Commentary on "Humanization of an Anti-VEGF Monoclonal Antibody for the Therapy of Solid Tumors and Other Disorders"

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The historical article describes the engineering of a molecule that for the first time allowed testing the hypothesis that blocking VEGF-mediated angiogenesis may confer a benefit on human patients (1). Twenty years later, the reagent described in our study, known today as bevacizumab or Avastin, represents a standard of therapy for multiple malignancies (2). It is noteworthy that VEGF-induced chronic hyperpermeability is, to a large extent, indirect and results from pathophysiologic changes in blood vessels after VEGF inhibition.

The history of VEGF reflects independent efforts, which resulted in the discovery of apparently unrelated biological activities. In 1983, Senger and colleagues reported the identification of vascular permeability factor (VPF) as a protein that rapidly increased vascular permeability when injected in the skin (3). However, VPF was not fully purified or cloned at that time (3), and thus, very limited progress in elucidating the significance of this activity was possible during the next several years. In 1989, we described the purification and cloning of VEGF, a novel endothelial cell–specific mitogen (4). After our initial work was published, an independent group reported the cloning of VPF, which proved to be the same molecule as VEGF (reviewed in ref. 2). Since then, numerous studies have sought to dissect the contribution of these two activities to the pathophysiologic roles of VEGF and to the consequences of its blockade.

VEGF as a Key Regulator of Angiogenesis

Many studies have documented the crucial role of VEGF in the development of embryonic and postnatal blood vessels (2). Loss of even a single vegf allele resulted in defective vascular development and early embryonic lethality in mice. Additional studies revealed the role of VEGF-mediated angiogenesis in key postnatal physiologic processes, including skeletal growth and cyclical development of the ovarian corpus luteum (2).

A major milestone was the identification of the two VEGF tyrosine kinase receptors, VEGFR1 and VEGFR2. It is now well established that VEGFR2 is the main signaling receptor that mediates both angiogenic and permeability-enhancing effects of VEGF (2). Two major tyrosine residues in human VEGFR2, Y1175 (Y1173 in the mouse) and Y951 (Y949 in the mouse), have been shown to differentially regulate angiogenesis and vascular permeability. Mice homozygous for the single substitution tyrosine to phenylalanine in position 1173 showed defective vasculogenesis and angiogenesis and died in utero around day 8.5 to 9.5 (5). Phosphorylated Y949/951 interacts with an adaptor that triggers formation of complexes between Src and VE-cadherin, resulting in the opening of interendothelial junctions (6). Inactivating mutations in this pathway largely abolished the initially reported (3) permeability-enhancing effects of VEGF in mice (6). Mutant mice were normal and fertile, indicating that this function of VEGF does not play major physiologic roles (6). However, a transient reduction in tumor edema and a decrease in the number of metastases were observed in the mutants, although primary tumor growth and blood vessel density were the same as in wild-type controls (6). It is noteworthy that VEGF-induced chronic hyperpermeability is, to a large extent, indirect and reflects growth of immature and structurally abnormal vessels that are inherently leaky due to a lack of pericytes and the development of microaneurysms (reviewed in ref. 2).

Anti-VEGF Antibodies Inhibit Pathologic Angiogenesis in Animal Models

To investigate the role of VEGF, we initially developed neutralizing mAbs (7). In 1993, we reported that the murine mAb A4.6.1, which neutralized all bioactive forms of human VEGF, inhibited the growth of human tumor cell lines implanted in immunodeficient mice (8). These findings were unexpected at that time, as it was widely believed that blocking tumor angiogenesis required inhibition of multiple factors and also provided the first direct evidence that tumor growth is dependent on angiogenesis. Further studies confirmed these findings and extended them to a variety of tumor models, including orthotopic models (9). Imaging of the tumor vasculature by intravital microscopy strongly supported the hypothesis that inhibition of angiogenesis was the mechanism of tumor growth inhibition (10). However, because mAb A4.6.1 does not block mouse VEGF, its use in human xenografts in mice resulted in underestimation of the contribution of VEGF to tumor angiogenesis, as several host stromal cell types produce VEGF (2).

By the mid 1990s, strong correlational evidence implicated VEGF in retinal ischemia-related neovascular syndromes secondary to diabetes, prematurity, or retinal vein occlusion (2). Intravitreal injection of mAb A4.6.1 resulted in a dramatic inhibition of neovascularization in a primate model of retinal vein occlusion,
thus providing proof of concept for the critical role of VEGF in this process (11).

**An Anti-VEGF Antibody for Clinical Trials**

mAb A.4.6.1 was selected as a candidate for humanization, a technology devised to prevent the immune response typically observed following administration of murine antibodies in humans (12). It consists of the transfer of the complementarity determining regions (CDR), involved in specific antigen binding, from the original antibody into a human framework (12). Humanizing an antibody was challenging and indeed as much an art as a science. A major problem was that CDR transfer alone rarely resulted in binding affinity comparable with the original antibody. A number of framework residues, not directly involved in antigen binding but required for proper conformation of the CDRs (13), needed to be mutagenized to the corresponding sequences in the original murine antibody to fully restore binding affinity. Even a single mismatched residue could significantly affect binding. Although modeling and prior experience were helpful in identifying such residues, each case was different and often multiple rounds of site-directed mutagenesis and testing of variants were required. It was fortunate that Leonard (Len) Presta joined this program. Len’s outstanding expertise and accomplishment in antibody engineering and humanization were crucial to the success of these efforts. The six CDRs from mAb A.4.6.1 were transferred into a normal human immunoglobulin framework (human heavy chain subgroup III and light chain k I) that had been successfully employed to humanize other therapeutic antibodies (1). As expected, the straight CDR transfer resulted in a variant with very poor binding affinity. Eight framework residues, seven in the variable heavy (VH) domain and one in the variable light (VL) domain, had to be mutagenized to the murine sequences to restore the binding affinity (1). The final humanized IgG antibody, bevacizumab, had essentially the same binding characteristics and biological properties as mAb A.4.6.1 (1). It inhibited VEGF-stimulated endothelial cell proliferation and in vivo growth of human tumor cell lines with the same potency and efficacy to the original murine mAb (1). This recombinant antibody was then selected for clinical development.

Subsequent studies explored the possibility that anti-VEGF antibodies with higher binding affinity for human VEGF than bevacizumab (Kd ~ 1 nM) might have greater antitumor efficacy. Engineering mice to express a mutant VEGF that is neutralized by anti-human VEGF antibodies made it possible to compare these variants not only for their ability to inhibit growth of human tumor xenografts but also for organ toxicity (14). Interestingly, systemically administered bevacizumab was as potent and efficacious as affinity-matured variants with much higher binding affinity. However, these studies revealed a correlation between VEGF-binding affinity and toxicity, especially for kidney and liver (14). Therefore, it appears that increasing binding affinity for VEGF beyond an optimal value has little or no therapeutic advantage and can actually be detrimental, at least when the antibodies are administered systemically.

**Subsequent Developments in the Field**

In January 1997, Genentech filed an Investigational New Drug application for bevacizumab, and phase 1 clinical studies in cancer patients began in April 1997. In that period, the angiogenesis field was experiencing tremendous excitement, to be followed by disappointment and skepticism. In 1997, Boehm and colleagues reported that endostatin (a fragment of collagen XVIII) had dramatic antiangiogenic effects, resulting in eradication of established tumors in mice, without inducing drug resistance (15). The effects were so striking that they generated hope that a potent new therapy for cancer would be soon available. However, subsequent human trials with recombinant endostatin did not show significant benefits.

Another disappointing development was the failure of a different class of antiangiogenic agents, the matrix metalloproteinase inhibitors (MMPI) in the late 1990s and early 2000s (16). In spite of strong preclinical data that convinced several pharmaceutical companies to develop MMPIs for multiple tumor types, none of the molecules tested showed clinical efficacy, for reasons that are not entirely clear (16). These and other setbacks, including a negative phase III study of bevacizumab in relapsed breast cancer patients in 2002, contributed to cast doubt on the validity of the antiangiogenic approach (17).

So, almost everyone was surprised by the presentation at the ASCO Annual Meeting in June 2003 of a phase III study showing that bevacizumab, in combination with cytotoxic chemotherapy, resulted in a significant increase in progression-free survival and overall survival in patients with previously untreated metastatic colorectal cancer, compared with chemotherapy alone (18). Bevacizumab was approved by the FDA for this indication in February 2004. Subsequent clinical studies confirmed the benefits of bevacizumab in colorectal cancer and extended them to additional malignancies, including non–small cell lung carcinoma (NSCLC), renal cell carcinoma, glioblastoma multiforme, ovarian cancer, and cervical cancer, resulting in regulatory approvals in the United States and in other countries (2). More than 2 million patients have been treated with bevacizumab, and today, this drug ranks among the most widely used therapeutics in oncology. Other inhibitors of the VEGF pathway, which include small-molecule VEGF receptor tyrosine kinase inhibitors, an antibody targeting VEGFR2, and a chimeric soluble VEGF receptor, have shown efficacy in several tumor types and have been approved by the FDA (reviewed in ref. 2).

It has been pointed out that the benefits conferred by anti-VEGF agents in oncology are not dramatic. However, markedly extending clinical benefits, especially survival, in patients with advanced cancer is still a major challenge for all anticancer approaches. In this context, 12 years after its initial approval, bevacizumab represents a standard of care for first and second line treatment of metastatic colorectal cancer (2). Although numerous clinical trials have been performed during these years, agents that significantly exceed the benefit conferred by bevacizumab in this setting have not yet emerged. Immunotherapy, which is so exciting today, results in striking benefits in melanoma, but only subsets of patients with other tumor types have substantial responses. Therefore, there are clearly unmet medical needs, and it is likely that bevacizumab and other anti-VEGF agents will continue to play important roles in cancer therapy. Most recently, benefits of bevacizumab treatment have been reported in pleural mesothelioma (19) and in EGFR-mutant NSCLC (20), leading to approval by the European Commission in June 2016 of bevacizumab in combination with erlotinib for advanced, EGFR-mutant NSCLC. Clearly, challenges and unresolved issues remain, including the identification of predictive biomarkers, establishing optimal combinations with inhibitors of other pathways as well as elucidating mechanisms of drug resistance (2).
However, one has to recognize that perhaps the most remarkable successes of anti-VEGF therapy have not been in cancer but instead in ophthalmology (2). Several studies established that intravitreal administration of ranibizumab, an affinity-matured Fab derived from bevacizumab-Fab (21), resulted in meaningful increases in visual acuity in patients with wet AMD or other intraocular vascular syndromes, while controls experienced vision loss (2). Afibercept, a chimeric soluble receptor, has also been approved by the FDA for multiple ocular indications (2). Even though bevacizumab was not developed for ophthalmologic indications, it is widely used off-label due to its low cost, and this has enabled many patients to have access to a therapy that resulted in vision improvement.

Very recent studies have reported the outcomes of 5-year treatment of wet AMD patients with bevacizumab or ranibizumab (22). Fifty percent of patients had good vision (20/40 or better). Before anti-VEGF agents were available, at 5 years, almost all wet AMD patients had severely impaired vision or were legally blind (22). These are dramatic advances, and the class of molecules described in the highlighted study played a seminal role in enabling them.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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