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Predictive biomarkers to anti-VEGF therapy: progress towards an elusive goal

Rekha Gyanchandani¹ and Seungwon Kim^{1,2}

¹ Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, Pennsylvania

² Department of Otolaryngology, University of Pittsburgh, Pittsburgh, Pennsylvania

Abstract

The search for reliable biomarkers predictive of response to anti-VEGF therapy has been elusive. VEGF-A, the therapeutic target of bevacizumab, is an intuitive candidate as a predictive biomarker to bevacizumab-based anticancer therapy. However, there remains much controversy in the utility of VEGF-A as a predictor of response to bevacizumab.

In this issue of *Clinical Cancer Research*, Hegde and colleagues report the results of their study that sought to determine the predictive utility of circulating vascular endothelial growth factor-A (VEGF-A) in bevacizumab-containing therapy (1). The authors analyzed the plasma levels in over 1800 in patients who were enrolled in multiple phase III clinical trials of bevacizumab-containing regimens in patients with various cancer types which were then correlated with clinical outcomes.

Angiogenesis is an essential component of carcinogenesis that is driven by multiple proangiogenic cytokines with the best characterized proangiogenic cytokine being VEGF-A. Although multiple therapeutic approaches have been developed to inhibit VEGF driven angiogenesis including small molecule inhibitor of VEGF receptor (VEGFR) tyrosine kinases and therapeutic antibodies against the ligand-binding portions of VEGFR, bevacizumab (a monoclonal antibody to VEGF-A) has shown greatest success in clinical development (Fig. 1). Bevacizumab has been approved by the FDA for use in non-small cell lung cancer (NSCLC), colorectal cancer (CRC), and renal cell carcinoma (RCC) (2–4). However, the excitement that surrounded the early development of bevacizumab has been dampened by the recent recognition that the clinical benefits of this agent is not as significant as first promised. This recognition is underscored by the recent decision by the FDA to rescind its approval of bevacizumab for use in metastatic or recurrent breast cancer. Bevacizumab was initially approved by the FDA for use in breast cancer in 2008 based on clinical trials which showed increases in progression-free survival (PFS) in patients with recurrent or metastatic breast cancer when bevacizumab was incorporated into the standard chemotherapy regimen (5). However, PFS obtained in subsequent trials were less than that observed in the trials prior to the FDA approval (6, 7). Moreover, significant rates of adverse effects were reported in these trials including a 1% mortality rate directly attributable to bevacizumab (6, 7). These factors lead the FDA to ultimately rescind its approval of bevacizumab for use in breast cancer. Lastly, the use of bevacizumab has resulted in

Corresponding author: Seungwon Kim, Eye & Ear Institute, 200 Lothrop Street, Suite 500, Pittsburgh PA, 15213. (412) 647-2117. kimsww2@upmc.edu.

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increased PFS in many clinical trials but increases in overall survival (OS) have been difficult to obtain.

While it is true that the limitations of anti-VEGF therapy is becoming more evident as our experience with these agents increases, it is also undeniable that a subset of cancer patients treated with bevacizumab do show objective clinical responses and improved survival. However, we have yet to identify predictive biomarkers that have been validated in multiple, independent studies and can reliably distinguish patients who are likely to respond from those who will not. The identification of such biomarkers will be critical in harnessing the full potential of anti-VEGF therapy and in minimizing the rates of adverse side effects. The design and implementation of clinical trials based on proven, predictive biomarkers should allow for the enrichment of proper patients cohorts and facilitate the understanding of therapeutic mechanisms behind anti-VEGF therapy.

Several cytokines have been proposed in the literature as a possible predictive biomarker for anti-VEGF therapy. However, VEGF-A, the target of bevacizumab, is the most intuitive candidate as a predictive biomarker in the case of bevacizumab therapy. The correlation between pretreatments levels of VEGF-A and response to bevacizumab therapy has been examined previously in multiple studies (8–11). One study with a positive finding between circulating cytokine and response to bevacizumab was reported by Bates et al (11). In this study, the authors found higher survival in patients with tumoral $\text{VEGF}_{165b}:\text{VEGF}_{\text{total}}$ ratio below the mean compared with patients with the ratio above the mean. VEGF_{165b} , a C-terminal splice variant of VEGF, has been shown to have antiangiogenic properties in animal models. It binds VEGFR2 with equal affinity as VEGF_{165} but does not activate downstream signaling proteins. The mechanism behind the association between lower VEGF_{165b} and improved response to bevacizumab is unclear as bevacizumab binds both VEGF_{165} and VEGF_{165b} with similar affinity. In contrast to the study by Bates et al, most studies reported in the literature failed to show a correlation between neither the tumor or plasma levels of VEGF-A and clinical outcomes. These studies were also not powered with enough sample size to allow for a robust biomarker analysis. Lastly, the heterogeneity in methods utilized for measuring plasma and tumor VEGF-A levels also prevents meaningful comparison of the results from these studies.

In this issue of *Clinical Cancer Research*, Hegde and colleagues strive to overcome these limitations by analyzing, with uniform methods, the blood and tissue specimens from over 1800 patients with mCRC, NSCLC, or RCC who were enrolled in four, independent phase III trials of bevacizumab-containing regimens. Although the plasma samples were available for this study from only a subset of patients in each trial, the demographic, clinical, and pathologic characteristics of the sampled patient group did not differ significantly from the patient group without plasma availability. The plasma levels of VEGF-A was measured using enzyme-linked immunoabsorbent assay (ELISA) which recognized all major isoforms of VEGF-A including VEGF_{121} , VEGF_{165} , and VEGF_{110} with equivalent sensitivity. Consistent with multiples studies in the literature, the plasma levels of VEGF-A were found to be prognostic and correlated with OS, regardless of whether bevacizumab was part of the treatments, in all but one clinical trial. More importantly, the authors' analysis did not reveal any statistically significant correlation between plasma VEGF-A levels and clinical response to bevacizumab including PFS and OS in any of the trials. Although this study is not without limitations, the data presented by the authors lends strong support to the hypothesis that VEGF-A does not have predictive values in patients undergoing bevacizumab treatment, at least for mCRC, NSCLC, or RCC. One possible explanation for this lack of correlation is that bevacizumab may be able to efficiently sequester plasma VEGF-A after administration regardless of the pretreatment levels. We have previously examined the plasma levels of VEGF in patients with recurrent or metastatic head and neck cancer before and after

administration of bevacizumab and have found over 90% decrease in plasma VEGF-A in all patients regardless of the pretreatment levels (12). An interesting finding by Hegde and colleagues that lend another level of complexity is the lack of correlation between the tumor expression of VEGF-A as measured by IHC and the plasma levels of VEGF-A as measured by ELISA. Clinical investigators often take it for granted that the tumor and plasma levels of a given cytokine will correlate with each other. However, the data according to Hegde and colleagues suggest that both the tumor and plasma levels of a given cytokines should be measured in order for biomarker analysis unless it is known which of the two types of expression reflects the true biological potential of that cytokine.

It is clear that antiangiogenic therapy has become an important element in our armamentarium against cancer. Our next challenge is to refine the indications for antiangiogenic therapy by defining the necessary biomarkers for pretreatment patient selection, and the study by Hegde and colleagues is a step in the right direction. Lastly, it should be kept in mind that the angiogenic potential of a given tumor is not determined by a single cytokine but by the sum-effects of many pro- and anti-angiogenic cytokines. Therefore, it may be too simplistic to expect that a single cytokine will be predictive of response to bevacizumab therapy. It remains to determine whether the response to bevacizumab can be predicted based on a single cytokine or by the collective effects of several cytokines.

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Figure 1. Current clinical agents targeting angiogenesis and their mechanisms of inhibition
A) Bevacizumab is a humanized monoclonal antibody directed at VEGF. B) IMC-1121B is a humanized monoclonal antibody targeting the VEGFR-2, thereby inhibiting ligand binding and activation of the receptor. C) TKIs are orally available agents that compete with ATP in the intracellular tyrosine kinase domain of the receptor. Figure adapted from Cristopolous et al (13) with permission from John Wiley & Sons, Inc.