

Ironically, these errors also have implications for improving computational methods for analysing protein and DNA sequences, because new algorithms are tested on current sequence information. Without knowing how often errors and inconsistencies occur in the databases, it becomes very difficult to improve these methods. "You can't develop [the next generation of functional annotation systems] unless you know where the errors are being made by current systems," Karp said.

Efforts to address the problem of erroneous and inconsistent data and to find ways to fix them are hampered by disagreement over who is ultimately responsible: the database curators, or database users. As a database user, Karp believes "It's both groups' responsibility, but ultimately the databases are the gatekeepers." Fraser disagrees, saying "the sequence depositors should be responsible", whereas SwissProt database developer Bairoch thinks "everyone has to feel responsible". Regardless of responsibility, database users and curators both seem to agree that most scientists underappreciate the problem of database errors. But Fraser confirmed that the existence of annotation errors is "considered serious by most genome centers and by many bioinformaticists."

Without further research, it is difficult to quantify the effect that errors have on database usefulness. However, as yet, no concerted effort has been made to specifically analyse public sequence databases. "We're investing huge amounts of money in the sequence databases, the entire scientific community relies on them, and yet we don't know some very basic things about their properties," Karp said. An assessment of database accuracy and reliability would also go some way towards educating the community about errors and would encourage debate about the problem. But this is likely to require additional funding, and the source of this funding is not clear. As Karp pointed out, "In some sense this is perhaps even a lower priority because it's not actually spending money on curating databases, it's spending money to check up on the people who curate the databases." Perhaps the first step in addressing the problem should be to educate the scientific community and encourage a greater collaboration in maintaining error-free resources. In their survey of quality-control procedures in archival databases, the CODATA Task Group on Biological Macromolecules concluded

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that the only possible solution is a dynamic annotation process, with the workload distributed among database curators and specialists (CODATA Task Group on Biological Macromolecules and Colleagues, 2000). Ultimately, database developers could find this specialist knowledge by appealing to the altruism of users. "Making people aware of errors is good and great; making people aware that they're responsible also for correcting errors is even greater," Bairoch said.

If one thing is certain, it is that the number of sequences in public sequence databases will continue to increase exponentially for the foreseeable future—as will the errors, most likely. As these databases constitute the foundations for advanced research in biology, their ability to maintain this role effectively has implications not just for bioinformatics and genomics, but for all fields of scientific research. If the strength of these foundations is not tested now, extracting useful information from databases may become even more difficult. "I think it's going to get much worse before it gets better. There's going to be an explosion in terms of heterogeneity of

resources and of people not finding what they want. Then people will complain and things will slowly get better," Bairoch predicted.

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## Less is more

**Research into anti-angiogenic therapies for treating cancer has finally had its first breakthroughs. But it may also influence the way in which classical chemotherapy is used for cancer treatment**

After many promises and failures, anti-angiogenic drugs finally seem to be making progress towards their clinical use. In May 2003, researchers at the meeting of the American Society of Clinical Oncology announced the first positive results with an anti-angiogenic therapy in a phase III cancer trial. The randomized, double-blind study of more than 900 patients with metastatic colon cancer showed that Avastin™, an antibody against vascular endothelial growth factor (VEGF),

combined with chemotherapy, extended overall survival beyond that achieved with chemotherapy alone and had a significantly improved response rate and duration of response. The US Food and Drug Administration reacted to the trial's positive result by granting Avastin™ 'fast track' drug-review status in June this year.

"This is an enormously important study and represents a very exciting step forward," said Leonard Saltz, a physician at New York City's Memorial Sloan-Kettering Cancer

### A SHORT HISTORY OF ANTI-ANGIOGENESIS RESEARCH

- 1971** Folkman discovers that tumours stop growing without a blood supply
- 1976** Several research groups find that blocking angiogenesis results in tumour dormancy
- 1982** Michael Klagsbrun isolates and characterizes basic fibroblast growth factor (bFGF)
- 1984** Discovery that tumours produce specific angiogenic proteins
- 1985** Folkman's research group identifies the first endogenous angiogenesis inhibitors—angiostatic steroids
- 1988** Discovery that angiogenic proteins are stored in the extracellular matrix
- 1989** Research shows that the 'angiogenic switch' converts non-angiogenic, microscopic dormant tumours to vascularized, growing and metastasizing tumours  
Discovery that recurrent tumours in humans can completely and durably regress by treatment with a single angiogenesis inhibitor, interferon- $\alpha$   
Napoleone Ferrara at Genentech purifies and clones vascular endothelial cell growth factor (VEGF)
- 1994** Research shows that primary tumours can suppress the growth of secondary metastases  
Michael O'Reilly identifies the first endogenous angiogenesis inhibitor—angiostatin
- 1995** Unifying hypothesis explains four patterns of metastasis by angiogenic regulation of dormancy or lack of it
- 1997** Michael O'Reilly purifies and characterizes endostatin  
Discovery that cryptic fragments of matrix proteins—for example, endostatin and angiostatin—function specifically as angiogenesis inhibitors  
Discovery that leukaemia is angiogenic and can be treated by anti-angiogenic therapy
- 2000** Discovery that low-dose, frequent or continuous (metronomic) chemotherapy is anti-angiogenic
- 2001** Optimum anti-angiogenic therapy is achieved by continuous, not intermittent, treatment with an anti-angiogenic inhibitor

Center (NY, USA). "Avastin™ represents a validated approach in solid tumours which will undoubtedly change the standard of care for colon cancer." His colleague Rakesh Jain, from Harvard Medical School in Boston (MA, USA), agrees: "The Avastin™ trial was just what the field has been waiting for." But the trial is just a first step. Although researchers in the field were not surprised at the good results, they agree that much more research is needed to determine the appropriate dosage of anti-angiogenic compounds and to test combinations with classic chemotherapy.

Researchers and patients have long hoped that angiogenesis inhibitors, such as endostatin, angiostatin, Thrombostatin™ and Herceptin®, would help overcome some intractable problems in cancer treatment, namely drug delivery, resistance and toxicity. As tumours are generally oxygen-starved at their core, many drugs cannot reach their innermost cells. Chemotherapy becomes less effective over time, as cancer cells mutate and develop drug resistance. Also, the high level of toxicity of these drugs often limits their effective dosage. "In the past two decades, effective cancer treatments have plateaued, in part because of the problem of drug resistance," said Robert Kerbel, Professor of Cellular and Molecular Biology at the University of Toronto, Canada. Angiogenesis inhibitors have long been thought to overcome these

problems because they do not target the tumour itself but the blood vessels that support it. Endothelial cells are recruited from the local environment and the bone marrow to form new blood vessels and these cells are genetically stable—unlike tumour cells—and are therefore less likely to develop drug resistance. In addition, tumour vasculature differs from mature blood vessels, so targeting dividing endothelial cells does not interfere with the normal angiogenic process in adults, that is, in wound healing, pregnancy and the menstrual cycle. And most angiogenesis inhibitors seem to be safe. These advantages of angiogenesis inhibitors have already sparked a new paradigm in cancer treatment that does not aim to destroy every cancer cell, but rather seeks to keep them under control. "If cancer could be stopped from growing, it could be possible for patients to live years with cancer as a manageable, chronic disease, like diabetes," explained Judah Folkman, from Boston's Children's Hospital (MA, USA), who pioneered anti-angiogenic research.

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Folkman developed this idea more than 30 years ago while at the National Cancer Institute (Bethesda, MD, USA), where he observed that tumour growth in isolated organs was limited without vascularization. "Tumours unable to induce angiogenesis remain dormant at a microscopic size: 1–2 mm<sup>3</sup> *in situ*," he wrote in 1971 in the *New England Journal of Medicine* (Folkman, 1971), so by attacking cancer's lifeline, it might be possible to starve tumours into regression. On the basis of his observation that metastases often start growing after the removal of the main tumour, he deduced that cancer cells produce pro- and anti-angiogenic factors. Michael O'Reilly, who was a postdoctoral fellow in Folkman's lab and is now Assistant Professor at the M.D. Anderson Cancer Center (Houston, TX, USA), eventually discovered the first three endogenous angiogenesis inhibitors in mouse tumours: angiostatin, endostatin and anti-angiogenic antithrombin. In the following years, Raghu Kalluri, Associate Professor at Harvard Medical School (Boston, MA, USA) found seven other endogenous inhibitors and found that they also acted as tumour suppressors. There are now 14 known endogenous angiogenesis inhibitors and more than two dozen anti-angiogenesis drugs in clinical trials (Table 1).

To prove his hypothesis about starving cancer, Folkman first treated intractable paediatric malignancies with interferon- $\alpha$ , and later treated patients with metastatic liver cancer and advanced pancreatic cancer—considered death sentences—daily, for about two years, with endostatin. They have regained their strength and are doing well, he says. But the medical establishment treated his ideas with scepticism. Some oncologists, including Larry Norton, head of the Division of Solid Tumor Oncology at the Memorial Sloan-Kettering Cancer Center, maintain that they are looking for a cure for cancer, not a treatment that causes dormancy. Other critics assert that angiogenesis cannot be the key component of every cancer, but represents only one part of an extremely complex picture that includes other, angiogenesis-independent factors. Others say that producing tumour dormancy is not sufficient. "Tumours will use other mechanisms to survive and grow if their blood supply is shut down," said Shahin Rafii, Professor of Medicine at Cornell University School of Medicine (New York, NY, USA).

Nevertheless, disease stabilization using angiogenesis inhibitors could become a valid

primary endpoint, “whereas with traditional chemotherapy drugs, ‘disease stabilization’ is considered tantamount to failure because cancer usually returns with those drugs and because patients have a poor quality of life with them,” Folkman countered. Moreover, the old paradigm of hitting tumours with high doses of chemotherapy seems to be crumbling. Several studies published in July 2003 in the *New England Journal of Medicine*

showed that high-dose chemotherapy combined with stem-cell transplantation—which allows extremely high doses of drugs—was no better, or at best only marginally more effective in prolonging life, than chemotherapy alone. “The rationale behind extremely high-dose chemotherapy and stem-cell transplantation for breast cancer was MTD [maximum tolerated dose], which two recent studies show to have little or no efficacy,” Kerbel

**“If cancer could be stopped from growing, it could be possible for patients to live years with cancer as a manageable, chronic disease, like diabetes”**

said. In addition, recent research shows that g-CSF, which is used to rebuild the immune system after high-dose chemotherapy, may accelerate cancer.

Most researchers in the field do indeed agree that angiogenesis inhibitors are effective at much lower doses than chemotherapy. Based on preclinical and clinical trials, the optimum biological dose for angiogenesis inhibitors was found to be much lower than the MTD, and thus toxicity will not be a limitation. But this leads to the next challenge: how to find reliable surrogate markers of efficacy. “Trials are examining the use of PET [positron emission tomography] scans and MRI [magnetic resonance imaging], levels of circulating endothelial precursor cells [CEPs] recruited from the bone marrow during tumour vascularization, and the analysis of tumour biopsies before and at specified points after treatment with anti-angiogenic drugs,” said Roy Herbst, Associate Professor at the M.D. Anderson Cancer Center. Other measures being studied include the quantification of drug–target interactions at the cellular level and levels of circulating angiogenic factors.

Many researchers believe that angiogenesis inhibitors are an important addition to surgery, chemotherapy and radiation, not a replacement. “Angiogenesis inhibitors are not magic bullets against cancer,” said O’Reilly, because tumours that might depend on up to six pro-angiogenic factors are not likely to be stopped by targeting one pathway. Indeed, earlier monodrug trials of Avastin™ in breast cancer, and British Biotech’s trials of a metallo-proteinase inhibitor, failed. Furthermore, tumours can become resistant to some angiogenesis inhibitors in the sense that targeting one growth factor might encourage a tumour to rely on a second, Kerbel said. And at the end of the day, most anti-angiogenic drugs are cytostatic, not cytotoxic, so to actually destroy cancer rather than pushing it into dormancy, cytotoxic drugs will still be needed.

To use angiogenesis inhibitors more effectively, researchers in the field are now investigating what makes one tumour quiescent and another grow and metastasize.

**Table 1 | Angiogenesis inhibitors**

Drug	Company
<b>Drugs that are exclusively anti-angiogenic</b>	
<i>In clinical trials</i>	
Angiostatin	Entremed (Rockville, MD, USA)
Avastin™	Genentech, Inc. (San Francisco, CA, USA)
Endostatin	Entremed (Rockville, MD, USA)
2-Methoxyestradiol (2-ME) Panzem	Entremed (Rockville, MD, USA)
Tetrahydrocortisol	–
TNP-470	Takeda Chemical Industries (Osaka, Japan)
Thrombospondin peptide	–
VEGF–Trap	Regeneron Pharmaceuticals (Tarrytown, NY, USA)
Vitaxin™	MedImmune, Inc. (Gaithersburg, MD, USA)
<i>Not yet in clinical trials</i>	
Canstatin	ILEX Oncology, Inc. (San Antonio, TX, USA)
Cleaved antithrombin III	–
DBP–MAF	–
PEDF	–
Tumstatin	ILEX Oncology, Inc. (San Antonio, TX, USA)
<b>Drugs that include anti-angiogenic activity</b>	
<i>FDA approved</i>	
Celebrex® (celecoxib)	Pfizer (New York, NY, USA)
Herceptin®	Genentech (San Francisco, CA, USA)
Iressa®	AstraZeneca (London, UK)
Avandia® (rosiglitazone)	GlaxoSmithKline (Uxbridge, UK)
Taxol®	Bristol-Myers Squibb Co. (New York, NY, USA)
Thalidomide	Celgene (Warren, NJ, USA)
Velcade™	Millennium Pharmaceuticals (Cambridge, MA, USA)
Zometa® (zoledronic acid)	Novartis (Basel, Switzerland)
<i>In clinical trials</i>	
Erbitux™	ImClone Systems (New York, NY, USA)
Combretastatin	OXIGENE (New York, NY, USA)
Interferon-α	Hoffmann LaRoche (Switzerland)
NM-3	ILEX Oncology, Inc. (San Antonio, TX, USA)
Tarceva™	OSI Pharmaceuticals (Melville, NY, USA)
PTK787	Novartis (Basel, Switzerland)
SU5416	SUGEN, Inc. (San Francisco, CA, USA)
SU6668	SUGEN, Inc. (San Francisco, CA, USA)
SU11248	SUGEN, Inc. (San Francisco, CA, USA)
Thalomid® (thalidomide)	Celgene (Warren, NJ, USA)
2-Methoxyestradiol (2-ME) Panzem	Entremed (Rockville, MD, USA)

DBP–MAF, vitamin-D-binding protein–macrophage-activating factor; FDA, US Food and Drug Administration; NM-3, an isocoumarin derivative; PEDF, pigment-epithelium-derived factor; VEGF, vascular endothelial growth factor.

**Many researchers believe that angiogenesis inhibitors are only an important addition to surgery, chemotherapy and radiation, not a replacement**

size. "Using multiple approaches, including proteomics and molecular 'fingerprinting', we are looking at factors that might influence tumour dormancy," O'Reilly said. Raffi is studying the factors that mobilize bone-marrow-derived endothelial precursor cells and pro-angiogenic haematopoietic stem cells to form new blood vessels. He believes that inhibiting these vascular precursors from migrating or being released from the bone marrow might be a strategy that can be used to treat or prevent cancer. For example, the initial growth of lymphomas seems to be dependent on CEPs, whereas the early growth of lung carcinomas and breast cancer is only partly dependent on these cells. Kalluri is focusing much of his research on the basement membrane, which also sequesters growth factors responsible for recruiting endothelial cells. He predicts that within five years, stroma-targeted therapies, in combination with low-dose chemotherapy and anti-angiogenic drug therapy, will help to change many cancers into manageable illnesses. O'Reilly, Kalluri, Folkman and others believe that, ultimately, treatment will be determined by and tailored to the phenotype of a particular cancer.

However, there is still a resistance problem with angiogenesis inhibitors. Folkman designates anti-angiogenic drugs as being either 'direct' or 'indirect' inhibitors, with the latter being more likely to produce drug resistance. Direct inhibitors—such as Avastin™, Thrombospondin, TNP-470, Vitaxin™, endostatin and angiostatin—target endothelial cells, whereas indirect inhibitors—Iressa®, Tarceva™, Erbitux™ and Herceptin®—inhibit the tumour from synthesizing or using angiogenic proteins. In addition, "a second wave of angiogenesis may occur as surviving cancer cells proliferate and produce angiogenic factors," according to Jain. "Blood vessels of a relapsed tumour can begin to exhibit abnormalities reminiscent of those of the untreated tumour." Combination therapy is therefore also needed to delay the second wave of angiogenesis by continuing to cut off vessels.

The research on angiogenesis inhibitors might also lead to a change in classical chemotherapy. Whereas Kalluri, O'Reilly and others are focusing on discovering new endogenous inhibitors, Kerbel is studying the anti-angiogenic activity of known chemotherapeutic drugs. He has shown that low-dose, continuous therapy, called 'anti-angiogenic metronomic chemotherapy', can have anti-angiogenic activity even in patients whose tumours have previously been treated with the same drug at high doses and have become resistant. "We are interested in how this can be exploited to enhance treatment efficacy and reduce the degree of toxicity associated with the use of such drugs," Kerbel said. He tested the feasibility of metronomic oral chemotherapy in mice with various tumour types and saw both delays in tumour growth and regression (Man *et al.*, 2002). These effects were enhanced in a metastatic breast cancer model when the drug was combined with an anti-VEGF receptor 2 antibody, ImClone's DC101. Combinations of new angiogenic inhibitors, older chemotherapeutic drugs given continuously in low doses, and inhibitors such as DC101, Iressa® and Celebrex®, are now in phase II cancer trials in Canada, the USA, Japan and Europe.

**Although it is still very much in the experimental phase, the anti-angiogenesis paradigm may be making inroads towards changing how oncologists treat patients**

"From the accumulated clinical experience with cancer chemotherapeutics administered at the MTD, it could be concluded that in contrast to the popular 'more is better' philosophy, more is not necessarily better. In fact, there is a clear lack of association between the extent of treatment-induced tumour reduction—the goal of MTD chemotherapy—and extension of life," Kerbel noted. "One possible outcome of continuous, low-dose chemotherapy may be prolonged stable disease and a positive impact on patient survival." Such low-dose metronomic chemotherapy will also avoid or delay drug resistance, which develops as a result of mandatory drug-free breaks. These breaks are necessitated by

high-dose chemotherapy, which depletes patients' bone marrow.

When Kerbel tested chronic, uninterrupted administration of these drugs at very low doses (30% of the MTD), he found that they are well tolerated and effective in mouse models and in humans. In one trial in which a high proportion of breast and ovarian cancer patients had stopped responding to the MTD of a taxane (a Taxol-related drug) given once every three weeks, giving the same drug weekly at one-third of the dose produced a response. Another trial, conducted by M. Colleoni, who is based in Italy (European Institute of Oncology, Milan, Italy), showed that continuous low-dose cyclophosphamide and methotrexate were minimally toxic, and effective, in breast cancer patients pre-treated with high doses of these drugs (Colleoni *et al.*, 2002). More than 30% of the 64 patients showed some clinical benefit after 24 weeks.

Although it is still very much in the experimental phase, the anti-angiogenesis paradigm may be making inroads towards changing how oncologists treat patients. A phase III breast cancer trial reported in April 2003 (Citron *et al.*, 2003) showed that shortening the resting periods between chemotherapy treatments from three to two weeks increased survival rates. Called 'dose-dense' chemotherapy by its originator, Larry Norton, the regimen was developed using a mathematical model of cancer growth and death, not an angiogenesis model, he said. Even so, the idea of shorter rest periods between treatments reflects a cardinal principle of anti-angiogenic therapy.

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