Predictive Biomarkers for Bevacizumab: Are We There Yet?

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Translational Relevance

Although VEGF targeted therapies are now approved for use in patients with advanced stage disease in a number of different cancer types, biomarkers for patient selection remain elusive. A recent publication reported on investigations regarding the utility of two putative biomarkers that may be of benefit in selecting patients with advanced stage gastric cancer for therapy including bevacizumab. In this *Perspective*, we critically evaluate these biomarkers and discuss the potential path forward that will be necessary to validate these markers in gastric cancer as well as other malignancies.

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 oncologist decide who should, and more importantly, who should not receive VEGF-targeted therapies. However, there has been renewed interest in predictive biomarkers for VEGF-targeted therapies especially in light of the fact that the US 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. In this perspective, we provide a critical evaluation of the utility of these markers, and the need for validation in prospective clinical trials.

Perspective

In 2003 that we saw the first evidence that targeting Vascular Endothelial Growth Factor (VEGF) led to clinical benefit. Since it was first demonstrated that Bevacizumab (BEV), when added to chemotherapy, improved progression-free survival (PFS), response rate (RR), and overall survival (OS) in patients with metastatic colorectal cancer (mCRC)(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 BEV beyond mCRC included unselected patients with advanced lung, renal cell, and metastatic breast cancers (mBrCa), and patients with glioblastoma.(3-6) All signs pointed to even more indications for BEV, such as pancreatic neuroendocrine tumors. However, the United States Food and Drug Administration (FDA) subseqiuently withdrew its approval for patients with mBrCa. This decision came after much debate but was less political than it was numerical: the lack of a survival benefit in confirmatory trials in patients with mBrCa that were mandated as a provision of the original accelerated approval of BEV in that disease.(7)

Despite the objective data -- in mBrCa, and in two adjuvant colorectal cancer studies showing no benefit of the addition of Bev to chemotherapy(8, 9), and some marginal results in other trials -- many oncologists believe that most patients benefit from the addition of BEV to chemotherapy, and the inclusion of BEV has become the default standard for many oncologists treating patients with various malignancies. The lack of the ability to identify the patients who will benefit *a priori*, i.e. 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 BEV and other VEGF inhibitors has come up empty.(10-13) For example, in the pivotal mCRC 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 BEV to chemotherapy was identified.(10) In fact, the futile search for a biomarker for BEV was the subject of a JCO editorial in 2006.(14)

These results could be interpreted in vaious ways, depending on one's vantage point: 1) there is no biomarker because BEV is effective in all subgroups of patients; or 2) a predictive biomarker for BEV 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 BEV changed drastically when the FDA withdrew its approval for BEV in patients with mBrCa. This regulatory decision coupled with the negative results of the adjuvant CRC trials and multiple negative trials studying VEGF receptor kinase inhibitors in several solid malignancies dashed the premise that VEGF-targeted therapies would be efficacious in patients across the board.

In a recent publication in the Journal of Clinical Oncology (JCO), Van Cutsem and colleagues report that circulating VEGF-A and tumor expression of neuropilin-1, a co-receptor for VEGF-A, could select for patients most likely to benefit from the addition of BEV to chemotherapy in patients with advanced or metastatic gastric cancer in the AVAGAST trial(15). Patients with high baseline plasma VEGF-A levels showed a trend toward improved overall survival (hazard ratio [HR], 0.72; 95% CI, 0.57 to 0.93) versus

patients with low VEGF-A levels (HR, 1.01; 95% CI, 0.77 to 1.31; interaction P = .07). Patients with low baseline expression of neuropilin-1 also showed a trend toward improved overall survival (HR, 0.75; 95% CI, 0.59 to 0.97) versus patients with high neuropilin-1 expression (HR, 1.07; 95% CI, 0.81 to 1.40; interaction P = .06). For both biomarkers, subgroup analyses demonstrated 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 was 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 and non-small cell lung cancers, and had limited predictive value in renal cancer, although, a clear prognostic effect was observed.(17) The hazard ratio (HR) for PFS for patients treated with chemo and BEV was <1 in all four 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 anti-coagulants (citrated tubes vs EDTA collection tubes), etc. 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 BEV to chemotherapy is intuitive and makes biologic sense, robust validation in a multicenter prospective trial is necessary.

Question number one regarding these findings from AVAGAST: “Have we found the predictive marker(s) for BEV?” Not yet. The predictive value of circulating VEGF-A was neither a primary nor secondary endpoint in the preplanned biomarker analysis of the original AVAGAST protocol (that accompanied the manuscript submission to JCO, 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 +/- BEV 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 BEV for patients with mBrCA, 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 study is listed under clinicaltrials.gov.

The second question: what are the relative values and reproducibility of the two 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 multi-parametric chip technique (IMPACT) is an established ELISA platform, the optimal sample processing requirements, analytical validation separate from the discovery set, and, importantly, the development of a universal standardized cut-off value for plasma VEGF-A levels for its use by 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 cut-point distinguishing high from low levels. For example, in the study by Van Cutsem et al.[7] the cut-off was determined *after the fact*. In practice, the cut-off must be *pre-determined* so that any bias in cut-off 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 cut-off for patients must be determined prior to trial initiation but the cut-off (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 where high levels were predictive, low levels of NRP-1 predicted for benefit of BEV. There are also technical concerns, since it is unclear whether the antibody used to

stain NP1 detects the biologically-relevant domain. The staining pattern is cytoplasmic and/or membranous in tumor cells and the impact of staining two different cellular compartments may be important to assess. In addition, NP-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 NP1 was performed internally at the reference laboratory. The validation methodology would need to include steps to off-set the influence of variability in fixation (that may occur in different geographic regions), tumor cell density in different types of specimens, and variability in NP1 staining in primary vs. metastatic sites. The authors utilized an 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 were not included. For a marker like NP1 it would be ideal for the immunohistochemistry quantitation to be fully automated by an image analysis followed by a grading system which is reproducible for wide-spread 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) showed 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.

Due to the above issues with NP1 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 BEV be enough to warrant re-evaluation of BEV by regulatory agencies? Will investigators, regulatory agencies, and the study sponsors (Roche/Genentech) be able to identify a cut-off 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 TKIs? And finally and most importantly, how would this play out in the clinic? Will oncologists and/or insurers use this assay if there is still a chance that some patients with low VEGF may still benefit from BEV, or vice versa, as this assay does not provide an all or none outcome?

The use of the novel ELISA 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 BEV. 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 BEV 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 the those patients unlikely to benefit from Bev and allow 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 there is more work to be done before we can claim victory in the search for the elusive predictive biomarker for BEV. We may be on the road but we are not there yet.

References

1. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med. 2004;350:2335-42.
2. Korpanty G, Smyth E. Anti-VEGF strategies - from antibodies to tyrosine kinase inhibitors: background and clinical development in human cancer. Curr Pharm Des. 2012;18:2680-701.
3. Escudier B, Pluzanska A, Koralewski P, Ravaud A, Bracarda S, Szczylik C, et al. Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. Lancet. 2007;370:2103-11.
4. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med. 2006;355:2542-50.
5. Friedman HS, Prados MD, Wen PY, Mikkelsen T, Schiff D, Abrey LE, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol. 2009;27:4733-40.
6. Miller K, Wang M, Gralow J, Dickler M, Cobleigh M, Perez EA, et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med. 2007;357:2666-76.
7. Lohmann AE, Chia S. Patients with metastatic breast cancer using bevacizumab as a treatment: is there still a role for it? Curr Treat Options Oncol. 2012;13:249-62.
8. Allegra CJ, Yothers G, O'Connell MJ, Sharif S, Petrelli NJ, Colangelo LH, et al. Phase III trial assessing bevacizumab in stages II and III carcinoma of the colon: results of NSABP protocol C-08. J Clin Oncol. 2011;29:11-6.
9. de Gramont A, Van Cutsem E, Schmoll HJ, Tabernero J, Clarke S, Moore MJ, et al. Bevacizumab plus oxaliplatin-based chemotherapy as adjuvant treatment for colon cancer (AVANT): a phase 3 randomised controlled trial. Lancet Oncol. 2012;13:1225-33.
10. Jubb AM, Hurwitz HI, Bai W, Holmgren EB, Tobin P, Guerrero AS, et al. Impact of vascular endothelial growth factor-A expression, thrombospondin-2 expression, and microvessel density on the treatment effect of bevacizumab in metastatic colorectal cancer. J Clin Oncol. 2006;24:217-27.
11. Ince WL, Jubb AM, Holden SN, Holmgren EB, Tobin P, Sridhar M, et al. Association of k-ras, b-raf, and p53 status with the treatment effect of bevacizumab. J Natl Cancer Inst. 2005;97:981-9.
12. Jubb AM, Miller KD, Rugo HS, Harris AL, Chen D, Reimann JD, et al. Impact of exploratory biomarkers on the treatment effect of bevacizumab in metastatic breast cancer. Clin Cancer Res. 2011;17:372-81.
13. Jubb AM, Harris AL. Biomarkers to predict the clinical efficacy of bevacizumab in cancer. Lancet Oncol. 2010;11:1172-83.
14. Bergsland EK. When does the presence of the target predict response to the targeted agent? J Clin Oncol. 2006;24:213-6.
15. Van Cutsem E, de Haas S, Kang YK, Ohtsu A, Tebbutt NC, Ming Xu J, et al. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a biomarker evaluation from the AVAGAST randomized phase III trial. J Clin Oncol. 2012;30:2119-27.
16. Jayson Gd, Delmar P, Miles DW, Shah MA, Van Cutsem E, Carmeliet P, et al. Evaluation of plasma VEGFA (pVEGFA) as a pan-tumour biomarker (BM) for the

treatment of patients (pts) with bevacizumab (bev). 2011 European Multidisciplinary Cancer Congress; 2011; 2011.
17. Hegde PS, Jubb AM, Chen D, Li NF, Meng G, Bernaards C, et al. Predictive Impact of Circulating Vascular Endothelial Growth Factor in 4 Phase III Trials Evaluating Bevacizumab. Clin Cancer Res. 2012.
18. Jubb AM, Strickland LA, Liu SD, Mak J, Schmidt M, Koeppen H. Neuropilin-1 expression in cancer and development. J Pathol. 2012;226:50-60.
19. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet. 2010;376:687-97.

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