

The Search for Factor X

How a team approach to discovery and development led to the first anti-VEGF drug in ophthalmology.

BY ANTHONY P. ADAMIS, MD



Ventures in Translation features innovators in the field of vitreoretinal disease. Successful translation of scientific ideas to useful medical treatments and technologies requires many elements. Each of the innovators featured here has a story to tell that often combines an exceptional understanding of disease, foresight, perseverance, and an ability to obtain funding for an unrecognized technology. The stories featured here will provide a snapshot of what it has taken to bring these breakthroughs to our patients. The authors range from scientists to clinicians to bankers to venture capitalists, and some will do a little of all.

In this installment, Anthony P. Adamis, MD, narrates the research and development in antiangiogenesis that led to drug development for retinal diseases. The journey from the initial work in antiangiogenesis by Judah Folkman, MD, to the approval of the first antivascular endothelial growth factor agent for ophthalmic use was long and involved many different players. In this article, Dr. Adamis outlines the progression of research from the laboratory to the patient and provides insight into one of the most exciting areas of posterior segment drug development in our history.

-Elias Reichel, MD

Judah Folkman, MD, the originator of antiangiogenesis for cancer,¹ always believed the method would also prove useful in ophthalmic disease. To work on the problem, he accepted ophthalmologists and vision scientists into his laboratory.

Soon after joining the Folkman laboratory, I was introduced to David Shima, PhD, who was a graduate student in the lab of Patricia D'Amore, PhD. Because we shared a mutual interest in ocular angiogenesis, Dr. Folkman suggested that we work together. The Folkman and D'Amore lab meetings, open to all interested parties, were rich in data and ideas. Participants included Joan Miller, MD; Lois Smith, MD, PhD; and Robert D'Amato, MD, PhD. As Dr. Folkman was fond of saying, the weekly gatherings served to "marinate" us in angiogenesis research.

Rosalind Rosenthal, PhD, was purifying a new angiogenesis factor secreted by sarcoma 180 cells in the Folkman lab. Around this time, Napoleone Ferrara, MD, published his discovery of vascular endothelial growth factor (VEGF), a secreted endothelial mitogen produced by bovine pituitary cells.² Dr. Folkman suspected his lab was purifying the same factor and contacted Dr. Ferrara. Within 6 months, they were able to show that the sarcoma 180 factor was indeed VEGF.³

One floor above the Folkman lab, Stella Kourembanas, MD, a neonatologist interested in pulmonary hyperten-

sion, was studying platelet-derived growth factor B (PDGF-B). At the weekly vascular biology conference (another open meeting), Dr. Kourembanas presented data showing that PDGF-B production was stimulated by hypoxia. Dr. Kourembanas' results suggested that some growth factors could be regulated by oxygen levels.⁴

FIRST LINK TO OPHTHALMOLOGY

At the 1992 Association for Research in Vision and Ophthalmology meeting, Anne Hanneken, MD, presented the first preliminary evidence for the presence of the newly described VEGF in retina.⁵ Leaving the poster session, Dr. D'Amore, Dr. D'Amato, and I talked about the role VEGF may play in ocular neovascularization. Because the cDNA sequence for VEGF showed significant homology with PDGF-B, and PDGF-B was upregulated by hypoxia, it stood to reason that VEGF might be stimulated by retinal ischemia and play a role in ocular neovascularization. Unlike basic fibroblast growth factor (bFGF), VEGF contained a signal peptide sequence for secretion into the extracellular space and therefore could be freely diffusible in ocular fluids.

Back in Boston, we approached Dr. Kourembanas to request the use of her apparatus for growing cells in variable oxygen concentrations, and she generously obliged. We stayed up late finishing the initial experi-

ments. It was worth the wait. Strong VEGF upregulation was observed in the hypoxic retinal cells. The plan was to confirm the results and to submit them for publication as soon as possible. Two weeks later, a paper from the lab of Eli Keshet, PhD, showed the hypoxic upregulation of VEGF in tumor cells.⁶ “Your results have been confirmed...even before publication!” was a legendary Folkman saying in such instances.

Dr. Miller and Evangelos Gragoudas, MD, were initiating their work on photodynamic therapy at the Massachusetts Eye and Ear Infirmary (MEEI) when they joined the team. Dr. Miller had modified the model of retinal ischemia of Sohan Hayreh, MD, and was able to consistently induce iris neovascularization, a model that made possible key experiments. Dr. Gragoudas provided valuable leadership in the design and conduct of the studies. At the same time, Folkman helped facilitate a collaboration with the lab of Harold Dvorak, MD.⁷ We wished to utilize the sensitive VEGF assay Dr. Dvorak’s team had developed, which was one of only two in existence at that time, for the planned monkey and human studies. He readily agreed.

FIRST STUDIES

With these tools in hand, the primate studies began. The results showed that retinal ischemia increased VEGF expression in retina, and that VEGF protein levels in the aqueous strongly correlated with the presence of iris neovascularization. The in vitro VEGF-hypoxia data and the in vivo primate experiments were presented at the 1993 Association for Vision and Ophthalmology meeting^{8,9} and were published shortly thereafter.^{10,11}

Donald D’Amico, MD, proved crucial to the team. Vitreous samples collected from Dr. D’Amico’s and Dr. Miller’s surgical patients at MEEI strongly linked VEGF to the presence of diabetic retinal neovascularization.¹² Around the same time, Lloyd P. Aiello, MD, PhD; Robert Avery, MD, and coworkers published an important paper confirming and extending the observation to other conditions characterized by retinal ischemia and neovascularization.¹³

In 1993, the team grew to include Dr. Ferrara, who was developing the first VEGF inhibitors at Genentech, Inc. (South San Francisco, CA). An early version of bevacizumab was injected into the vitreous of monkeys to see if neovascularization could be inhibited. Iris neovascularization was suppressed with the first injection, and eventually in all animals.¹⁴ In separate experiments, VEGF injections into the vitreous of normal eyes induced iris and retinal neovascularization, as well as retinal vascular leakage, microaneurysms, intraretinal microvascular abnormalities and ischemia.^{15,16}

Separately, Drs. Aiello and Smith were able to show that VEGF blockade could prevent retinal neovascularization in the mouse model of oxygen-induced retinopathy.¹⁷ Taken together, these data provided direct evidence that VEGF behaved like the hypothetical “Factor X” described by Michaelson,¹⁸ Ashton,¹⁹ and Wise.²⁰ VEGF was secreted by the ischemic retina and was necessary and sufficient for neovascularization of the iris and retina.

INTEREST FOR OPHTHALMOLOGY BUILDS

Interest in antiangiogenesis for ophthalmic disease was building. Interferon alfa-2b, an US Food and Drug Administration (FDA)-approved drug for certain blood cell tumors, had recently been shown by Dr. Folkman and coworkers to be antiangiogenic in children with hemangiomas.²¹ In 1991, these findings prompted ophthalmologists, led by Wayne Fung, MD, to begin using interferon off-label to treat wet age-related macular degeneration (AMD).²² David Guyer, MD, a MEEI retina fellow at the time, joined the team to learn if interferon-alfa-2b would prove effective for wet AMD in a large randomized trial.²³ With Dr. Folkman’s assistance, manufacturer Roche (Basel, Switzerland) was approached about conducting a prospective trial. Working with Drs. Guyer (co-chair), Gragoudas, and Miller, as well as Larry Yannuzzi, MD, Jason Slakter, MD, and others, we designed and conducted the Roche-sponsored interferon trial. Denis O’Shaughnessy, PhD, helped lead the trial at Roche. When tested in a large population of wet AMD patients, interferon proved ineffective.²⁴ But the experience taught us how to conduct well-controlled AMD trials, and ultimately led to multiple contacts with companies interested in developing an AMD therapy.

ANTI-VEGF AGENTS IN DEVELOPMENT

At the time, only two companies were developing anti-VEGF drugs: Genentech, Inc., and Nexstar (Boulder, CO), a small biotechnology company. Of the two, Nexstar was persuaded to test their anti-VEGF aptamer, pegaptanib, in wet AMD. Daniel Martin, MD, treated the first patient; however, after six patients were enrolled, Nexstar was purchased by Gilead Sciences, Inc. (Foster City, CA), and the study was halted.

At MEEI, the preclinical work continued. Using the primate model of choroidal neovascularization in Dr. Miller’s lab, the team showed that experimental choroidal neovascularization was associated with the expression of VEGF and its receptor,²⁵ and that intravitreal injection of Genentech’s ranibizumab potently suppressed choroidal neovascularization.²⁶

Frustrated by the lack of clinical development, we (Drs. Guyer and myself; Samir Patel, MD; Martin Glick; and John McLaughlin) founded Eyetech Pharmaceuticals (New York, NY) in April 2000. The capital markets were receptive in the spring of 2000, and Eyetech was able to obtain financing to license pegaptanib from Gilead. Dr. Guyer served as Chief Executive Officer, and many members of the original team worked with Eyetech in various capacities. Esteemed ophthalmologists, including Emmett T. Cunningham, MD, PhD, MPH, and Barrett Katz, MD, MBA, also joined the full-time team. Mr. Glick and Mr. McLaughlin, seasoned biotech executives, provided key assistance from the outset. From 2000 to 2004, Eyetech partnered with Pfizer, Inc. (New York, NY), completed the phase 3 AMD trials, filed a new drug application with the FDA, and became a public company. Shortly after Eyetech was founded, Genentech initiated the ranibizumab clinical development program in AMD.

FIRST FDA APPROVAL

In 2004, pegaptanib (Macugen) became the first anti-VEGF drug in ophthalmology to receive FDA approval. Although pegaptanib showed a 50% reduction in mean vision loss,²⁷ the biggest gains were seen the following year when the phase 3 ranibizumab results were reported.²⁸ For the first time, an AMD therapy was shown to produce a net gain in vision, and in 2006, ranibizumab (Lucentis) was approved by the FDA. Shortly thereafter, phase 2 trials in diabetic retinopathy^{29,30} also showed positive results. Since then, new VEGF inhibitors have entered clinical development, including VEGF-Trap (Tarrytown, NY).³¹

At the 2003 American Academy of Ophthalmology (AAO) meeting, Dr. Folkman was invited to be the keynote speaker at the event where the pegaptanib phase 3 data were first presented. In 2007, 2 months before his death, his contributions to ophthalmology were recognized at the opening session of the AAO meeting. Dr. Folkman gave an inspired keynote address and received a well-deserved, sustained standing ovation. ■

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