Abstract

Therapy targeting VEGF has become the standard of care in several solid malignancies. Early investigations attempting to identify predictive markers for the efficacy of therapy failed to identify any predictive markers that could help oncologists decide who should—and, more importantly, who should not—receive VEGF-targeted therapies. However, interest has been renewed in predictive biomarkers for VEGF-targeted therapies, especially in light of the fact that the U.S. Food and Drug Administration withdrew approval for use of bevacizumab, an antibody to VEGF, in patients with metastatic breast cancer. In a recent publication in the *Journal of Clinical Oncology*, investigators identified circulating VEGF and tumor neuropilin-1 expression as potential predictive biomarkers for bevacizumab. From this perspective, we provide a critical evaluation of the use of these markers and the need for validation in prospective clinical trials. *Clin Cancer Res; 19*(11): 1–4. ©2013 AACR.

In 2003, we saw the first evidence that targeting VEGF led to clinical benefit. Since it was first shown that bevacizumab, when added to chemotherapy, improved progression-free survival (PFS), response rate (RR), and overall survival (OS) in patients with metastatic colorectal cancer (1), many (~50%) phase III trials with VEGF-targeted therapies have been deemed “successful,” reaching the predefined primary endpoints and, in some cases, changing treatment paradigms (2).

The expanded use of bevacizumab beyond metastatic colorectal cancers included unselected patients with advanced lung, renal cell, and metastatic breast cancers and patients with glioblastoma (3–6). All signs pointed to even more indications for bevacizumab, such as pancreatic neuroendocrine tumors. However, the U.S. Food and Drug Administration (FDA) subsequently withdrew its approval for patients with metastatic breast cancers. This decision came after much debate but was less political than it was numerical: the lack of a survival benefit in confirmatory endpoints and, in some cases, changing treatment paradigms (2).

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Despite the objective data—in metastatic breast cancers, and in 2 adjuvant colorectal cancer studies showing no benefit with the addition of bevacizumab to chemotherapy (8, 9), and some marginal results in other trials—many oncologists believe that most patients benefit from the addition of bevacizumab to chemotherapy, and the inclusion of bevacizumab has become the default standard for many oncologists treating patients with various malignancies. The lack of ability to identify patients who will benefit *a priori*, that is, the absence of a predictive biomarker, meant that they treated nearly every patient with the antibody, without knowing which patients would be more likely to derive benefit.

In the past, the search for validated predictive biomarkers for bevacizumab and other VEGF inhibitors has come up empty (10–13). For example, in the pivotal metastatic colorectal cancer trial, tissue and plasma samples were studied for various mediators of angiogenesis, including VEGF and its upstream mediators, but no subgroup of patients who were more or less likely to benefit from the addition of bevacizumab to chemotherapy was identified (10). In fact, the futile search for a biomarker for bevacizumab was the subject of a *Journal of Clinical Oncology* editorial in 2006 (14).

These results could be interpreted in various ways, depending on one’s vantage point: (i) there is no biomarker because bevacizumab is effective in all subgroups of patients or (ii) a predictive biomarker for bevacizumab had not yet been identified. To cynics, the failure to find a biomarker could be viewed as inadequate resolve, given the commercial success of the drug, and the inherent conflict facing drug manufacturers, whose identification of a biomarker would likely shrink the market for the agent. Regardless of our past views, the pressure to find a biomarker for bevacizumab changed drastically when the FDA withdrew its approval for bevacizumab in patients with metastatic breast cancers. This regulatory decision, coupled with the negative results of the adjuvant colorectal cancer trials and multiple negative trials studying VEGF receptor kinase inhibitors in several solid malignancies, destroyed the premise that VEGF-targeted therapies would be efficacious in patients across the board.

In a recent publication in the *Journal of Clinical Oncology*, Van Cutsem and colleagues (15) report that circulating...
VEGF-A and tumor expression of neuropilin-1 (NRP-1), a coreceptor for VEGF-A, could select for patients most likely to benefit from the addition of bevacizumab to chemotherapy among patients with advanced or metastatic gastric cancer in the AVAGAST trial. Patients with high baseline plasma VEGF-A levels showed a trend toward improved OS [HR, 0.72; 95% confidence interval (CI), 0.57–0.93] versus patients with low VEGF-A levels (HR, 1.01; 95% CI, 0.77–1.31; \( P_{\text{interaction}} = 0.07 \)). Patients with low baseline expression of NRP-1 also showed a trend toward improved overall survival (HR, 0.75; 95% CI, 0.59–0.97) versus patients with high NRP-1 expression (HR, 1.07; 95% CI, 0.81–1.40; \( P_{\text{interaction}} = 0.06 \)). For both biomarkers, subgroup analyses showed significance only in patients from non-Asian regions. The circulating VEGF-A data are the most interesting. This new ELISA developed by Roche/Genentech scientists preferentially detects smaller VEGF isoforms in plasma after preservation in EDTA. Circulating VEGF-A was also featured at the 2011 meeting of the European Society of Medical Oncology, where data using this assay were presented showing its potential predictive role in breast, pancreatic, and gastric cancers (16). In other large phase III studies, VEGF-A analyzed using an ELISA that recognizes all isoforms of plasma VEGF-A with equivalent sensitivity was not predictive in patients with colorectal cancer and non–small cell lung cancer and had limited predictive value in renal cancer, although a clear prognostic effect was observed (17). The HR for PFS for patients treated with chemotherapy and bevacizumab was <1 in all 4 trials regardless of baseline serum VEGF-A levels. Re-analysis using the isoform sensitive assay again did not yield predictive value in these indications. These inconclusive findings may very well be due to technical reasons such as older samples, multiple freeze–thaw cycles, different anticoagulants (citrated tubes vs. EDTA collection tubes), and so forth. Alternatively, differences may be due to different mechanisms of action of VEGF inhibition in these disease types.

The investigators should be commended for mandating and then collecting tissue and plasma in the vast majority of patients. In addition, the development of a VEGF ELISA that may be more sensitive for smaller isoforms of VEGF than other ELISAs provides a new tool to test the hypothesis that VEGF may be a predictive marker. Although the concept that patients with high circulating VEGF levels may benefit from the addition of bevacizumab to chemotherapy is intuitive and makes biologic sense, robust validation in a multicenter prospective trial is necessary.

Question number one about these findings from AVAGAST is as follows: Have we found the predictive marker(s) for bevacizumab? Not yet. The predictive value of circulating VEGF-A was neither a primary nor a secondary endpoint in the preplanned biomarker analysis of the original AVAGAST protocol (that accompanied the manuscript submission to Journal of Clinical Oncology, a requirement of the journal). Thus, the findings of biomarker evaluation from the AVAGAST trial can only be considered hypothesis generating in gastric cancer, even though the samples were collected prospectively and were available for most patients. Validation, as always, is necessary if we are to consider circulating VEGF as a predictive biomarker.

Optimal validation would require a prospective trial, randomizing or stratifying patients based on VEGF levels to standard therapy ± bevacizumab across different tumor types, given the inherent differences on the impact of VEGF in distinct tumor microenvironments. Indeed, as part of its appeal to the FDA regarding the withdrawal of bevacizumab for patients with metastatic breast cancers, Roche/Genentech pledged to conduct a prospective clinical trial in patients with breast cancer that would replicate a prior registration study but would use circulating VEGF-A as a factor in stratifying patients into VEGF-high and VEGF-low cohorts; this study is currently enrolling patients (NCT01663727). Similarly, there has been discussion about repeating the AVAGAST study with a biomarker-driven stratification, but at the time of writing this editorial, no such study is listed under clinicaltrials.gov.

The second question is this: What are the relative values and reproducibility of the 2 putative biomarkers identified in this study? Both have their merits and flaws. VEGF-A should ultimately be easy to quantitate once the new proprietary ELISA is made available to investigators and oncologists, but the interpretation of the result will need further work. Although the immunologic multiparametric chip technique (IMPACT) is an established ELISA platform, the optimal sample processing requirements, analytic validation separate from the discovery set, and, importantly, the development of a universal standardized cutoff value for plasma VEGF-A levels for its use by Clinical Laboratory Improvement Amendments (CLIA)–certified clinical laboratories will have to be determined. Furthermore, because this is not a dichotomous variable, such as a mutated oncogene, or copy number by FISH analysis, much will depend on the cutoff point distinguishing high from low levels. For example, in the study by Van Cutsem and colleagues (15), the cutoff was determined after the fact. In practice, the cutoff must be predetermined so that any bias in cutoff point can be avoided and, obviously, must allow for an oncologist to make a decision in the here and now. In future studies, the appropriate cutoff for patients...
must be determined before trial initiation, but the cutoff (median) may vary in patients from different geographic regions and for patients with different subtypes of gastric cancer. This is no small challenge across a range of cancers. For instance, gastric cancers are characterized by diverse demographics, pathobiology, and histologic subtypes. Given that the role and regulation of VEGF in each tumor type is unique, the median VEGF-A levels may be tumor type dependent.

The second biomarker identified in this study was tissue expression of NRP-1, which poses even greater challenges to validate. NRP-1 is a co-receptor for VEGF, but its biologic function remains to be completely elucidated (17). In contrast to circulating VEGF-A, for which high levels were predictive, low levels of NRP-1 predicted for benefit of bevacizumab. Technical concerns are also an issue, as it is unclear whether the antibody used to stain NRP-1 detects the biologically relevant domain. The staining pattern is cytoplasmic and/or membranous in tumor cells, and the impact of staining 2 different cellular compartments may be important to assess. In addition, NRP-1 immunohistochemistry stains the endothelium and pericytes of the blood vessels as well as tumor cells, and the contribution of each component may differ (18). Thus, for example, strong staining of tumor cells with weak staining of blood vessels may lead to different outcomes (both prognostic and predictive) than alternative staining patterns.

The immunohistochemistry validation for NRP-1 was conducted internally at the reference laboratory. The validation methodology would need to include steps to offset the influence of variability in fixation (that may occur in different geographic regions), tumor cell density in different types of specimens, and variability in NRP-1 staining in primary versus metastatic sites. The authors used a histologic (H)-score for grading the staining of tumor cells but specifics as to the counting methods (manual or by automated image analysis); if done manually, interobserver variability of staining interpretation was not included. For a marker like NRP-1, it would be ideal for the immunohistochemistry quantitation to be fully automated by an image analysis, followed by a grading system that is reproducible for widespread use. Immunohistochemistry as a technique for biomarker assessment is challenging even for an established marker like HER2. The most recent data from the ToGA trial (19) revealed a sizable number of patients with tumor samples showing discrepancy between immunohistochemistry and FISH amplification. In addition, the criteria being applied for immunohistochemical quantitation of HER2 are different in gastric cancer than in breast cancer.

Because of the above issues with NRP-1 staining and the lack of any data that this biomarker adds to the value of circulating VEGF-A, we are not confident that this biomarker will be clinically useful. In any case, if this work can be validated in patients with gastric cancer (or in other solid malignancies), more questions arise. Will the difference in OS in patients with high VEGF levels treated with bevacizumab be enough to warrant reevaluation of bevacizumab by regulatory agencies? Will investigators, regulatory agencies, and the study sponsors (Roche/Genentech) be able to identify a cutoff for high and low VEGF levels that can be beneficial in predicting response? What is the reproducibility of the assay in different populations over time? Will circulating VEGF be of predictive value for the use of VEGFR tyrosine kinase inhibitors? Finally and most importantly, how would this play out in the clinic? Will oncologists and/or insurers use this assay if a chance still exists that some patients with low VEGF may benefit from bevacizumab, or vice versa, as this assay does not provide an all or none outcome?

The use of the novel ELISA that is more sensitive for smaller VEGF isoforms may represent an important advance, and it is exciting to observe that translational research has revealed a potential biomarker for bevacizumab. However, it is difficult to know how much closer we are to personalizing its use. We appear to have hit the ceiling with the use of bevacizumab in multiple tumor types, and one way to break through this ceiling is to identify predictive biomarkers. We hope that clinical studies will be undertaken with the prospective use of biomarkers for patient selection that will allow us to target the appropriate patients for therapy, offer alternatives to those patients unlikely to benefit from bevacizumab, and enable us to avoid the toxicities and costs for patients who will not benefit. We look forward to seeing the initiation of clinical trials that would prospectively validate the interesting findings in this study because more work must be done before we can claim victory in the search for the elusive predictive biomarker for bevacizumab. We may be on the road but we are not there yet.

Disclosure of Potential Conflicts of Interest
D. Maru has a commercial research grant from Taiho Pharma USA Inc. A. P. Venook is a consultant/advisory board member of Genentech. L.M. Ellis is a consultant/advisory board member of Genentech/Roche

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