Clinical Application of Therapies Targeting VEGF

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This year's Lasker DeBakey Clinical Research Award goes to Napoleone Ferrara for the discovery of vascular endothelial growth factor (VEGF) as a major mediator of angiogenesis and for the development of an effective anti-VEGF therapy for wet macular degeneration, a leading cause of blindness in the elderly.

Many of us have been lured into a career in science by the hope that we would someday make a scientific discovery benefiting patients suffering from a previously incurable disease. Only as we progress in our careers do we realize how difficult and rare such a discovery is, not to mention how disconnected the actual scientific discovery often is from the development of a new therapeutic based on that discovery. Thus it is exceptionally rare that a single individual not only makes the seminal discovery but also helps to champion the development of an effective new class of therapeutics. Napoleone Ferrara, recipient of this year's Lasker DeBakey Clinical Reseach Award, provides a rare such example.

Ferrara's landmark scientific discovery involved the isolation and cDNA cloning of vascular endothelial growth factor (VEGF) as a mitogen for vascular endothelial cells. In large part due to Ferrara's subsequent efforts, we now know that VEGF is the most important driver in the body of normal as well as pathological blood vessel growth. We also now realize that VEGF not only induces vessel sprouting and growth but can also regulate vessel function in other ways, so as to regulate vascular tone and blood pressure, as well as vessel wall integrity and vascular permeability. The Lasker committee is recognizing Ferrara for the discovery of VEGF and for his specific contribution to the eye field, where he played a key role in the development of an anti-VEGF therapy for age-related macular degeneration (AMD), a leading cause of blindness in the elderly. Although not directly acknowledged in the current award, Ferrara made arguably even more exceptional contributions to the parallel development of a similar therapy for cancer.

Distinct Vascular Pathologies in Eye Diseases and in Cancer

The vasculature plays a critical role in a variety of eye diseases as well as in cancer growth. In AMD, the most severe vision loss occurs in patients who develop the "wet form" of the disease characterized by choroidal neovascularization (CNV). CNV refers to the growth of abnormal vessels originating from the choroidal vascular network, directly underlying the retina. The abnormal vessels do not usually invade the neural retina and thus do not directly disrupt the retina and its function. Instead, these abnormal vessels become excessively leaky, leading to retinal swelling and edema, which in turn impairs vision. Optical coherence tomography (OCT) can beautifully image the living retina and reveal the extent of swelling, including within the macula and its foveal region, the tiny central portion of the retina that is responsible for the "central vision" critical to important tasks such as reading and driving. OCT images demonstrate that patients with AMD can have marked swelling in their central retina to over three times normal thickness, resulting in severe vision loss (Figure 1).

As Ferrara himself has thoroughly reviewed, the observation that tumor growth is associated with increased vascularity was initially made over 100 years ago, and this observation was then followed by a series of classic papers over the following decades suggesting that tumors might produce a diffusible factor that stimulates angiogenesis, and that this angiogenesis could be required for tumor growth (Ferrara et al., 2004). The realization that the apparently disparate vascular pathologies in cancer and eye diseases had a common trigger, and thus potentially a related cure, awaited the discovery and cloning of VEGF.

The Discovery and Cloning of VEGF and VPF

In 1989, Ferrara and Henzel, working at Genentech, reported the purification and amino-terminal sequence of an endothelial-specific mitogen; they termed this protein VEGF. Shortly thereafter, Ferrara and colleagues described the molecular cloning of the cDNA encoding VEGF (Leung et al., 1989). While Ferrara and his colleagues focused on the endothelial growth properties of this new protein, a parallel effort was unknowingly trying to purify and clone the same protein, but with an eye toward a totally different biological function. In 1983, the Dvorak laboratory identified a tumor-derived factor, which they termed "vascular permeability factor" (VPF), that rapidly and potently induced microvascular permeability and fluid leak but for which they had no molecular sequence (Senger et al., 1983); I remember first hearing the VPF story directly from Dvorak in the mid-1980s at Cold Spring Harbor when he attended the cloning course that I was teaching, along with Fred Alt and Al Bothwell, in which Dvorak was trying to gain the expertise to clone this intriguing factor. Presumably because our training

of Dvorak was not sufficient, cloning of VPF was subsequently undertaken by the Monsanto Company, which published the amino-terminal protein sequence as well as the cDNA sequence in 1989 (Connolly et al., 1989; Keck, 1989).

Cloning of VEGF and VPF revealed that they were the same factor, and this convergence showed that this new factor had at least two fascinating biologic activities—not only could it induce endothelial cell proliferation, but it could cause vascular leak and edema. Over the next two decades, Ferrara was the clear world leader in further elucidating the biology and pathological roles of this new growth factor, helping drive more widespread adoption of VEGF as its name. Ferrara early on realized the value of using genetic inactivation in mice, as well as engineered biologics that could work in multiple species, as powerful tools. In 1996, he demonstrated that early mouse development depended on precise dosing of VEGF by showing that inactivation of even a single VEGF allele resulted in embryonic lethality due to severe vascular abnormalities. He cleverly developed and elegantly exploited biologics-based blockers (such as antibodies and soluble receptors) to show that VEGF is required for overall postnatal growth, and to define its roles in structures such as growing bones and the cycling ovary (Gerber et al., 1999a, 1999b). He also worked with collaborators to show that VEGF acted via an endothelial-specific receptor tyrosine kinase, further confirming that evolution had selected VEGF to act specifically on the vascular endothelium by limiting its receptor distribution to these cells.

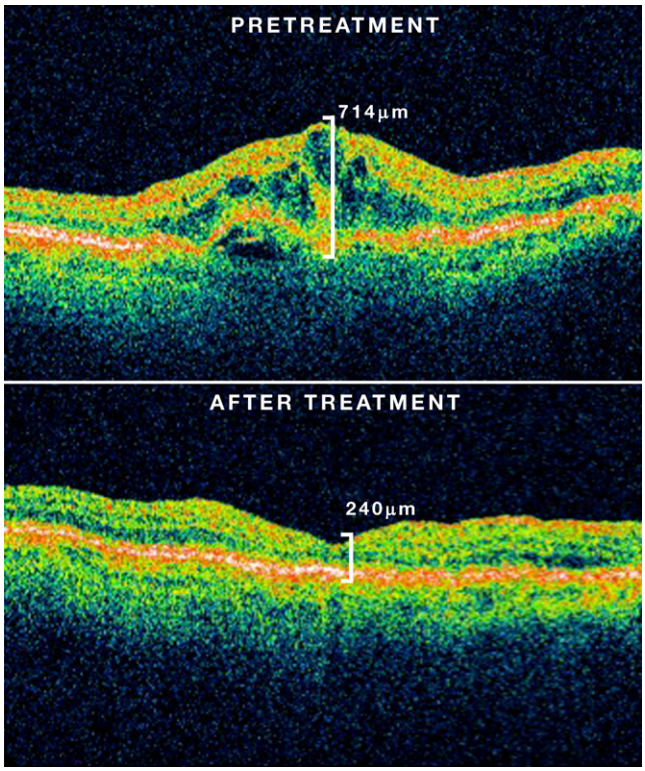

Figure 1. Anti-VEGF Therapy for Wet Age-Related Macular Degeneration

Swelling of the central retina in a patient with age-related macular degeneration, as seen by optical coherence tomography, is reduced by treatment with anti-VEGF therapy. Prior to treatment this individual could read 35 letters on a specialized "ETDRS" eye chart. After treatment, this improved to 66.

VEGF and Tumor Angiogenesis

As noted above, it had long been appreciated that neo-angiogenesis accompanies and might be required for tumor growth. Building on this background, Folkman was the first to propose that therapies designed to prevent such angiogenesis might provide a useful new way to combat cancer (Folkman, 1971). Folkman, however, also presented a rather complicated view of tumor angiogenesis in which there were myriad positive and negative regulators, almost all of which (such as fibroblast growth factors, transforming growth factors, collagen fragments known as endostatin, and plasminogen fragments known as angiostatin) served roles outside of the vasculature as well; Folkman suggested that tumor angiogenesis depended on a complex integration of these various positive and negative regulators but did not propose a specific angiogenic pathway nor a key trigger. In contrast, Ferrara showed that angiogenesis depended on a clear cascade of factors, with VEGF as the key initiator of most angiogenic processes; Ferrara's demonstration of the primacy of VEGF also pushed the field to realize that additional growth factors had also evolved to specifically regulate the endothelium by similarly utilizing endothelial-specific receptors, such as other members of the VEGF family as well as the more recently discovered angiopoietin family (Yancopoulos et al., 2000).

Diligently pursuing his focus on VEGF, Ferrara developed a mouse monoclonal antibody to block VEGF, termed A.4.6.1. It was initial experiments using this antibody in animal models that established the primacy of VEGF in tumor angiogenesis—Ferrara showed that the antibody could strongly inhibit tumor growth by limiting tumor-induced angiogenesis, not only providing the first convincing evidence that blocking tumor angiogenesis could indeed prevent tumor growth but simultaneously establishing VEGF as the critical target in the process (Kim et al., 1993); importantly, the results were reproduced in many laboratories using an assortment of VEGF-blocking reagents, including a clinical candidate termed the VEGF Trap that was developed in our laboratory.

Despite the results with VEGF blockade reported by Ferrara and others, the pharmaceutical industry did not immediately jump on VEGF as an exciting cancer target. In part, this had to do with prevailing views in the field that there were myriad potential targets to attack, and that no target was more important than others. Ferrara pressed on and next humanized A.4.6.1 so that it could be used in human trials. This humanized antibody, given the generic name bevacizumab and the brand name Avastin, first entered clinical trials in 1997. Bevacizumab ultimately achieved FDA approval in 2004 as a first-line treatment for metastatic colorectal cancer in combination with chemotherapy, based on its statistically and clinically meaningful benefits on progression-free survival and overall survival (Ferrara et al., 2004), and has since garnered additional approvals. The

bevacizumab story provides the definitive demonstration that, in man, specific antiangiogenesis blockade can provide useful tumor control in multiple cancer settings and is a testimonial to the efforts and persistence of Ferrara, and it still remains the standard for angiogenesis-based therapeutics.

Kinase inhibitors that target the VEGF receptor signaling pathway have since been approved in cancer but do not display as widespread activity while also exhibiting broader toxicities. There appear to be several reasons for this. Biologics-based therapies such as bevacizumab are naturally selected to have high affinity and great specificity for their target and also have the benefit of long-circulating half-lives following injection, allowing for rather complete and long-term blockade with little if any off-target activity, which has proven more difficult to achieve with small-molecule kinase inhibitors. Probably due to the confusion that marked the field a few years ago, few biologics-based VEGF-targeted therapies are in late-stage clinical trials in cancer; it remains to be seen whether either of the two biologicals in phase III trials (that is, the VEGF Trap or Lilly's ramucirumab that targets the VEGF receptor) will provide similar or even greater benefit than bevacizumab.

Anti-VEGF Therapy for Eye Diseases

Ferrara played a key role in the development of anti-VEGF therapies for eye diseases, an endeavor that depended on the contributions and influence of several key collaborators as well as independent groups. First of all, it should be pointed out that most believe it is the permeability-inducing activity of VEGF, first described by Dvorak, that leads to the retinal swelling and edema that cause vision loss in wet AMD; other eye diseases (such as proliferative diabetic retinopathy) do exhibit the profound pathologic neovascularization that we now know is also driven by VEGF. It was in the latter type of settings that the first definitive link between VEGF and human eye disease was made, simultaneously in 1994 by Adamis and colleagues as well as Aiello and King working in collaboration with Ferrara (Adamis et al., 1994; Aiello et al., 1994); both groups showed marked increases in VEGF levels in the eyes of patients suffering from intraocular neovascularization. Shortly thereafter, both groups worked in collaboration with Ferrara to show the benefit of blocking VEGF in animal models of ocular neovascularization; Ferrara provided the critically required anti-VEGF blocking reagents for these seminal studies.

The introduction of anti-VEGF therapies into the clinic for eye diseases came from a completely unexpected source, a small company named NeXstar Pharmaceuticals. This company was based on Larry Gold's "aptamer" technology, which was being used to develop small synthetic RNAs as a new class of drugs, and one of their scientists, Nebojsa Janjic, was developing an anti-VEGF aptamer with cancer in mind; however, this aptamer was ineffective when systemically administered in animal tumor models. Stimulated by Adamis' paper, Janjic reasoned that his aptamer might work better if directly injected into the eye. Toward this end, Janjic met in 1996 with Adamis and Guyer, who helped Janjic design a clinical development plan for AMD. The aptamer, termed Macugen, entered clinical trials in 1999. In the meantime, Adamis and Guyer decided to try to start their own venture and searched for the best available VEGF inhibitor they could license for use in the eye; it was at this point that I met the pair as they became interested in our VEGF Trap, and I became convinced by their compelling rationale. Unfortunately, the VEGF Trap was then entangled in a collaboration with the Proctor & Gamble Health Care group, which was not interested in either developing it or out-licensing it for the eye, and thus Adamis and Guyer had to look elsewhere; several years later, we were independently able to progress the VEGF Trap into the clinic for eye diseases. By 2000, Adamis and Guyer had started a company called Eyetech and, not having other options, licensed Macugen and continued its clinical development. In phase III, Macugen produced rather modest results, somewhat slowing the progressive visual decline of AMD but was nevertheless approved by the FDA in 2004; Pfizer entered into the mix and paid a huge premium to obtain rights to this innovative therapeutic.

Although temporally behind the Macugen story, and certainly spurred by the competition, Ferrara and Genentech had far superior VEGF blockers at their disposal. Because of concerns that a full-length antibody might not diffuse efficiently into the retina when injected into the vitreous, Ferrara and his colleagues decided to engineer a humanized Fab variant of A.4.6.1 for use in the eye that was ultimately given the generic name ranibizumab and the brand name Lucentis (Ferrara et al., 2006). Ranibizumab had other advantages over bevacizumab, most notably a much higher affinity that allowed it to be active at lower concentrations, which Ferrara felt might be important in terms of allowing for maintained activity when the drug would drop to low levels between monthly injections into the eye. Genentech initially dosed patients with ranibizumab in 2000 and received FDA approval for the treatment of wet AMD in 2006. The efficacy results were quite stunning, especially when compared to those obtained with the poorer blocker, Macugen. Instead of merely slowing vision loss, patients on average gained vision and maintained these gains if dosed on a monthly schedule. Ranibizumab has since been studied in other eye diseases and recently gained approval for retinal vein occlusion. Worldwide, Lucentis is now being used to treat about a quarter million patients a year. It perfectly fits the definition of pharmaceutical blockbuster, in terms of providing enormous clinical benefit to many patients while simultaneously producing enormous revenues. However, there are emerging issues. In part frustrated by the cost of ranibizumab, clinicians explored off-label use of intravitreal injection of bevacizumab for eye diseases and claimed to see similar benefit (Rosenfeld, 2006). While there are certainly concerns in terms of safety risks to patients of such off-label use, the National Eye Institute decided that the potential pharmacoeconomic value of a lower-priced alternative warranted running clinical trials directly comparing ranibizumab and bevacizumab in AMD; results are expected in 2011. In addition, because patients and physicians are very interested in decreasing the frequency of eye injections, there have been many attempts to study less frequent dosing paradigms; despite these efforts, current evidence supports the need for regular if

not monthly injection of ranibizumab to optimize its benefit. Early studies with other biologics blockers raise the possibility that an even higher-affinity blocker, perhaps at higher doses, could provide further visual gains or allow for longer interval dosing.

In many ways, Ferrara's career represents the fulfillment of every drug discoverer's dream, and the Lasker Award could not be going to a more worthy recipient. Ferrara not only made a seminal scientific discovery, but then he and his colleagues at Genentech built on this discovery to spearhead the development of an entirely new class of therapeutics with major applications in two previously distinct clinical arenas—vascular eye diseases and cancer. Although Ferrara's VEGF antibody is now being used to treat about 250,000 cancer patients a year, the current award may have avoided specifically acknowledging Ferrara's contribution to the cancer field because of questions regarding the degree of clinical benefit of bevacizumab in cancer. Because bevacizumab represents an entirely new way of attacking cancer, utilization of this approach is still a work in progress and may require new treatment paradigms to optimize benefit. Traditional treatment paradigms in which the anticancer therapy is stopped after a short treatment period when tumor killing is thought to be completed, or after tumor progression when the tumor is thought to have become chemo-resistant, make little sense for an antiangiogenesis approach: the point is not to try to wipe out the tumor initially but instead to provide ongoing control by limiting host support; any benefit would be expected to dissipate as soon as such therapy is stopped. Ferrara's colleagues at Genentech have nicely demonstrated this point in very recent animal studies (Bagri et al., 2010), as well as in recent clinical studies including one in ovarian cancer using an innovative "maintenance design" carried out by the Gynecological Oncology Group (GOG-0218). Data from this study can be used to make several important points. First, this study shows that, at least in this setting, bevacizumab does not primarily work by allowing more efficient delivery of chemotherapy (as had been proposed by others), given that the gained benefit is at least as good during the monotherapy maintenance stage as during the prior combination stage. Moreover, the study convincingly shows that continued maintenance with anti-VEGF therapy is necessary to prevent loss of clinical benefit. In addition to maintenance approaches or treatment-through-progression strategies, the benefit of anti-VEGF therapy may also be improved by combining with agents targeting other angiogenic pathways; notably, several companies are in trials combining anti-VEGF agents with other antiangiogenic agents, such as those targeting Angiopoietin-2. Chemotherapeutics may also be developed that work better on tumors made hypoxic via antiangiogenic therapy. Although antiangiogenesis approaches in cancer are likely to be further optimized as the community learns better how to take advantage of this approach, there is little doubt that anti-VEGF treatments pioneered by Ferrara and his colleagues will long remain the foundation of such efforts. Thus, it can be hoped that this well-deserved Lasker award for the discovery of VEGF and the development of a treatment for AMD is a harbinger of prestigious accolades to come that would also include specific recognition of Ferrara's contributions to tumor biology and cancer treatment.

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G.D.Y. works at Regeneron, which is developing anti-VEGF therapeutics.

REFERENCES

Adamis, A.P., Miller, J.W., Bernal, M.T., D'Amico, D.J., Folkman, J., Yeo, T.K., and Yeo, K.T. (1994). Am. J. Ophthalmol. *118*, 445–450.

Aiello, L.P., Avery, R.L., Arrigg, P.G., Keyt, B.A., Jampel, H.D., Shah, S.T., Pasquale, L.R., Thieme, H., Iwamoto, M.A., Park, J.E., et al. (1994). N. Engl. J. Med. *331*, 1480–1487.

Bagri, A., Berry, L., Gunter, B., Singh, M., Kasman, I., Damico, L.A., Xiang, H., Schmidt, M., Fuh, G., Hollister, B., et al. (2010). Clin. Cancer Res. *16*, 3887–3900.

Connolly, D.T., Olander, J.V., Heuvelman, D., Nelson, R., Monsell, R., Siegel, N., Haymore, B.L., Leimgruber, R., and Feder, J. (1989). J. Biol. Chem. *264*, 20017–20024.

Folkman, J. (1971). N. Engl. J. Med. *285*, 1182–1186.

Ferrara, N., Hillan, K.J., Gerber, H.-P., and Novotny, W. (2004). Nat. Rev. Drug Discov. *3*, 391–400.

Ferrara, N., Damico, L., Shams, N., Lowman, H., and Kim, R. (2006). Retina *26*, 859–870.

Gerber, H.P., Hillan, K.J., Ryan, A.M., Kowalski, J., Keller, G.A., Rangell, L., Wright, B.D., Radtke, F., Aguet, M., and Ferrara, N. (1999a). Development *126*, 1149–1159.

Gerber, H.P., Vu, T.H., Ryan, A.M., Kowalski, J., Werb, Z., and Ferrara, N. (1999b). Nat. Med. *5*, 623–628.

Keck, P.J. (1989). Science *246*, 1309–1312.

Kim, K.J., Li, B., Winer, J., Armanini, M., Gillett, N., Phillips, H.S., and Ferrara, N. (1993). Nature *362*, 841–844.

Leung, D.W., Cachianes, G., Kuang, W.J., Goeddel, D.V., and Ferrara, N. (1989). Science *246*, 1306–1309.

Rosenfeld, P.J. (2006). Am. J. Ophthalmol. *142*, 141–143.

Senger, D., Galli, S., Dvorak, A., Perruzzi, C., Harvey, V., and Dvorak, H. (1983). Science *219*, 983–985.

Yancopoulos, G.D., Davis, S., Gale, N.W., Rudge, J.S., Wiegand, S.J., and Holash, J. (2000). Nature *407*, 242–248.