Case Report/Case Series

Somatic p.T771R KDR (VEGFR2) Mutation Arising in a Sporadic Angioma During Ramucirumab Therapy

Young H. Lim, BS; Ian D. Odell, MD, PhD; Christine J. Ko, MD; Keith A. Choate, MD, PhD

IMPORTANCE Inhibition of angiogenesis is an effective anticancer strategy because neoplasms require a rich blood supply. Ramucirumab, approved by the US Food and Drug Administration in 2014 to treat gastric adenocarcinomas and non–small cell lung carcinomas, targets vascular endothelial growth factor 2 (VEGFR2). We identified a patient prescribed a regimen of irinotecan hydrochloride, cetuximab, and ramucirumab for metastatic rectal cancer (diagnosed in November 2013 and treated through early January 2015) who developed a new-onset, expanding vascular lesion on his right leg. Via exome sequencing, we found that the lesion contained a single somatic mutation in KDR (encodes VEGFR2), possibly in response to ramucirumab. Vascular tumors are not a known complication of antiangiogenic therapeutics.

OBSERVATIONS Exome sequencing of the well-demarcated, blanching vascular lesion on the lateral right shin revealed a somatic p.T771R mutation in KDR, without evidence of other somatic mutations or loss of heterozygosity. Histological features included lobules of small vessels within the dermis, resembling a tufted angioma.

CONCLUSIONS AND RELEVANCE A potential adverse effect of ramucirumab in combination therapy is the development of sporadic angiomas. The p.T771R mutation was previously implicated in autophosphorylation of VEGFR2 and reported in angiosarcomas alongside other driver mutations. Our observations suggest that this mutation confers a proliferative advantage in the setting of ramucirumab therapy. Patients receiving ramucirumab should be monitored for the development of new vascular lesions.

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ngiogenesis is a hallmark of cancer. Growing tumors recruit networks of new vasculature to meet their increased demand for oxygen and nutrients, as well as for efficient removal of metabolic waste products.1,2 The identification of diffusible vascular endothelial growth factors (VEGFs) and VEGF receptors (VEGFRs) as key regulators of angiogenesis and the observation that tumors enhance the expression and secretion of these factors and receptors paved the way for novel anticancer drugs inhibiting this pathway.2

There are 7 VEGF members in mammals, including VEGFA, VEGFB, VEGFC, VEGFD, VEGFE, and VEGFF and placenta growth factor (PIGF).3 These growth factors each bind to 1 or more of 3 VEGFR tyrosine kinases.1,3 The first is VEGFR1 (FLT1 [OMIM 165070]), which binds VEGFA, VEGFB, and PIGF to negatively regulate embryonic angiogenesis. The second is VEGFR2 (KDR [OMIM 191306]), which binds VEGFA to drive embryonic and tumor angiogenesis. The third is VEGFR3 (FLT4 [OMIM 136352]), which is predominantly expressed in lymphatic endothelial cells and binds VEGFC and VEGFD to regulate lymphangiogenesis. Among the VEGFRs, VEGFR2 is the primary mediator of physiologic and pathologic VEGF-induced angiogenesis and demonstrates the most potent tyrosine kinase activity.1 Consequently, its signaling cascade has been extensively studied, and VEGFA inhibitors, such as bevacizumab and other VEGFR2 inhibitors, have emerged as agents for antiangiogenesis therapy in cancer.2,4

Ramucirumab is an intravenously administered human monoclonal antibody (IgG1) that selectively binds the extracellular domain of VEGFR2, blocking access of VEGF ligand.4 In mice, a murine VEGFR2 monoclonal antibody (DC101) was found to strongly inhibit tumor growth,5 and ramucirumab has recently been found to have efficacy in gastric and gastroesophageal junction adenocarcinomas.4 The most common adverse effects of ramucirumab (shared among all antiangiogenic agents) include hypertension due to a decrease in VEGF-dependent nitric oxide production and proteinuria due to an inhibition of VEGF-dependent interactions between podocytes and the glomerular endothelium.1,4 With promising results in its phase 3 trials, ramucirumab gained approval from the US Food and Drug Administration in April 2014 for use in patients with advanced or metastatic gastric and gastroesophageal junction cancer with disease progression during or after pretreatment with platinum-containing or fluoropyrimidine-containing chemotherapy.4 On-
going clinical trials are studying the efficacy of combining ramucirumab with other distinct antitumor agents. For example, trial E7208 studies changes in outcome of irinotecan hydrochloride and cetuximab therapy with vs without ramucirumab.6

### Report of a Case

Our study was approved by the Yale University Human Investigation Committee and complied with the principles of the Declaration of Helsinki. We describe a 109-kg man in his 40s diagnosed in November 2013 as having stage IV KRAS (OMIM 190070) wild-type rectal adenocarcinoma, with pulmonary and liver metastases. In addition to irradiation to the rectum (4500 centigray [cGy] total, with a boost of 640 cGy), he received 12 cycles of standard-dose folinic acid, fluorouracil, and oxaliplatin, with the addition of bevacizumab from cycles 5 to 12. Completion of treatment in April 2014 was followed by 10 cycles of a maintenance regimen of folinic acid, fluorouracil, and bevacizumab (oxaliplatin was withheld because of neuropathy) from May to October 2014. Restaging imaging throughout maintenance therapy revealed progression of pulmonary metastases and stable disease in the liver.

Because of disease advancement, the patient was enrolled in December 2014 in trial E7208 at the Smilow Cancer Hospital at Yale-New Haven and was randomized into the irinotecan and cetuximab with ramucirumab (IC + R) treatment group to receive irinotecan hydrochloride (150 mg/m² [340 mg]), cetuximab (400 mg/m² [916 mg]), and ramucirumab (6 mg/kg [655 mg]) every 3 weeks. In early January 2015 after 2 cycles of the trial, he developed a single, well-defined, blanchable bright red plaque densely composed of telangiectasias on his lower right lateral shin, which grew to 4 cm (Figure 1A). There was no associated pain or pruritus. Histopathological analysis showed discrete clusters of small-caliber vessels, most resembling a tufted angioma (Figure 1B and C).

To determine the genetic basis of this new vascular tumor, we used paired whole-exome sequencing of affected tissue (labeled ANGIO100) and the patient’s blood. After alignment of exome data to the Human Genome version 19 reference genome, tissue-specific somatic variants were annotated, and nonsynonymous mutations were filtered for variants absent in normal control data sets, including the Exome Aggregation Consortium, the 1000 Genomes Project, and the National Heart, Lung, and Blood Institute (eAppendix in the Supplement). Using a Fisher exact test to rank somatic variants with a genome-wide significance, we identified a list of candidate mutations that were subsequently viewed using a software program (Integrated Genomics Viewer; https://www.broadinstitute.org/igv/) to exclude mismapped, identifying a heterozygous KDR c.C2312G, p.T771R mutation as the only somatic variant. B. The mutation was verified via targeted Sanger sequencing of DNA isolated from laser-microdissected vessels. The mutation was absent in blood and laser-microdissected epidermis, implicating the mutation as tumor endothelium specific. Ref indicates reference allele.

## Figure 1. Clinical and Histological Features of a Vascular Lesion Arising in the Setting of Ramucirumab Therapy

<table>
<thead>
<tr>
<th>Image</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>Lower right lateral shin</td>
</tr>
<tr>
<td>B</td>
<td>Histological findings</td>
</tr>
<tr>
<td>C</td>
<td>Higher magnification</td>
</tr>
</tbody>
</table>

A, A single, well-demarcated, blanchable red plaque, measuring 4 cm in diameter. The plaque breaks up into smaller punctate macules at the periphery. B and C, Superficial, small lobules of small-caliber vessels are noted on histology, and the findings are reminiscent of a tufted angioma. The epidermis is normal (hematoxylin-eosin, original magnification ×4 [B] and ×20 [C]).

## Figure 2. Whole-Exome Sequencing and Sanger Sequencing Identification of a Somatic p.T771R Mutation in the Vascular Lesion Endothelial Cells

### Patient
tumor

<table>
<thead>
<tr>
<th>Somatic Mutation</th>
<th>No. of Reads in Tissue Ref</th>
<th>No. of Reads in Tissue Non Ref</th>
<th>No. of Reads in Blood Ref</th>
<th>No. of Reads in Blood Non Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANGIO100 KDR, c.C2312G, p.T771R</td>
<td>87 6</td>
<td>97 0</td>
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<td></td>
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</tbody>
</table>

### Sanger sequencing

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<th></th>
<th>V</th>
<th>G</th>
<th>T/R</th>
<th>V</th>
<th>G</th>
<th>T</th>
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<tbody>
<tr>
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<td>G</td>
<td>&lt;Ref&gt;</td>
<td>&lt;Ref&gt;</td>
<td>G</td>
<td>&lt;Ref&gt;</td>
</tr>
<tr>
<td>ANGIO100 Blood</td>
<td>&lt;Ref&gt;</td>
<td>G</td>
<td>&lt;Ref&gt;</td>
<td>&lt;Ref&gt;</td>
<td>G</td>
<td>&lt;Ref&gt;</td>
</tr>
</tbody>
</table>

A, Following alignment of exome data and filtration against control data sets, nonsynonymous somatic variants with at least 6 nonreference reads (Non Ref.) in tissue and fewer than 2 Non Ref. reads in blood were isolated. The resulting variants were visualized using the Integrated Genomics Viewer (https://www.broadinstitute.org/igv/) to exclude mismapping, identifying a heterozygous KDR c.C2312G, p.T771R mutation as the only somatic variant. B. The mutation was verified via targeted Sanger sequencing of DNA isolated from laser-microdissected vessels. The mutation was absent in blood and laser-microdissected epidermis, implicating the mutation as tumor endothelium specific. Ref indicates reference allele.
We identified the p.T771R mutation in the captured vasculature, which was absent in blood and epidermis (Figure 2 and eFigure 3 in the Supplement).

At the time of writing this case report, the patient is continuing the E7208 IC + R trial regimen. The size and appearance of the vascular lesion remain unchanged.

Discussion

The development of new benign or malignant tumors in response to chemotherapy is a known phenomenon. These second cancers may originate from genotoxic therapy-induced chromosomal instability or from paradoxical activation of a pathway targeted by the treatment, such as that seen in patients with melanoma receiving the BRAF (OMIM 164757) inhibitor vemurafenib who develop keratoacanthomas with activating HRAS (OMIM 190020) mutations.8,9

Given the rapid appearance after the initiation of therapy and the time line of our patient’s vascular lesion, we believe the mutation arose in response to the E7208 trial regimen. KDR p.T771R was previously identified in 4 patients with angiosarcoma12 in conjunction with other mutations listed in the Catalogue of Somatic Mutations in Cancer (http://cancer.sanger.ac.uk/cosmic), and expression studies of angiosarcoma revealed upregulation of KDR, with approximately 10% of patients harboring KDR mutations.14 In addition to p.T771R, p.N717V and p.A1065T were identified in other cases of angiosarcoma.12,13 In vitro studies have shown that these mutations lead to autophosphorylation of the KDR tyrosine kinase, suggesting that these variants are activating mutations.12

The mechanism of mutagenesis remains unclear. For vemurafenib-induced keratoacanthomas, activating HRAS mutations are postulated to have occurred before therapy, while on exposure to vemurafenib the inhibition of BRAF leads to trans-activation of CRAF (OMIM 164760) and upregulated downstream MAPK (OMIM 610389) signaling.14 Similarly, our patient carry a somatic mosaic for the autoactive KDR, with the mutant endothelial cells gaining growth advantage on systemic inhibition of VEGFR2. Paradoxical angiogenesis may be promoted by perturbing one branch of the VEGF axis, as seen in patients receiving bevacizumab who demonstrate elevated plasma levels of PIGF, VEGF, and VEGF.15 In the context of ramucirumab, increased expression of VEGFs and activation of the other VEGFRs may synergize with an autoactive VEGFR2 to stimulate proliferation of mutant endothelium. Furthermore, complementary non-VEGF-mediated angiogenic pathways (eg, involving fibroblast growth factors) are also upregulated in the context of antiangiogenic therapies. Fibroblast growth factors have demonstrated an even greater proangiogenic effect than VEGFs, with an ability to act synergistically with VEGFs to enhance endothelial cell survival and proliferation.11

Conclusions

To our knowledge, there are no previous reports of vascular tumors arising in the setting of ramucirumab treatment or any other VEGF or VEGFR inhibitor therapy. We describe a patient who developed a spontaneous, proliferative angiomatous lesion with a somatic activating VEGFR2 mutation when being treated with a VEGFR2 inhibitor. Researchers have suggested implementing antiangiogenic therapeutics against vascular and endothelial cancers,15 and one study demonstrated the efficacy of bevacizumab against an infantile hemangioma that underwent malignant transformation to become an angiosarcoma. Our findings highlight the need for surveillance of patients treated with VEGF and VEGFR inhibitors for the development of new-onset vascular lesions. We suspect that such lesions may represent paradoxical VEGFR activation analogous to that seen in HRAS-mutant keratoacanthomas in patients treated with BRAF inhibitors. Further investigation of the pathways involved is needed to understand the pathogenesis of these lesions.
Conrad Jobst
Great Inventor and Stocking Maker

Walter H. C. Burgdorf, MD; Vincent A. Muscarella, MD; Leonard J. Hoenig, MD

Most dermatologists have some familiarity with Jobst stockings as a mainstay of therapy of chronic venous insufficiency, but how many of us know anything about the man who invented them?

Conrad Jobst (1889-1957) was a German tool and die maker, not a chief engineer for Ames-Bonner Brush Company in Toledo, Ohio. Here he helped revolutionize an item everyone uses daily—the toothbrush. Up until this point, toothbrushes were hand-made, often with bone handles and pig bristles. Jobst used newly developed celluloid for the entire product and designed machines to shape and bore the handle as well as automatically insert the many rows of uniform artificial bristles. The modern toothbrush was born. Jobst would have no trouble recognizing today's toothbrushes or the machines used to make them.

He also manufactured the first retractable hardtop convertible, modifying a 1933 Auburn cabriolet by incorporating a 4-piece retractable chrome-plated hardtop. His prototype plans were patented. Peugeot produced the 402B retractable hardtop in 1938, while the first American car to incorporate such technology was the 1957 Ford Fairlane 500 Skyliner.

Jobst suffered from varicose veins and had all the problems of chronic venous insufficiency, including stasis dermatitis and venous ulcers. He noticed that when he stood in his swimming pool, he obtained almost immediate pain relief. After consulting with physicians at Henry Ford Hospital in Detroit, Michigan, and physiologists at the Wright Patterson Aeromedical Laboratory in Dayton, Ohio, he applied his engineer’s ingenuity to create a product that helped him and perhaps millions of other patients with similar problems.

He reasoned that the continuous and gradual decrease in pressure from the foot up the leg that one experiences in a pool was the most effective way to replace or support defective venous valve function. Using a variety of new elastic fabrics, careful measurements, and special machines, he came up with the Jobst Venous Pressure Gradient stocking (Figure). Initial studies suggested that a pressure of 40 to 50 mm Hg at the ankle gradually decreasing proximally was the ideal substitute for natural venous function and was associated with best clinical responses. Careful, customized measurement of each leg was required so the stocking could be individually designed and produced to create a uniform pressure gradient no matter what the shape of the limb. Jobst insisted that each patient be referred via prescription by a physician.

Pressure gradient stockings, pioneered by Conrad Jobst, were an important advance in the treatment of vascular disease and continue to help patients suffering from venous insufficiency often accompanied by cutaneous complications.

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Figure. Two Sketches From Conrad Jobst’s Patent Application No. 2574873

The sketch on the left illustrates the method of measuring to fit an individual surgical stocking. The sketch on the right shows a blank cut from elastic fiber. When sown together, there will be a gradual pressure gradient decreasing from ankle to calf as relatively more fabric becomes available. The same techniques are still in use. Figure courtesy of the US Patent and Trademark Office.