# IN THE SPOTLIGHT

# Anti-VEGF Therapy Revived by c-Met Inhibition, But Is c-Met the Answer?

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**Summary:** A new study by Sennino and colleagues demonstrates that selective VEGF inhibition via the use of an anti-VEGF antibody is sufficient to increase invasion and metastasis in a c-Met–dependent manner. Anti-VEGF therapy induced tumor hypoxia, hypoxia-inducible factor $1\alpha$, and c-Met activation in the RIP-Tag2 model of neuroendocrine pancreatic cancer. Selective c-Met inhibition was sufficient to block these effects, providing a potential mechanism for and solution to overcome increased invasion in the face of anti-VEGF therapy. *Cancer Discovery; 2(3); 211–3. ©2012 AACR.*

*Commentary on Sennino et al., p. 270 (8).*

## INTRODUCTION

VEGF induces endothelial cell proliferation, survival, and migration and is a potent vascular permeability factor. VEGF-induced angiogenesis, or blood vessel sprouting from an existing vascular network, is relatively rare in most adult tissues and occurs normally only during wound healing and menstruation (1). Angiogenesis, however, is required for the growth of solid tumors, making it an attractive target for tumor therapy. Many current antiangiogenic therapies target the VEGF pathway, the predominant driver of angiogenesis.

The anti-VEGF antibody bevacizumab (Genentech) is approved by the U.S. Food and Drug Administration for patients with metastatic colorectal, non–small cell lung cancer, glioblastoma, and metastatic renal cell carcinoma. Notably, approval for the use of bevacizumab in combination with paclitaxel for the treatment of metastatic breast cancer was rescinded by the U.S. Food and Drug Administration when recent phase III trials showed only modest clinical efficacy and significant risks associated with therapy (2). Small-molecule receptor tyrosine kinase inhibitors (RTKi) are less specific for the VEGF pathway and are currently approved for the treatment of renal cell carcinoma, gastrointestinal stromal tumors, and hepatocellular carcinoma. Given the extensive clinical use but modest clinical efficacy of anti-VEGF agents, many groups are currently investigating changes in the tumor microenvironment after anti-VEGF therapy.

In 2009, authors highlighted in 2 articles increased invasion and metastasis in multiple preclinical tumor models after therapy with strategies that block VEGF activity (3, 4).

These studies carefully documented a change in tumor phenotype after anti-VEGF therapy that had been noted previously (5, 6) and in so doing have set the field of antiangiogenic therapy on edge. Ebos and colleagues (3) reported accelerated metastasis and decreased survival after short-term treatment with RTKi targeting the VEGF pathway in preclinical models of breast cancer and melanoma. In a complimentary study, Paez-Ribes and colleagues (4) reported increased local tumor cell invasion and distant metastasis in models of neuroendocrine pancreatic cancer (PNET) and glioblastoma after either prolonged or short-term VEGF inhibition by using various strategies (RTKi, monoclonal antibodies, or a genetic strategy to ablate VEGF in tumor cells).

Several possible mechanisms for these phenomena have been proposed. The reduction of tumor vasculature increases tumor hypoxia, possibly selecting for a population of more aggressive tumor cells. Furthermore, hypoxia might stimulate tumor cell invasion through the induction or activation of extracellular proteases, induction of alternative signaling growth factor pathways, or by triggering an epithelial-to-mesenchymal transition (EMT)–like phenotype (7). Furthermore, data presented by Ebos and colleagues (3) suggest that anti-VEGF therapy may condition distant organs to permit tumor cell extravasation and metastatic colonization. The reality is that the combination of these proposed mechanisms is likely responsible for increased invasion and metastasis observed after anti-VEGF therapy, although as the current data from Sennino and colleagues (8) suggest, one specific mechanism may dominate in certain tumor model systems.

## STUDY RESULTS

In this issue of *Cancer Discovery*, Sennino and colleagues (8) present significant evidence that continuous anti-VEGF therapy reduces primary tumor growth and increases overall animal survival but also increases tumor cell invasion and metastasis in two independent models of pancreatic cancer (8). This study follows up on a previous study from the same group (9) that demonstrated a multi-RTKi (XL880), which inhibits VEGFRs and c-Met among others, reduced primary tumor growth and blocked tumor cell invasion

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and metastasis. The present study expands on these results and addresses the hypothesis that c-Met is a major driver of increased tumor invasion after anti-VEGF therapy in a model of murine PNET. The authors propose that anti-VEGF therapy increases intratumoral hypoxia, which in turn drives a genetic program resulting in elevation of c-Met expression and activity and EMT-mediated tumor cell invasion. The study design uses the unique features of the RIP-Tag2 model to evaluate tumor cell phenotype, invasion, and metastasis. The model, which progresses in a predictable manner, is not typically grossly invasive until the animals are moribund. In addition, tumor cells can be tracked by the expression of SV40 T antigen. Furthermore, acinar cells, which are distinct from tumor cells, can be imaged by evaluating amylase expression. These characteristics facilitated documentation of liver metastases and the development of a primary tumor invasion index to gauge the effect of anti-VEGF therapy on tumor phenotype.

c-Met has been implicated previously as a driver of EMT and metastasis. In the present study, c-Met expression and activity was increased significantly on tumor cells in mice receiving anti-VEGF therapy, confirming the known relationship between hypoxia and c-Met. To determine whether the increased tumor invasion and metastasis observed following anti-VEGF therapy was dependent on c-Met activity, c-Met inhibition was combined with anti-VEGF therapy in mice bearing either RIP-Tag2 or Panc1 orthotopic pancreatic tumors. The results of combination therapy were striking; combination therapy completely abrogated the increase in invasion and metastasis observed after anti-VEGF therapy. In fact, combination therapy reversed invasion in the RIP-Tag2 model, where fewer intratumoral amylase positive cells were present after 3 weeks of therapy than at therapy onset. Furthermore, c-Met inhibition significantly reduced liver metastasis alone and in combination with VEGF inhibition in the RIP-Tag2 model, where the combination of c-Met inhibition with sunitinib was most effective. Finally, the authors used a dual VEGFR2 and c-Met RTKi (XL184). After treatment with XL184, tumor cell invasion and metastasis was significantly reduced in the RIP-Tag2 model, mimicking the results seen previously when combinations of anti-VEGF and c-Met targeted therapies were used.

It is important to note that anti-VEGF therapy alone extends the survival of RIP-Tag2 animals despite increasing invasion and metastasis, which reflects the overall balance between primary tumor growth, invasion, metastasis, and morbidity in these animals. However, dual inhibition of VEGF and c-Met signaling provides a significant survival benefit compared with single pathway inhibition. The overall model supported by Sennino and colleagues (8) is one whereby anti-VEGF therapy prunes the vasculature and increases intratumoral hypoxia. Hypoxic conditions trigger increased c-Met expression and activation, ultimately leading to an EMT-like phenotype and increased tumor invasion and metastasis.

## REMAINING QUESTIONS

Although hypoxia-induced activation of c-Met can explain the increase in metastasis in these 2 models of

pancreatic cancer, this might not be a universal phenomenon in every tumor model system. Recently, Couto and colleagues (10) reported that short-term or long-term exposure to a monoclonal anti-VEGF antibody displayed therapeutic benefit but did not alter the incidence of metastasis in 4 genetically engineered mouse models, which included models of pancreatic ductal adenocarcinoma and small cell lung cancer. These studies and numerous previous reports describe reduced metastatic burden after anti-VEGF therapy in models of melanoma (11), colon and gastric cancer (12, 13), prostate cancer (14, 15), and renal cell carcinoma (16), suggesting that all tumor models do not invade and metastasize in response to anti-VEGF therapy. However, anti-VEGF therapy was given as a continuous regimen in many of these studies, and the effect of short-term therapy on metastatic outcome may have been different. Notably, increased invasion and metastasis has only been documented after long-term anti-VEGF therapy in models of PNET, pancreatic ductal adenocarcinoma, and glioblastoma. Sennino and colleagues (8) report the elevation of c-Met expression on tumor cells after anti-VEGF therapy. The effect of anti-angiogenic therapy on other cells (e.g., pericytes, immune cells, and fibroblasts) and components (e.g., ECM) of the tumor microenvironment should be considered.

This is especially relevant given that changes in cytokines, including TGF-β, which as reported by Sennino and colleagues (8) can be elevated more than 10-fold after anti-VEGF treatment, can have pleiotropic effects on multiple players in the metastatic cascade. For example, TGF-β can directly regulate macrophages and the deposition of collagen. Prometastatic macrophages are central players in metastasis in many murine models of cancer, yet how they are affected by anti-VEGF therapy remains to be elucidated. In addition, elevation of TGF-β as a result of anti-VEGF induced hypoxia could alter the deposition of collagen or other ECM proteins, which although long thought to be static scaffolds, might participate directly in tumor cell survival and invasion. However, these open questions do not detract from the clear advantage demonstrated by combinatorial inhibition of VEGF and c-Met pathways, which has significant therapeutic potential and should be pursued vigorously.

Whether inhibition of c-Met quiets the debate concerning anti-VEGF–induced tumor cell invasion or intensifies it remains to be seen. One thing is for certain: Investigation into the biology of antiangiogenic therapy has entered a new phase because investigators now understand that anti-VEGF therapy has profound effects on the tumor microenvironment as a whole and are diligently working to characterize the mechanisms behind these effects and counteract them, if necessary.

## Disclosure of Potential Conflicts of Interest

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