agent trial serves primarily as a proof of principle and the basis for further investigation.

The magnitude of the clinical benefit of bevacizumab in this trial was small. The differences in the time to the progression of disease between the high-



dose bevacizumab group and the placebo group was only a few months. Nevertheless, the likelihood is high that this difference was due to true biologic activity. The lack of an overall survival benefit in this trial and the small size of the increase in the time to progression may reflect the crossover design and the rigorous indications for declaring progression and removing a patient from the study (an increase in diameter of any single lesion by as little as 12 percent could constitute tumor progression). Some patients left the study with only small new lesions or mixed responses, but often with minimal or no increase in the size of preexisting tumors. In fact, 23 patients treated with high-dose bevacizumab showed no net increase in the size of index lesions from base line to the time of tumor progression. Tumor progression in these patients was typically based on the appearance of small new lesions or an increase in the size of some lesions that was offset by regression in other lesions. It would be worthwhile to determine survival in patients allowed to continue to receive bevacizumab despite tumor progression.

Future treatments for renal cancer that target angiogenic mechanisms should consider pathways other than that mediated by vascular endothelial growth factor. There are other proteins in the local microenvironment of some tumors that can promote angiogenesis. For example, fibroblast growth factor 5, which has angiogenic activity, is secreted

by most renal cancers,¹⁹ suggesting that combinations of bevacizumab and inhibitors of members of the fibroblast growth factor family may have promise for treatment of this disease. It is likely that the future of antiangiogenic therapy will require a rational combination of inhibitors, directed by a bet-

ter understanding of the biology of each individual type of cancer.

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