When Napoleone Ferrara was a medical student in Italy in the 1970s, he became interested in reproductive endocrinology. Although his training never specifically focused on angiogenesis, he is recognized today for shaping the field through the discovery of its core signaling molecule, VEGF, and for exploiting VEGF for the first successful clinical treatments of wet age-related macular degeneration (AMD) and cancer. On September 21, 2010, the Albert and Mary Lasker Foundation announced they will present Ferrara (Figure 1) with the 2010 Lasker~DeBakey Clinical Medical Research Award for his work on VEGF, which led to an effective therapy for neovascular AMD. Ferrara spoke with the JCI about his journey from discovery to treatment. Revving the endocrine engine Ferrara grew up in the shadow of the most active European volcano in Catania, Sicily. His father was a judge, and others in his family were in the legal profession, but his interest in the life sciences started even in high school: “To me,” Ferrara said, “it was much more interesting than the law.” He followed his passion to medical school, where he felt fortunate to land in the laboratory of Umberto Scapagnini. Ferrara noted that the University of Catania was much better known for its clinical focus than for its research, but “along came this dynamic young pharmacology professor who had trained for […]
Napoleone Ferrara receives the 2010 Lasker~DeBakey Clinical Award for breakthroughs in angiogenesis research

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Revving the endocrine engine

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Scapagnini introduced Ferrara to Richard Weiner at UCSF. After finishing his medical training in Italy, Ferrara began a postdoctoral fellowship in Weiner’s laboratory, studying the dopaminergic regulation of prolactinomas. He remembered from his reproductive endocrinology training that cyclical growth of blood vessels in the ovaries is essential for fertility. In addition, the literature that he studied revealed numerous examples of an overgrown vasculature lying at the heart of the disease pathology. But Ferrara pursued the system that he knew best, and the endocrine cells that controlled ovarian function were located in the pituitary gland; in Weiner’s lab, he began to study how they grew and functioned.

He isolated and cultured follicular cells, a population of non–hormone-secreting cells from the anterior pituitary of cows (1) that Ferrara called “very strange, and still after decades . . . very obscure.” The follicular cells had cytoplasmic projections in inti-mate contact with the perivascular spaces, which suggested they might have a function in regulating the pituitary vasculature. One day, Ferrara tried adding conditioned media from cultures of follicular cells to endothelial cells. The endothelial cells proliferated more rapidly, and this planted the seed in Ferrara’s head that the follicular cells could be secreting an angiogenic agent.

Discovering VEGF

In 1988, Ferrara decided to move on from his postdoctoral fellowship. Although he had the opportunity to go back to Italy, he said, “my heart wanted to stay in the United States and keep going with work I thought was important.” The biotechnology company Genentech in South San Francisco provided just the opportunity he was looking for. Genentech not only was known for ground-breaking science, but had also cultivated an atmosphere of academic discovery, encouraging employees to pursue pet projects in their free time as long as the assigned work was done. Ferrara was hired to work on relaxin, an experimental treatment to ease childbirth, but relaxin ended up a failure in clinical trials, and that project was soon abandoned. But during his time working on relaxin, the angiogenic seed planted in Weiner’s lab began to sprout: Ferrara noted, “We had some partially purified proteins with promising activity, but there was certainly a long way to go.” He spent nights and weekends collecting and processing supernatants from cow pituitary cultures and exploring the resultant proteins in the hopes of isolating the one that made endothelium proliferate.

In 1989, he finally isolated the angiogenic protein specific for vascular endothelial cells, which had an amino acid sequence that matched that of no known substance; he termed it vascular endothelial growth factor (2). Around the same time, an independent group uncovered the gene (3) for vascular permeability factor (VPF), a protein that Harold Dvorak had identified in 1983 (4) on the basis of its ability to elicit blood vessel leakage. However, VPF had not been fully purified, sequenced, or cloned, and little was known about this factor. Sequence comparison later showed that the VPF and VEGF genes were identical. Further testing determined that VEGF was crucial for tumor angiogenesis, and we now know that of the more than 200 kinds of human cancer, greater than half produce VEGF. “This work started as really basic research about angiogenesis in the pituitary,” Ferrara said, but he knew about Judah Folkman’s original hypothesis about choking tumors by shutting off their blood supply (5) – “we knew this could go...
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beyond the basic research and solve a mystery which could go much broader, like to tumors or reproduction.”

Ferrara credits the culture at Genentech for giving him the time and resources to dedicate to the discovery of VEGF: “At the very beginning, this was a purely discretionary project. No one really thought this would be therapeutic, but Genentech always had their liberal policy to allow scientists to explore. There are so many promising molecules that don’t go on to have any relevance to what you’re looking for.”

Harnessing VEGF for therapy

Ultimately, Ferrara knew he was employed by a company, and that he needed to turn his attention to exploiting VEGF as a therapeutic target. He and other Genentech scientists initially developed a murine anti-VEGF monoclonal antibody that exerted a potent inhibitory effect on the growth of three tumor cell lines injected subcutaneously in nude mice (6). After being treated with the antibody, tumors derived from G55 glioblastoma multiforme, SK-LMS-1 leiomyosarcomas, and A673 rhabdomyosarcomas stopped growing, and some animals lived for months. The antibody was not effective at stopping the growth of these cancer cell lines in vitro, which proved that blocking angiogenesis was the mechanism of tumor suppression in vivo. Ferrara remembered the results as “really surprising. It was thought that one would need to block many factors to inhibit angiogenesis.”

All the while, Ferrara and his collaborators continued their basic science discoveries about VEGF and its receptors. Over the years, five VEGF-related genes have been identified (VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E), some acting in different endothelial compartments. By the end of 1989, Ferrara reported the isolation of cDNA clones for one bovine VEGF and three human VEGF-A isoforms (7). He also was involved in determining the various isoforms’ differential interactions with their related receptor tyrosine kinases, VEGFR-1 and VEGFR-2 (also known as Flt1 and Flk-1, respectively). Ferrara’s laboratory showed that VEGFR-1 is the high-affinity VEGF receptor (8). He also had a hand in demonstrating that VEGFR-1 expression is responsive to hypoxia via a hypoxia-inducible factor–dependent mechanism (9) and that VEGFR-1 is also a receptor for the proangiogenic agent placental growth factor (10). In 1996, Ferrara and Peter Carmeliet published back-to-back articles in *Nature* (11, 12) showing an essential role of VEGF in embryonic vasculogenesis in the mouse: deletion of a single VEGF allele resulted in embryonic lethality between days 11 and 12. Ferrara said, “These genetic experiments truly validated the ideas about the central role for VEGF in angiogenesis.” While Ferrara explored the basic biology of VEGF and its mechanisms of action, given how well the murine antibody worked, Genentech was eager to develop a version that could be used to treat humans. Ferrara worked closely with Leonard Presta, at that time Genentech’s authority on antibody humanization, to develop what ultimately became bevacizumab (Avastin). Like its murine counterpart, bevacizumab binds and neutralizes all human VEGF-A isoforms and bioactive proteolytic fragments, but does not interact with mouse or rat VEGF (13). Ferrara explained that the antibodies have been “engineered in such a way that approximately 7% of the murine residues, including the complementary determining regions involved in antigen binding, are inserted in a human antibody framework. The purpose of humanization is to avoid the immune responses that are frequently associated with the administration of murine and sometimes chimeric antibodies.” He added, “The beauty of a monoclonal antibody is its specificity. Small-molecule therapies can sometimes [interact] with other molecules, especially at higher doses, and cause side effects from activity unrelated to the targeted molecule.” The only thing Ferrara didn’t have a hand in was choosing the name. “The company hired some firm that comes up with these bizarre potential names. But the name Avastin itself was suggested by the pathologist Frank Peale, who helped us a lot with the histopathology of anti-VEGF in tumors.”

The Avastin clinical trials

The antibody looked good; the mouse studies were promising; it was time to take the antibodies to humans. Genentech tried a scattershot approach in its clinical trials, testing the drug against many different cancers at the same time. In 1997, Genentech started phase I clinical trials with Avastin, showing that the antibody as a single agent was safe to administer (14). And that adding it to a standard chemotherapy regimen did not exacerbate chemotherapy-associated toxicities (15). Initial clinical trial results did look good for Avastin — until September 2002, when it failed to meet its primary efficacy endpoint of survival in a phase III breast cancer trial. Ferrara was disappointed, but still had some hope. “It did not increase the survival, but there was some evidence that the treatment shrank some tumors in the trial. Also, these patients were in third-line therapy,” meaning they had already been treated by two other methods that had failed. “This is a very high bar for a trial. The patients were in a much more advanced stage and very sick.”

Happily, preliminary results from a phase II renal cell carcinoma trial showed that Avastin did meet its primary efficacy endpoint (16). That trial was the first step toward FDA drug approval. Trials were also already ongoing in 925 patients diagnosed with previously untreated metastatic colon cancer. Comparison of the median survival time of patients treated with irinotecan/5-fluorouracil/leucovorin (IFL) chemotherapy in combination with Avastin and patients treated with IFL and a placebo showed that patients receiving Avastin had a median endpoint survival time greater than 4 months longer than those receiving placebo. This exceeded the primary efficacy endpoint and was also the greatest difference in median survival time reported in a phase III trial. When asked how he felt when the FDA approved Avastin for colorectal cancer treatment on February 26, 2004, Ferrara said, “If I say I was delighted, it will be an understatement.”

Some criticism, however, has been leveled at Genentech (now combined with the larger company Roche) about the costs associated with Avastin. When Avastin was approved in 2004, Genentech set the price at $2,200 for an average dose, to be taken every two weeks; a year’s treatment for colon cancer costs about $50,000. With insurance coverage, patients may have copayments of $10,000 to $20,000 annually. Genentech says that they and Roche have spent more than $2.25 billion to bring Avastin to market starting with Ferrara’s original work; this figure reflects expenditure on research, clinical trials, and filing for regulatory approval. In response to criticism that the 4- to 5-month extension in survival is not worth the cost, Genentech argues that the high cost pays for the expensive and risky research needed to develop new drugs. Also, Ferrara notes that to the best of his knowledge, no biologic agent has yet exceeded the benefit conferred by Avastin on colon cancer patients, emphasizing the challenge in further extending patient survival and the need to perform more research.
Regardless, Ferrara created a blockbuster: in 2008, sales of Avastin were nearly $2.7 billion, according to the Genentech annual report. Ferrara notes that he has met several patients over the years who have been treated with drugs he has developed, and “hearing from them that their lives have been extended or that their sight has been restored is the most gratifying experience of all.”

Turning an eye to Lucentis
Ferrara knew that there were other disorders marked by excessive vascular proliferation and thought that there could be additional clinical indications for anti-VEGF therapy. A prime candidate for study was the eye, where the appearance of proliferative vessels can mean rapid and sometimes irreversible vision loss.

He started with a VEGF-neutralizing chimeric protein injected directly into the eyes of mice with ischemic retinopathy. The treatment resulted in a near-complete block of retinal angiogenesis (17). The next year, Ferrara and his colleagues used non-human primates with retinal ischemia-associated iris neovascularization. Intravitreal injection of the murine precursor of bevacizumab completely abrogated any pathological angiogenesis in primate eyes (18). Their success with interfering with VEGF in the eye led Ferrara and his Genentech colleagues to consider one of the more devastating retinal diseases of excess neovascularization: wet AMD.

AMD is the leading cause of severe vision loss in individuals more than 50 years old in the developed world. While wet AMD accounts for 10%–20% of cases of AMD, it is responsible for more than 80% of the severe vision loss associated with AMD. Wet AMD occurs when growth of abnormal and fragile vessels, which have a propensity to bleed and leak, starts behind the retina under the macula (Figure 2). The blood and fluid from these vessels lift the macula from its normal place at the back of the eye. Damage to the macula causes loss of the ability to see straight ahead and may make it more difficult to read, drive, or perform other daily activities.

Ferrara and his colleagues had concerns about systemic delivery of bevacizumab — using an agent that interferes with blood vessel formation seemed risky in a population with a high risk of cardiovascular disease. Direct intravitreal injection seemed more a prudent delivery method. With that goal in mind, Ferrara and colleagues, including Henry Lowman, chose to engineer a smaller derivative of the anti-VEGF antibody, ranibizumab (Lucentis), which they predicted would penetrate the retina better than its parent, be more potent, and carry a lower risk of certain inflammatory responses. Ferrara notes, “Ranibizumab is an affinity-matured version of the bevacizumab Fab [antigen-binding fragment]. They come from the same original murine antibody.”

In 2000, Genentech and investigators at University of Miami Miller School of Medicine Methodist Hospital in Houston began wet AMD clinical trials with Lucentis. The encouraging preliminary data led to multicenter trials on more than 1,000 patients. Lucentis has been shown to not only stop vision loss, but also improve
sight in many patients after a year of monthly injections (19). All other previously available therapies have not been able to restore vision. In a candid moment, Ferrara said, “When Lucentis worked, we were even more stunned, honestly, than when Avastin worked, because the magnitude of benefit was huge. Treating patients with metastatic cancer, and in particular extending their survival, represents a formidable challenge, and Avastin gave a nice survival benefit to patients with advanced cancer — but it was months, not years. Lucentis can actually restore visual acuity in a large number of patients, which is just stunning.” On June 30, 2006, the FDA approved Lucentis for the treatment of wet AMD, and Ferrara and his Genentech colleagues rejoiced again.

**Where to from here?**

Ferrara is humble, but gratified, about what he has accomplished in his 22 years at Genentech. Although VEGF is now his full-time pursuit, he still encourages those in his lab to pursue their pet projects. “Honestly, I don’t know how easy it is going to be to find another VEGF,” he cracked, “but who knows? They should try.”

Ferrara was quick to acknowledge what an honor it is to receive this year’s Lasker–DeBakey Clinical Medical Research Award. “I feel extremely grateful to the Lasker Foundation, and I don’t really have any words to express how honored and proud I am to have won this year.”

Since 1945, the Albert and Mary Lasker Foundation Awards Program has recognized the contributions of physicians, scientists, and public servants who have made major advances in the understanding, diagnosis, treatment, cure, and prevention of human disease. Ferrara will receive his award at a luncheon in New York City on October 1 along with a $250,000 honorarium.

**Ushma S. Neill**