Innovations

Starving cancer into submission

EntreMed, Inc.

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Cut off a tumor's blood supply — it's source of nutrients and growth factors — and the tumor should perish. EntreMed, Inc., of Rockville, Maryland, is one of many companies trying to put this simple theory of anti-angiogenesis into practice. By licensing anti-angiogenic molecules discovered by Judah Folkman (Children's Hospital, Boston, Massachusetts), EntreMed has acquired perhaps the hottest intellectual property in cancer research today, and become the consummate middle-man. The company does the pre-clinical grunt-work for which Folkman does not have the time or resources, and provides the big pharmaceutical companies with drug candidates they cannot resist.

Twenty five years in the wilderness

Attacking angiogenesis, the process by which blood vessels sprout and invade new areas, was not always so popular. "When Folkman first proposed attacking cancer by inhibiting angiogenesis he was quite alone," says Robert Auerbach (University of Madison, Wisconsin). "He recognized the importance of it and made it a real research goal. In that sense he's a real pioneer."

Folkman first put forth his thesis in a 1971 article in the *New England Journal of Medicine*. If angiogenesis is not switched on, said Folkman, a tumor will grow to be only the size of a pea. This state is maintained by balanced proliferation and cell death, until a combination of hypoxia, inactivation of tumor suppressor genes, and activation of tumor promoter genes results in the production of angiogenic molecules (candidates include basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF)).

Folkman went on to provide evidence for the importance of angiogenesis in tumor growth. But what he needed was a potent inhibitor — both to prove his theory and as a possible cancer cure. He characterized numerous inhibitors, including one from cartilage, one from the vitreous area of the eye, protamine, and various steroids.

The most promising early compound was a re-discovered natural product called fumagillin (Table 1). But the first of Folkman's potential drugs to attract the attention of EntreMed came from a database. Folkman and postdoctoral fellow Robert D'Amato thought about the possible side-effects of an anti-angiogenic drug and came up with two: problems with menstruation and birth defects (limb development may depend in part on the pathways laid down by blood vessels). Only six drugs in their database had both side-effects; the stand-out was thalidomide.

Thalidomide was first used in Germany in 1957 as a sedative for mothers with morning sickness. In the next four years it was used in over 40 countries, although it was rejected in the United States in 1961 by the Food and Drug Administration (FDA) because it also causes mild peripheral neuropathy. In that same year other countries began banning its use, but not before ~12,000 babies were born with deformations including flipper-like arms and stumps for legs.

In the same year that he reported on thalidomide's activity, Folkman found that 2-methoxyestradiol and a protein called angiostatin were anti-angiogenic. Also in 1994, David Cheresh of Scripps Research Institute in La Jolla, California, found that integrin $\alpha_v\beta_3$ was needed to mediate a survival signal for endothelial cells (which line blood vessels). Antagonists to $\alpha_v\beta_3$ caused tumor regression *in vivo*, and are now in clinical trials (Table 1).

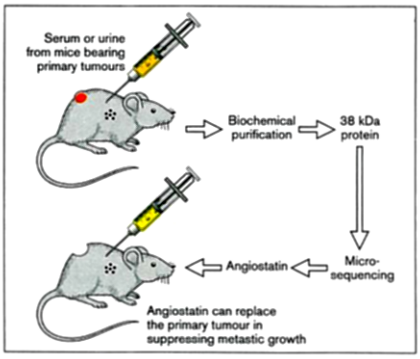
Postdoctoral fellow Michael O'Reilly waded through over 40 liters of mouse urine to purify a few micrograms of angiostatin, using the strategy depicted in Figure 1. Surgeons had known for many years that removal of a primary tumor can cause secondary metastases to suddenly vascularize and enter a growth spurt. Folkman hypothesized that the primary tumor was producing a circulating anti-angiogenic factor, which would be found in the serum, or urine, of mice with primary tumors. The primary tumor might overcome the effect of the inhibitor(s) by making locally active angiogenic factors, which the metastases cannot yet produce.

Table 1

A partial list of companies interested in anti-angiogenesis therapies.

Company	Product
TAP Holdings, Inc.	TNP-470, fumagillin derivative (pediatric phase I; adult phase III)
British Biotech plc	Marimastat, a small molecule matrix metalloproteinase inhibitor (phase III)
IXSYS, Inc.	Humanized anti-$\alpha_v\beta_3$-integrin antibody (finishing phase I)
Merck Darmstadt	Small molecule inhibitors of integrin $\alpha_v\beta_3$ (preclinical)
Genentech, Inc.	Anti-VEGF mononclonal antibody (phase I)
SUGEN, Inc.	Small molecule inhibitor of VEGF receptor Flk-1/KDR (phase I)
Agouron Pharmaceuticals, Inc.	Small molecule matrix metalloproteinase inhibitor (phase II/III); small molecule VEGF receptor inhibitor (preclinical)
Repligen Corp.	Small molecule inhibitors of bFGF and VEGF (preclinical)
Boston Life Sciences, Inc.	Troponin I (preclinical)
Hybridon, Inc.	Antisense VEGF (preclinical)
NeXstar Pharmaceuticals, Inc.	Aptamer antagonist of VEGF (preclinical)

Figure 1



Strategy for isolating angiostatin. Primary tumors can suppress the development of metastases by producing a circulating factor. Serum or urine from mice bearing a primary tumor can substitute for the primary tumor in producing this result, and fractionation yields a pure protein, angiostatin, that has the same activity.

Folkman's latest and most exciting inhibitor, endostatin, was purified from an endothelial tumor cell line. Both protein inhibitors are fragments of larger proteins: angiostatin is derived from the blood-clotting protein plasminogen, and endostatin from collagen XVIII, a component of the extracellular matrix.

A company for hire

EntreMed is not called AngioMed because in 1991, the year it was founded, it was open to any good concept. "The basis for the company was that we would foster the growth of promising technology discovered at academic institutions," says John Holaday, chief executive officer. Since 1994, the projects that EntreMed first licensed have taken a back seat to angiogenesis, although a cell permeation technology may enter phase I trials in the next year. EntreMed researchers will electroporate inositol hexaphosphate (IHP) into red blood cells so that the IHP binds hemoglobin. The altered hemoglobin can release three of its oxygen molecules (rather than just one) when it reaches an area low in oxygen, such as heart blood vessels during an attack of angina.

The breakthrough for EntreMed came in October 1993, when the company signed a contract with Folkman and Children's Hospital for rights to Folkman's work in return for royalties and $2 million per year of research funding. At this stage Folkman had good evidence for thalidomide's efficacy in mice, and was close to isolating angiostatin. Hoffmann-La Roche had decided against a collaboration with Folkman, but meanwhile D'Amato (who did the thalidomide work) had been talking to Holaday, his mentor from an earlier time at Walter Reed Army Institute of Research in Washington, D.C. "Life is a process of personal relationships," says Holaday, and this personal relationship gave his company a focus.

A cash infusion came in late 1995 from Bristol-Myers Squibb, when EntreMed had only 60 days of money in hand. Thalidomide trials sponsored by the two companies and the National Cancer Institute began in April 1996, and on the strength of this work EntreMed went public.

Interim results of the phase II thalidomide trials are promising: a 50% response rate for glioblastomas, gliomas and Kaposi's sarcoma. Phase II trials of thalidomide for age-related macular degeneration are also underway, as this form of adult-onset blindness is characterized by excessive blood vessel growth in the retina. If thalidomide is effective, its approval process may be reasonably straightforward. Stringent safety guidelines have already been worked out by Celgene Corp. (Warren, New Jersey), who are awaiting final FDA approval of thalidomide for the treatment of leprosy sores.

Bring on the big guns

Thalidomide may, however, be merely a warm-up for the protein inhibitors angiostatin and endostatin. EntreMed has now produced active human proteins from cultures of the yeast *Pichia pastoris*, and trials may begin in 1999.

The most spectacular preclinical data have come from endostatin. In the 27 November 1997 issue of *Nature*, Folkman reported that endostatin could repeatedly shrink tumors. Once the tumors shrank, Folkman stopped endostatin treatment, restarting only when the tumors had re-grown to 1–2% of total body mass. Each round of shrinkage occurred at the same rate, suggesting that the genetically stable endothelial cells do not become drug resistant. After 2–6 repetitions, depending on the cancer type, the cancers failed to grow back at all.

"We were pleasantly surprised [by these results]," says Edward Gubish, EntreMed's vice president for research. The high rate of endothelial cell turnover in tumors may explain why endostatin can shrink tumors rather than just halt their growth. "It could be the Hayflick experiment *in vivo*," says Craig Crews of Yale University. "The endothelial cells may have senesced." Alternatively, regrowth of new cells may be halted by increasing amounts of inhibitor, which may accumulate in the matrix as the endothelial cells remodel their surroundings.

EntreMed's weakness is that it doesn't know the precise mechanism of action for any of its three leading drug candidates, which makes the FDA approval process and the design of analogs more challenging. It also has many competitors, although some of the drugs proposed by these companies suffer from a lack of specificity (the broad spectrum matrix metalloproteinase inhibitors) or the possibility of drug resistance (tumors may make alternative angiogenic molecules when others such as VEGF are blocked).

If Folkman's proteins can make the leap from mice to humans his early vision will find ultimate vindication. "Folkman was fighting against a whole line of thinking: that you have to kill all the cancer cells directly," says Cheresh. Instead, he proposed that killing the support cells would work. "I think he is right," says Cheresh. "I think it is possible."

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