How Well Do Angiogenesis Inhibitors Work? Biomarkers of Response Prove Elusive

By Vicki Brower

In 2007, the late Judah Folkman noted “a major obstacle” to the development of drugs targeted at angiogenesis, or new blood vessel growth, in tumors: the lack of noninvasive biomarkers for identifying patients most likely to respond to the drugs. Now, with three approved angiogenesis inhibitors (AIs) on the market—bevacizumab, sunitinib, and sorafenib—and hundreds of trials for others under way, the need for accurate, inexpensive, and noninvasive biomarkers has become even more pressing.

“Among researchers, there is a consensus that we need to step back and better understand how angiogenesis inhibitors work, and don’t work as well as we’d hoped,” said Curzio Ruegg, M.D., professor and head of experimental oncology at Switzerland’s University of Lausanne. “Better biomarkers should also help us answer the nagging question why AIs often improve progression-free survival but don’t result in better overall survival.”

AI biomarkers that are detectable in blood or serum are especially intriguing because they would be relatively low cost and accessible, in contrast to the advanced imaging tools used to gauge response. And they could aid in diagnosis, staging, prognosis, and treatment selection, said Sam Gambhir, M.D., Ph.D., director of the molecular imaging program at Stanford University.

For all these reasons, AI biomarker research is booming. Much of the research has focused on cytokines, including the protein targets of AI drugs, such as vascular endothelial growth factor (VEGF). But intense interest also surrounds biological markers such as circulating endothelial cells (CECs), and a subset of those cells, circulating endothelial progenitors (CEPs).

Cytokine Markers
Although the three approved AIs target VEGF and its receptor pathway, using VEGF as a biomarker has been problematic. “While measuring blood levels of VEGF, a biological driver of angiogenesis, seems logical to gauge drug activity, it has not consistently predicted drug response in trials,” Ruegg said.

One reason may be that when VEGF is inhibited, the levels of other angiogenic proteins, such as fibroblast growth factor and interleukin 6, are temporarily increased, enabling the angiogenesis process even when VEGF is blocked.

Another obstacle is that different cancers may react differently to the VEGF-targeted drugs, said Rupal Bhatt, M.D., Ph.D., who is testing sorafenib and sunitinib in mice to identify biomarkers of resistance. Bhatt, along with many other researchers, is doubtful that one biomarker to work for all cancers will be found. She believes that each tumor type is governed by a set of pro- and antiangiogenesis molecules specific to that tumor type.

“Every researcher in this field is looking for a universal biomarker, but no one has found one yet, and it is unlikely they will,” she said.

But researchers have had some success with individual cancers. In January, John Heymach, M.D., Ph.D., of the University of Texas M. D. Anderson Cancer Center in Houston, reported that it was possible to predict which tumors would progress in colorectal cancer patients treated with bevacizumab. Progression occurred in patients with steeply rising levels of two cytokines, basic fibroblast growth factor and placental growth factor. The researchers found elevated levels of these proangiogenic cytokines before radiological evidence revealed disease progression.

Measuring basic fibroblast growth factor and placental growth factor could help physicians predict resistance to anti-VEGF therapy, Heymach said at the American Society of Clinical Oncology’s Gastrointestinal Cancers Symposium in San Francisco. Having this information would enable a rapid change of treatments instead of waiting months for radiological evidence, he said.

Platelet Proteome
Other new research may help explain why VEGF and other angiogenic proteins in serum and blood have not consistently proven to be accurate biomarkers of angiogenesis. Giannoula Klement, M.D., pediatric oncologist at Children’s Hospital in Boston, and colleagues have found that platelets actively store and release pro- and antiangiogenic proteins in two distinct compartments in response to the body’s signals for wound healing and cancer development. “Only in response to these signals do platelets, the body’s first responders in a range of conditions, deliver angiogenic proteins locally,” Klement said.

A few years ago Folkman and Klement proposed measuring the “platelet proteome” of cancer patients to determine whether specific patterns of angiogenic proteins might yield important information. This proteomic readout from platelets would be patient- and disease specific, rather than yielding one universal pattern, they theorized.
Simultaneously, they had identified a host-secreted protein, platelet factor 4, which acts as a master switch to modulate angiogenic signaling—a potential universal marker that, together with the platelet proteome, could help detect cancer and its recurrence.

In 2004, the team began testing these hypotheses by measuring platelet factor 4 and the platelet proteome of 40 colorectal cancer patients. In 2007 and 2008, they began testing breast and renal cancer patients as well. They are monitoring each patient for 2–3 years and hope to identify a different pattern of biomarkers for each of the three cancer types. “As patterns emerge, we will be able to make rational choices and choose multiple inhibitors for each patient based on their tumor proteome,” said Klement, who holds a patent on the use of platelets as markers of early tumors that has been licensed to Ortho Clinical Diagnostics in Rochester, N.Y.

Circulating Endothelial Cells
Researchers are also studying mature CECs and a subset of these cells, bone marrow–derived CEPs, as markers of cancer growth, drug efficacy, and prognosis. CEPs, thought to be more immature, express one extra marker—a stem cell marker—that is not expressed on the more mature CECs. The hypothesis, Bhatt said, is that CECs are shed from existing vessels and that CEPs are released from the bone marrow and recruited to areas where new vessels are being formed.

“Of all potential biomarkers, scientists are perhaps most excited about these cells,” Bhatt said. She presented evidence at the 2008 American Society of Clinical Oncology annual meeting that CECs and CEPs can be used to monitor angiogenesis in patients with sporadic kidney cancer and that the CEC/CEP ratio may also enable early detection of the disease in patients with a rare, genetic form of the disease.

CECs and CEPs are present in low levels in most cancers—higher in breast and lower in lung cancer—and overall, their quantities change in response to treatment. VEGF and other angiogenic cytokines mobilize CEPs from the bone marrow to form new tumor blood vessels. (Scientists believe that AIs work, at least in part, by interfering with CEP mobilization, not only by suppressing local blood vessel sprouting.)

High baseline levels of both cell types predicted response to antiangiogenic therapies in animal models treated with a range of drugs and helped define the optimal biological dose of these drugs, according to Franco Bertolini, M.D., Ph.D., of Milan’s European Institute of Oncology.

In his studies, patients taking metronomic chemotherapy—continuous, low-dose chemotherapy—and bevacizumab had high baseline levels of pretreatment CECs, which were associated with clinical response. For patients given regular-dose chemotherapy and bevacizumab, high-level baseline CEPs also are associated with a good response, Bertolini said.

He said that he and his colleagues have found that some cytotoxic drugs mobilize CEPs and restart neoplastic angiogenesis after therapy, suggesting that AI drugs should be given along with chemotherapy to improve their efficacy. Conversely, patients with high CECs have active cancer vessel-wall turnover—shedding of mature and dying endothelial cells that compose blood vessel walls—and benefit from metronomic chemotherapy and AIs. “This theory is corroborated by the observation that relapse after metronomic chemo plus bevacizumab is associated with a dramatic drop in CECs, most likely reflecting a new type of cancer vascularization,” Bertolini said.

Using CEPs and CECs as biomarkers was, until recently, impractical because of their scarcity, disagreement among researchers about how best to identify them, and the lack of a simple detection method. Flow cytometry may lead to a standardized method for detecting them, according to recent research by Heymach and Bertolini. Nevertheless, a lot more research is needed to determine how they can be used as biomarkers and for which cancers, Bertolini said.

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