

Lost in migration

George S Mack and Andrew Marshall

Combinations of cytostatic treatments and chemotherapies currently in clinical practice offer limited hope for patients whose cancers have spread. But increasing understanding of the processes underlying metastasis may one day provide other therapeutic options.

In the United States alone, over 565,000 people will die from cancer this year according to the American Cancer Society. As invasive and expansive as primary tumor masses might be, they account for only one out of ten cancer fatalities. For those diagnosed with solid tumors, occult metastases in such organs as the bone marrow, lung, liver or brain account for the vast majority of cancer deaths.

Over the past century, drug developers and clinicians have honed surgical techniques and introduced an increasing array of poisons, including radiotherapies or cytotoxic chemotherapies, as well as molecularly targeted cytostatic drugs in the battle against cancer. After diagnosis, if a metastasis has not already occurred, in many cases removal of the primary tumor can restore a patient to long-term health. But all too frequently, metastatic colonies, after multiple rounds of standard-of-care therapies, can reactivate and become life threatening—sometimes months, sometimes years after the initial diagnosis.

Although cancer biology has recently made progress in understanding how cancer spreads, our understanding of the molecular mechanisms of metastasis remains rudimentary. Basic research lacks animal models that closely resemble the course and pattern of clinical disease in different cancers. Controversy continues concerning the origin and contribution in various tumors of cells from the primary mass and disseminated tumor cells (DTCs, which, because of their progenitor-like qualities, have been termed ‘cancer stem cells’). And the molecular similarities between primary tumors and their metastases remain unclear; indeed, the combination of genomic instability and differing selective pressures and environ-

mental cues at sites distant from the primary tumor (metastatic speciation) can make secondary tumors a moving target for therapeutic intervention. The clinical complexities of selecting cancer patients who respond to treatment with novel targeted agents, the advanced stage of disease that many of these patients are in and the lack of definitive regulatory criteria to measure the effectiveness of treatments all combine to make drug development against metastatic cancer a daunting challenge.

An emerging picture

The metastasis field has arrived at an important crossroads. Once a murky and incomprehensible disease process, cancer is beginning to yield to recent advances in cancer biology that throw light on not only the mechanisms by which primary tumors disseminate through the body and then colonize a secondary organ but also the interactions between tumor cells, stromal cells and immune cells involved throughout this metastatic cascade.

Cancers spread when tumor cells escape from the primary cancer into the surrounding tissue, invade the vasculature (a process termed intravasation), voyage through the vascular system (blood or lymph) to a distant location and, reversing the process, exit from the vasculature (extravasation) into an organ remote from the original site and establish a secondary tumor (Fig. 1). It is these secondary tumors that cause the majority of cancer deaths, either through direct organ compromise or through complications as a result of last-ditch treatments.

The metastatic process is by its very nature inefficient—and ideas on which part of the cascade is the rate-limiting step have varied over the years. Whereas it was once assumed that metastasis occurred only after the primary tumor had achieved a certain size (that is, the ‘linear progression model’), recent

work tracking tumor cells as they disseminate throughout the body suggests that at least for some cancers (e.g., ductal carcinoma), seeding of cells into distant organs happens early on¹. What’s more, single tumor cells disseminated to the bone marrow and peripheral blood can often be detected years before the occurrence of clinically detectable metastases. In other cases (e.g., breast cancer), a significant fraction of patients with detectable DTCs never develop distant metastases. Conversely, in 5% of the hospitalized cancer population, patients have metastatic disease without diagnosis of the primary tumor (termed CUP; cancer of unknown primary). This suggests that in certain cancer types, DTCs sometimes acquire the capacity to proliferate and colonize early on, even if epithelial tumor cells at the primary site fail.

What’s more, colonization of the distant tissue to establish a secondary tumor, which requires both a permissive microenvironment as well as a migratory malignant cell receptive to the growth-promoting signals of the destination organ—otherwise known as the ‘soil and seed’ hypothesis first promulgated by the late 19th century British surgeon Stephen Paget—appears to be a key, rate-limiting step. The emergence of a secondary cancer is so rare, at least relative to the number of tumor cells set free into circulation, that as few as 0.01% of circulating cells ultimately survive to form new viable distant colonies of tumor tissue, according to cancer biologist Isaiah (Josh) Fidler of the University of Texas MD Anderson Cancer Center (Houston). A one-cubic-centimeter tumor mass can contain a billion cells and shed a million cells per day into circulation. “And if only ten cells survive, that means there are ten chances that a metastasis will occur every day,” he says. However, excluding dormant secondary lesions that may never be detected or do any harm, statistics tell investigators that at the

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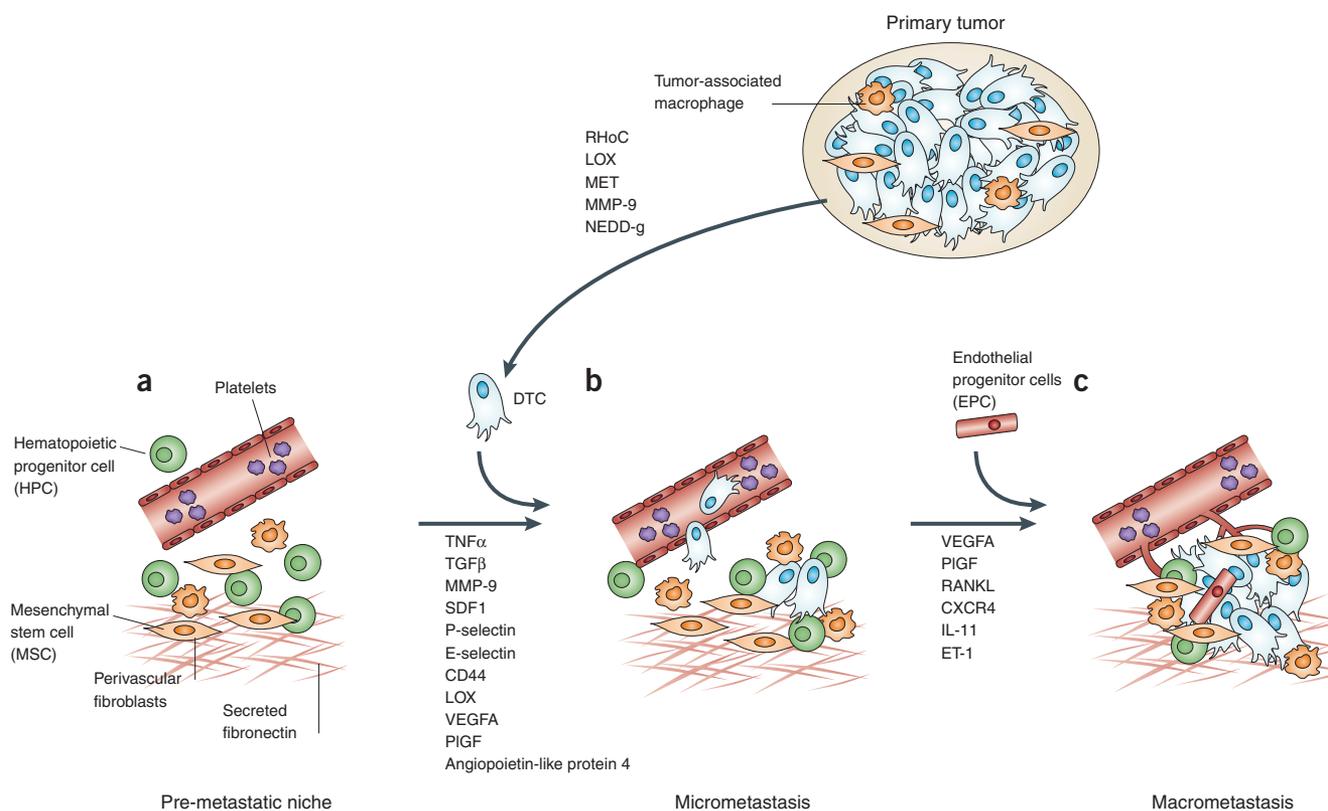


Figure 1 Steps to metastasis formation. (a) In response to factors secreted by the primary tumor, including RHoC, LOX, MMP-9, inflammatory S100 chemokines and serum amyloid A3 (SAA3) are upregulated in pre-metastatic sites leading to clustering of bone marrow-derived hematopoietic progenitor cells (HPCs). HPCs secrete a variety of pre-metastatic factors, including tumor necrosis factor α (TNF- α), MMP9 and TGF β . Activated fibroblasts, possibly derived from mesenchymal stem cells (MSCs), secrete fibronectin, an important adhesion protein in the niche and LOX expression is increased, modifying the local ECM. (b) DTCs engraft the niche to populate micrometastases. The site-specific expression of adhesion integrins on activated endothelial cells such as P-selectin and E-selectin may enhance DTC adhesion and extravasation at these sites, and cell-cell interactions such as CD44 ligation in the metastatic niche may promote DTC survival and enable proliferation. (c) Recruitment of endothelial progenitor cells (EPCs) to the early metastatic niche mediates the angiogenic switch and enables progression to macrometastases and colonization. Adapted from ref. 28.

time of solid tumor diagnosis, roughly half of all patients have a metastatic lesion already in place, and some of these may have been growing for 2 to 3 years.

Another intriguing clinical observation is that some cancers (e.g., lung and pancreas) metastasize very soon after primary tumor cells progress through the tumor boundary,

whereas others spread much later—as much as decades later in the case of breast cancer, prostate cancer and ocular melanoma. In addition, particular cancers tend to metastasize to particular organs (Table 1, Box 1 and Fig. 2), which cannot be fully explained by organ accessibility or vascular traffic patterns. Thus, although the latency period for breast cancer

metastases in the brain (which is protected by the blood-brain barrier) is longer than that for bone marrow (where there are large fenestrations in blood vessels), lung adenocarcinoma and other highly invasive cancers are still capable of rapidly spreading to both locations irrespective of accessibility. The molecular events underlying these different proclivities remain poorly understood, although molecular signatures associated with tropism to different distant sites are coming into focus² (Box 2). Vital imaging has allowed researchers to see tumor cells move throughout the body of experimental animals (Box 3 and Fig. 3), and some of the properties of the ‘pre-metastatic niche’ are coming to light.

Breaking out of the basement

Once a primary tumor has acquired initiating mutations that proffer unlimited proliferation potential, repress apoptotic mechanisms, redirect cell metabolism and accelerate genomic instability and aneuploidies, the next step in

Table 1 Tropisms of common cancers

Tumor type	Principal sites of metastasis
Breast	Bones, lungs, liver and brain
Lung adenocarcinoma	Brain, bones, adrenal gland and liver
Skin melanoma	Lungs, brain, skin and liver
Colorectal	Liver and lungs
Pancreatic	Liver and lungs
Prostate	Bones
Sarcoma	Lungs
Uveal melanoma	Liver

Source: ref. 2.

Box 1 Bone—a model for drugs targeting metastases in specific organs?

Most cancer treatments in clinical practice currently focus on the primary tissue of origin, but another approach is to treat malignancies from the perspective of the site of metastasis. This would include both the migrating tumor cells and the stromal cells at the organ to which they spread; thus, breast cancer cells that metastasize to lungs express such factors as angiopoietin-like 4, COX-2 and MMP-1, whereas those that spread to bone express parathyroid hormone-related peptide, tumor necrosis factor α , IL-6 and IL-11 and respond to stromal-cell derived factor 1 produced in the bone marrow.

A current example of a cancer therapy where the concept of targeting a specific metastatic niche is being realized is the use of agents that inhibit osteoclast activity in bone metastasis (Fig. 2). The balance between osteoblast activity and osteoclast activity is important in bone metastasis as bone matrix must be destroyed to create a niche for tumor cell colonies to grow. Novartis' bisphosphonate Zometa (zoledronic acid), a small molecule that binds to hydroxyapatite in the bone matrix, was approved in early 2002 and marketed for multiple myeloma and to manage or delay bone metastasis from lung, breast and prostate cancers (as well as to treat hypercalcemia of malignancy). Bisphosphonates promote apoptosis of osteoclasts, and, accordingly, Zometa delays or prevents skeletal-related events, such as bone deterioration, fractures and metastatic bone pain. Amgen's humanized monoclonal antibody Prolia (denosumab; formerly AMG 162) binds to receptor activator of NF- κ B ligand (RANKL) on the cell membrane of osteoclasts and prevents RANKL interaction with its receptor RANK, which reduces bone resorption by inhibiting osteoclast activity. In a head-to-head phase 3 trial, Amgen released data last July showing that Prolia demonstrates a clear advantage over Zometa on postponement of time to the first metastatic skeletal-related events. The initial disease indication for Prolia is post-menopausal osteoporosis, and the product could be a \$2.5 billion–\$3.0 billion per year product, according to Wells Fargo (San Francisco) senior biotech analyst Aaron Reames, who anticipates no major roadblocks to FDA approval.

Three other companies with programs in proof-of-concept trials targeting the bone metastatic niche are Merck, Novartis and Acceleron Pharmaceuticals (Cambridge, MA, USA). Merck is investigating a small molecule targeting lysosomal cysteine protease cathepsin K, an enzyme expressed by osteoclasts during bone resorption and metastasis. In a randomized, double-blind phase 2 trial of 43 patients with breast cancer and bone metastases, the drug, odanacatib (a [2,2,2-trifluoro-1-(biphenyl-4-yl)ethyl]-4-fluoroleucine derivative of 4-fluoroleucine), has shown robust, sustained and reversible antiresorptive activity, with no demonstrable effect on off-target cathepsins. Novartis (under license from Morphosys (Martinsreid, Germany)) is investigating

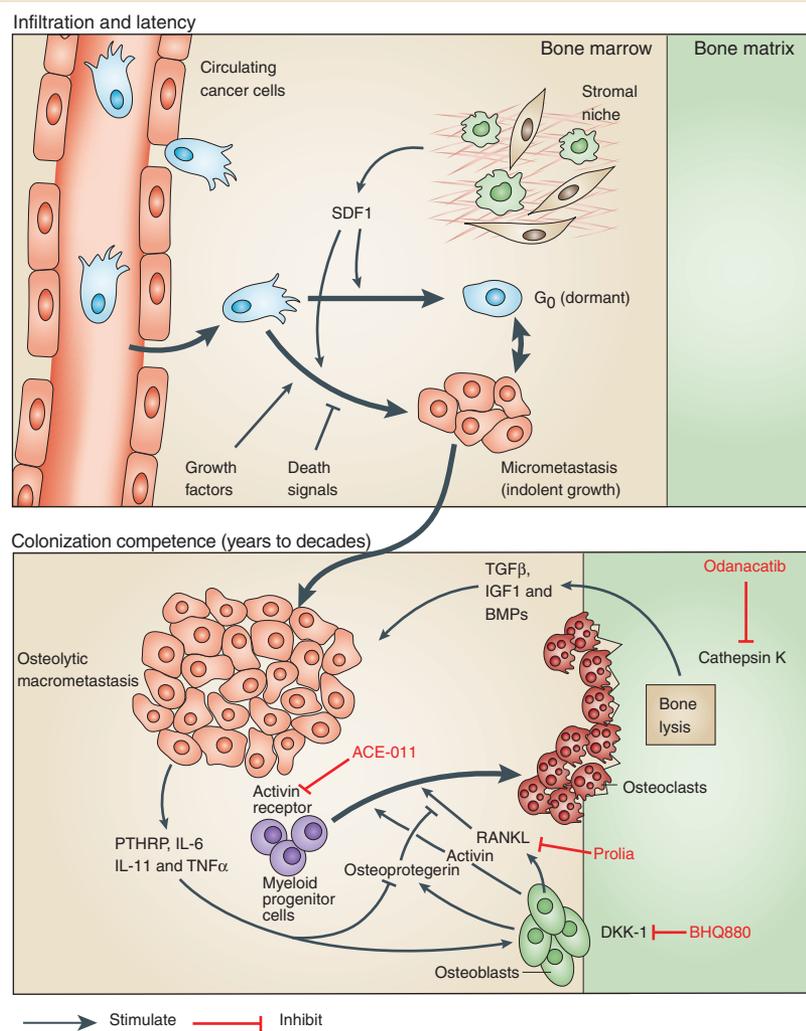


Figure 2 Cellular pathways associated with bone metastases and associated therapies. Drugs are shown in red. Adapted from ref. 2.

BHQ-880, an intravenous neutralizing human mAb against the soluble endogenous Wnt inhibitor Dickkopf-1 (DKK1) that stimulates osteoblastogenesis, in phase 1b/2 trial of myeloma patients with bone metastases. And Acceleron is developing otercept (ACE-011), a fusion protein containing a soluble form of the activin IIa receptor and an Fc fragment of IgG1, which was reported in December to result in remission in 7 out of 22 multiple myeloma patients with osteolytic lesions.

Therapies specifically targeting lung, liver and brain metastases are further in the future, although several startups are attempting to address the difficulty of getting cancer drugs to penetrate the blood-brain barrier to access secondary tumors there. Thus, by conjugating Taxol to angiopep-2, a 19-mer peptide mimetic of the N-terminal sequence of apolipoprotein B that binds the lipoprotein receptor-related protein on the blood-brain barrier, AngioChem (Montreal) hopes to facilitate delivery to malignant gliomas—a phase 1 trial is currently ongoing. And, in The Netherlands, to-BBB Technologies (Leiden) is developing liposomes coated with glutathione-conjugated PEG to deliver Adriamycin to brain tumors.

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cancer progression involves the loss of cellular polarity and detachment from the basement membrane. In addition, alterations are induced in the interactions between tumor epithelial cells and the extracellular matrix (ECM). Enzymes, such as matrix metalloproteinases (MMP-1, MMP-2, MMP-3 and MMP-13), epilysin and the transmembrane serine protease TMPRSS4, are secreted from or upregulated on tumor epithelial cell surfaces and into the ECM to trigger detachment, allowing cells to migrate away from the primary tumor mass.

MMPs are zinc-dependent endopeptidases capable of breaking down the elements of ECM (that is, collagens, fibronectin, laminin, elastin and basement membrane glycoproteins), which interact with tumor cell surface proteins, such as $\beta 4$, $\alpha 5\beta 1$ and $\alpha V\beta 6$ integrins and cadherins, and they are involved in making space for the angiogenesis and lymphogenesis that enables nourishment and progression of tumor cells outside the tumor margins.

From the drug developer's point of view, the accessibility of the ECM and cell membrane proteins compared with intracellular targets could be advantageous, particularly in terms of dose-limiting toxicities. Thus far, however, little success has been achieved in targeting these enzymes. In the 1990s, British Biotech Pharmaceuticals (Oxford, UK) developed two MMP inhibitors, batimastat for injection and marimastat, for oral use, both of which are hydroxamic acid derivatives. Because these were nonselective MMP inhibitors, patients receiving the drugs experienced severe muscle and joint pain, and the reduced dosages employed lacked efficacy in trials for glioblastoma, pancreatic, gastric, breast and ovarian cancers. Several other MMP inhibitor flops in cancer followed, including Bayer's (Leverkusen, Germany) tanomastat, Novartis's (Basel) MM1 270 and Agouron's prinomastat (AG3340). These early disappointments in targeting MMPs have had the effect of discouraging other drug developers from targeting these enzymes.

"Let's say they were broad spectrum and not specific for certain MMPs," says molecular cell biologist Reuven Reich of Hebrew University of Jerusalem. "That's the reason these kinds of drugs failed, we think." Recently Reich and his collaborators Amnon Hoffman and Eli Breuer, both also of Hebrew University, published findings from *in vivo* work they had been doing in murine models with a new MMP inhibitor, *cis*-2-aminocyclohexylcarbamoylphosphonic acid (*cis*-ACCP)³. They believe their small orally bioavailable molecule could be useful as an antimetastatic agent. It's a more specific compound than marimastat targeting only MMP-2, and it has less affinity for iron than

the hydroxamic acid products, that in all probability combined with iron from myoglobin to precipitate out into the tissues and cause severe pain in those taking the British Biotech drug marimastat. But because of its specificity, *cis*-ACCP is a much milder inhibitor and is soluble in water, which is ideal for extracellular targeting and also elimination through the kidneys. Thus far, their research group has gathered data using two cancer models in mice—murine melanoma cells injected into the tail veins and an orthotopic human prostate cancer cell model. The data show solid tumor metastases in mouse models reduced by ~90%. There was no toxicity detectable, and in rats it was shown that 84% of intravenously administered *cis*-ACCP was eliminated, unchanged, in the urine. Reich is now looking to "partner with a pharmaceutical company to help us to take it to a higher level."

Time will tell whether the early excitement in MMPs as drug targets in metastasis is justified. What is clear is that the expression of MMP subtype varies with cancer stage and type. Thus, non-small cell lung cancer (NSCLC) overexpresses MMP-11 and MMP-14 rather than MMP-2, which likely means that agents, such as *cis*-ACCP, are unlikely to be efficacious in this disease. With this in mind, further advances in our understanding the complexities of MMP functions *in vivo* might be required before targeted agents become a reality.

Another family of ECM proteins linked with metastatic processes is the S100 proteins, especially S100 calcium-binding protein A4 (S100A4, formerly known as MTS1). Evidence is accumulating that S100A4, when upregulated in the ECM, disrupts cell-to-cell adhesion and facilitates the process of break-

Box 2 Early diagnosis?

Ultimately, the ability to keep one step ahead of cancer may depend on having the ability to detect metastases earlier in the process, rather than attempting to treat advanced, established disease. As it is the ethos of clinicians to insist on seeing evidence of disease before treating a patient, improved diagnostic tests, both molecular and imaging, that exploit increasing understanding of the molecular events that predict critical mitogenic and metastatic alterations will be critical to improvement in cancer patient outcomes.

In 2006, molecular cell biologists Bruce Zetter and Marsha Moses, both of Children's Hospital Boston and Harvard Medical School, co-founded Predictive Biosciences (Lexington, MA, USA) to develop noninvasive tests that can make sense of certain biomarkers, notably MMPs and a disintegrin and metalloproteinases, both of which have been shown to contribute to ECM degradation and associated tumor cell detachment. The idea is to develop algorithms around these enzymes so patients can be monitored for recurrence and progression of diverse types of malignancies, somewhat like the prostate-specific antigen test that warns against advancing prostate cancer. Because these enzymes are detectable in urine, patients can be tested as frequently as necessary. Predictive Biosciences has a urine bank of over 8,500 samples that they are using to validate a library of biomarkers licensed from Children's Hospital of Boston and affiliated institutions. The company's first target is bladder cancer survivors, who undergo painful cystoscopies every few months to look for the recurrent disease. A recent study of 530 patients, 84 with bladder cancer, shows that levels of MMP-9 are effective in discriminating disease-free patients from those suffering a recurrence 42% of the time¹⁵. In addition, the Moses laboratory has described a biomarker, lipocalin 2, which is associated with progressive breast cancer and which can be measured in urine¹⁶.

Another way to get at metastases early is looking for the presence of DTCs. Although originally accomplished by identifying cells with epithelial markers present in the bone marrow that is predominantly mesenchymal in origin, this approach is now being tested in the peripheral blood. One such system has been offered since 2004 by Veridex (a Johnson & Johnson company located in Raritan, NJ, USA). The technology, called CellSearch, pulls circulating tumor cells from the blood of colon, breast and prostate cancer patients using magnetic nanoparticles coated with mAbs against EpCAM.

Researchers at Massachusetts General Hospital (Boston) have also developed a microfluidic device, the CTC (circulating tumor cell) chip, which has 78,000 antibody-coated microposts on a surface the size of a business card that can trap DTCs expressing EpCAM from whole blood. Mehmet Toner, professor of biomedical engineering at Harvard-MIT Division of Health and Science Technology, and his colleagues have shown that their device is capable of detecting DTCs in the blood of patients with five different tumor types with 99% accuracy¹⁷. Commercialization has been handed off to a startup, On-Q-ity (Waltham, MA, USA).

Laura DeFrancesco and GM

Box 3 Modeling metastasis

Almost all drugs tried in humans work against subcutaneous xenografts in mice. The problem is this hardly ever translates to the clinic. According to a 2004 study, as few as 3.8% of patients in phase 1 cancer drug trials between 1991 and 2002 achieved an objective clinical response¹⁸. Placing tumor cells under the skin, although commonly used in drug testing settings because the tumors are easy to establish and measure, does not take into account tumor tropism—the predilection for tumors to grow in only certain environments. In seminal work conducted over 20 years ago describing the behavior of tumor cells in different microenvironments, MD Anderson's Fidler confirmed this principle and went on to develop orthotopic models for studying metastasis, in which cells derived from a variety of human tumors were implanted into correct anatomical sites in nude mice¹⁹. The difference is night and day. Measuring metastasis from tumors planted subcutaneously, Fidler reported zero successes in 700 tries, whereas orthotopic placement of tumor tissue in mice produced metastases in every tumor type attempted.

Echoing that sentiment is Robert Hoffman, president and CEO of AntiCancer (San Diego) and professor of surgery at the University of California at San Diego. “An intact tumor microenvironment is necessary for a good cancer model,” he says. Hoffman's company has commercialized the concept by creating a set of mice in which tumor tissue is implanted orthotopically at various sites to follow tumor progression and dissemination²⁰. Hoffman, along with his then post-doc Takashi Chisima, in 1996 had the idea to make the tumors fluorescent so that imaging could be used to follow tumor cells to distant tissues and organs. It would be the first time cancer metastases would be observed through expression of green fluorescent protein²¹. Today, the company offers a set of organ-specific animal models, called MetaMouse, which have tumor tissue implanted in different organs (e.g., breast, brain, prostate), that allow stromal cells and tumor cells to be labeled with different fluorescent labels so that interactions between the two cell types can be studied *in vivo*. Instead of killing and opening the animal

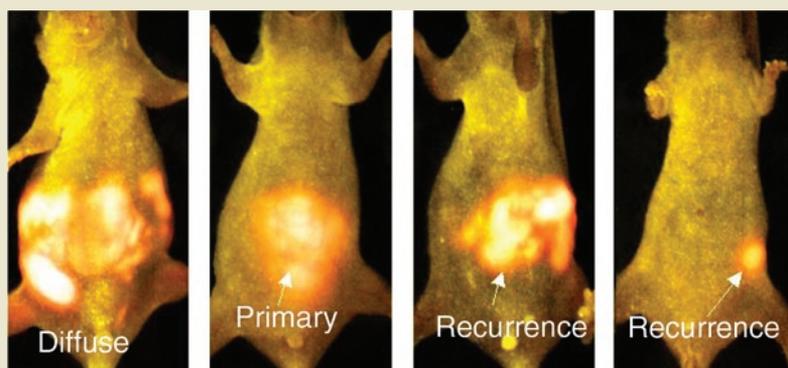


Figure 3 AntiCancer's MetaMouse allows visualization of tumors in whole animals following orthotopic implantation of human pancreatic tumor cells expressing red fluorescent protein. The mice in the different panels received different treatments. Reprinted with permission from ref. 29.

to see where tumors developed, researchers can see the tumors and their progress in real time with an imaging system (Fig. 2).

Taking a different approach to dissecting metastasis, the Canadian company Innovascreen (New Glasgow, Newfoundland, Canada) has developed an *in vivo* system to observe and quantify tumor cell behavior and treatment response using a shell-less (*ex ovo*) avian embryo system. Innovascreen founders John Lewis, the CEO, and CSO Andries Zijlstra originated an intravital imaging system while at Scripps Clinic (La Jolla, CA, USA)²², which is sold as a service by Innovascreen. By injecting tumor cells into the chorioallantoic membrane where they form a tumor, or into the vasculature of the chorioallantoic membrane, they are able to monitor the migration of cells away from the primary tumor, the invasion of the vasculature by tumor cells as well as extravasation from the vessels using three-dimensional time-lapse photography. The imaging system can visualize as many as six different molecules, including fluorescently labeled therapeutics that target vasculature and fluorescent proteins transduced into the tumor cells. Using this direct observation approach, preclinical drug candidates can be quickly assessed for their ability to affect each step in metastasis. “These assays are all designed to be completed within 3 weeks,” Lewis says. “It allows for flexibility in planning and refinement of dosing and other experimental parameters to evaluate investigational drugs.”

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ing through basement membranes. Although S100A4 has been accepted for years as a useful prognostic biomarker—when it is not expressed aberrantly, many breast cancer patients can survive to live out a full life⁴—until recently, it hasn't attracted much attention as a therapeutic target.

This is set to change as Supratek Pharma (Montreal) pushes ahead with a S100A4-targeting drug that was originally approved two decades ago. Supratek has modified the anti-allergy product azaxanthone with an excipient that enhances solubility to create an orally bioavailable drug, SP-MET-X1. The intention is to administer SP-MET-X1 in larger doses than labeled for the allergy indi-

cation, but as the safety of azaxanthone has been long established, the company hopes that it will be a good candidate for long-term chronic treatment, administered several times daily, potentially for years, as a prophylaxis and maintenance therapy.

Thus far, supporting data from preclinical animal models show that the drug inhibits or delays metastasis formation, implantation and progression in animal models. Moreover, Supratek investigators believe SP-MET-X1 can be synergistic with other forms of therapy, such as chemotherapy. The company is planning to begin a phase 1 trial with SP-MET-X1 this year, but the first indication for which the company will file is not yet settled.

Inhibiting urokinase

Another enzyme that is able to proteolytically degrade the ECM and basement membrane around primary tumors is the secreted 54 kDa serine protease urokinase-type plasminogen activator (uPA). In research that originated at the Technical University of Munich two decades ago, investigators Manfred Schmitt, Viktor Magdolen, Nadia Harbeck and Olaf Wilhelm were searching for reasons why a subset of post-surgical breast cancer patients did particularly poorly in terms of survival. By studying patient plasma and tumor samples, they discovered that levels of uPA are inversely correlated with survival. Their findings ultimately revealed that uPA can trigger

a plasminogen proteolytic sequence in the ECM that under normal conditions serves to prevent clotting in the alveoli of the lungs and glomerular apparatus of the kidneys. But uPA can also degrade ECM, interact with MMPs and erode into the microvasculature, thereby allowing tumor cells to exit from a primary site and entry into the lymph or bloodstream. These DTCs also can generate uPA, enabling them to escape from the narrow lumina of microvasculature into new host sites where they break down connective tissues to create a nidus for potential metastatic colonies.

It is now known that uPA is produced and secreted as a single-chain proenzyme (pro-uPA) that binds to the cell surface uPA receptor (uPAR). On receptor binding, pro-uPA is cleaved (primarily by plasmin but also by kallikrein, blood coagulation factor XIIa and cathepsin B) into its two-chain, active form, targeting activity to areas of the cell surface containing uPAR, where it cleaves ECM components, such as fibronectin and laminin receptor (integrin $\alpha 6 \beta 1$). Besides its role in proteolysis, uPAR also regulates many cell surface proteins, such as integrins, growth factor receptors and G protein-coupled receptors, and activates the signaling of metastasis-promoting factors, such as basic fibroblast growth factor, vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF) β and hepatocyte growth factor/scatter factor (HGF/SF). The proteolytic activity of uPA is regulated by plasminogen activator inhibitor-1 (PAI-1) and PAI-2, the former of which induces internalization of the enzyme-receptor complex, leading to uPA degradation.

Developing agents that target uPA has been difficult, in part due to the problem of achieving specificity versus other serine proteases, such as thrombin or plasmin. Back in the 1990s and early 2000s, several companies were working on uPA inhibitor programs, including Abbott Laboratories (Abbott Park, IL, USA), GlaxoSmithKline (Brentford, UK) and Pfizer (New York), as well as smaller companies, such as Corvas International (acquired by Dendreon of Seattle in 2003) and 3-Dimensional Pharmaceuticals (acquired by Johnson & Johnson (New Brunswick, NJ, USA) in 2003). One reason these programs were largely ineffective is that compounds need to be very basic to bind uPA's active site, which almost invariably creates pharmacokinetic problems in making the drug bioavailable. One biotech company, however, founded by Wilhelm, Schmitt and Magdolen, is continuing development of a small-molecule pro-drug that binds uPA. Willex (Munich) is currently testing Mesupron (formerly

WX-671) in combination with chemotherapy in phase 2 trials as a first-line treatment for individuals with HER2-negative metastatic breast cancer (Table 2).

Willex researchers have addressed the pH problem by creating a pro-drug Mesupron, which has excellent bioavailability and can be given orally but is pharmacologically inactive. In the liver and other tissues, Mesupron is metabolized to the company's original formulation WX-UK1, a potent inhibitor of uPA. Because the drug is orally bioavailable and has shown acceptable toxicity in phase 1 studies in healthy volunteers, the company suggests their agent could potentially be used to prevent or depress metastasis formation on a chronic basis after tumor resection or other gold standards of care. Indeed, the US Food and Drug Administration (FDA), which typically wants to see new chemical entities tested in late-stage disease after other therapies have failed, approved a phase 2 trial of Mesupron with the chemotherapy Gemzar (gemcitabine), which began in July 2008, for locally advanced, non-operable, nonmetastatic pancreatic cancer to determine if there is improvement in response rate and progression-free survival and to see if time to metastasis and overall survival can be improved. Thus far, the results are promising; in some of these patients, mortality is lower and therapy is being continued longer than originally planned. "Our approach is somewhat different," says Willex head of R&D Paul Bevan. "We're not looking to get rid of metastases—we're looking to stop them from forming."

The receptor for uPA (uPAR) also represents an interesting target. With the exception of neutrophils and monocytes, quiescent cells almost never express uPAR. An initial report establishing that it is particularly enriched in metastatic lesions compared with primary tumors has since been confirmed by several other studies⁵. Moreover, immunohistochemistry of primary tumor masses shows that uPAR is more concentrated on tumor cells located at the invasive front or leading edge, which is consistent with the assertion that the receptor could be a metastatic driver. Investigators at the now-out-of-business biotech company Attenuon, which was in San Diego, and collaborators at the University of Texas MD Anderson Cancer Center have validated uPAR alone as a blockade target for preventing metastasis through a pleiotropic group of events that include downregulated expression of c-MET (mesenchymal-epithelial transition factor) and suppression of insulin-like growth factor 1 (IGF-1)-dependent tumor cell migration and invasion in both colon and pancreatic cancer cells. Former Attenuon CSO

Andrew Mazar, now entrepreneur in residence and member of the Robert H. Lurie Cancer Center at Northwestern University in Evanston, Illinois, has recently found a licensing partner for his former company's preclinical humanized monoclonal antibody (mAb) huATN-658, which targets uPAR. The antibody has not been tested in humans as yet, but mouse studies have demonstrated significantly suppressed human pancreatic tumor growth and liver metastases along with complete repression of retroperitoneal invasion. "I think the product still has a future," says Mazar. "Clearly, uPAR is an important and very selective target for a variety of different tumor types, but until now companies have never really figured out how to go after this particular receptor." Today, Mazar is working on a new project to inhibit metastasis by targeting the uPA using nanoparticles invented at Northwestern. "I think this approach also has the potential to broadly target a broad spectrum of tumor types," he says, "And in fact, the profile of tumors to be targeted will be similar to those targeted with the anti-uPAR antibody." It is worth noting that many clinicians view PAI-1 as one of the most informative prognostic markers in several cancers, high levels of the protein being associated with poor prognosis.

Cancer in transition

Alterations in the interactions between the primary tumor and ECM are also accompanied by the conversion of epithelial tumor cells into mesenchymal cells—the so-called epithelial-to-mesenchymal transition (EMT). This is associated with the expression of several small noncoding regulatory RNA molecules (Box 4). EMT is thought to be a pivotal event in metastasis, endowing tumor cells with their migratory, invasive and stem-like properties, their ability to suppress apoptosis and senescence and their capacity to dampen immune responses. It has long been known to be involved in embryogenesis, but its role in the spread of human cancer has become widely appreciated only recently, mainly because evidence of its occurrence in clinical samples was overlooked—individual mesenchymal cells originating during EMT are hard to differentiate from stromal cells or other tumor-associated fibroblasts in the vicinity.

An important initial step in EMT is the degradation by MMPs (e.g., MMP-3 or MMP-13) of the protein E-cadherin (epithelial calcium-dependent adhesion molecule), a molecule that maintains adhesion between cells and acts as a tumor suppressor in normal cells. When E-cadherin expression diminishes, the appearance of N-cadherin (neuronal calcium-dependent adhesion molecule) on tumor cell

Table 2 Select agents in late-stage development that target aggressive/metastatic cancers

Company	Target	Agent	Oncology indications	Development stage
Novartis	Hydroxyapatite of bone matrix	Zometa	Bone metastases in multiple myeloma and breast-to-bone metastasis	Marketed (February 2002)
Amgen	RANKL (RANK ligand)	Denosumab (humanized mAb)	Breast-to-bone metastasis and prostate-to-bone metastasis	Phase 3
	Angiotensin 2	AMG-386 (Fc fragment linked to 20-residue peptide that binds angiotensin-2)	Breast, ovarian and RCC	Phase 2
	IGF-1R	AMG-479 (a fully human mAb)	Advanced solid tumors	Phase 2
	c-MET	Rilotumumab (a fully human IgG2 mAb)	Metastatic colon cancer	Phase 2
Exelixis	pan-RTKs	XL-184 (small-molecule RTK inhibitor)	Thyroid cancer	Phase 3
	pan-RTKs	XL-184	Advanced solid tumors	Phase 2
OSI Pharmaceuticals	IGF-1R	OSI-906 (small-molecule RTK inhibitor)	Metastatic adrenocortical carcinoma	Phase 3
Adherex Technologies	N-cadherin	ADH-1 (a cyclic pentapeptide)	Melanoma	Phase 2
Antisense Pharmaceuticals	TGF β	Trabedersen (a phosphorothioate oligodeoxynucleotide)	Glioblastoma	Phase 2
ArQule	c-MET	ARQ-197 (a small-molecule RTK inhibitor)	Various advanced solid tumors	Phase 2
Bristol-Myers Squibb	SRC-family protein-RTKs	Dasatinib (orally bioavailable small molecule)	Breast-to-bone metastasis, breast cancer (triple negative), colorectal cancer and liver	Phase 2
Centocor (Johnson & Johnson)	$\alpha\beta 3/\alpha\beta 5$ integrin	Intetumumab (fully human mAb)	Metastatic melanoma	Phase 2
Genentech	c-MET	MetMAB (a humanized monovalent 5D5 Fab mAb)	NSCLC	Phase 2
	SMO	GCD-0449 (small-molecule inhibitor)	Metastatic basal cell carcinoma, colorectal cancer and ovarian cancer	Phase 2
Global TransBiotech	Heparanase	PI-88 (phosphomannopentaose)	Malignant melanoma	Phase 2 (completed)
GlaxoSmithKline	c-MET, VEGFR-2, AXL RTKs	Foretinib (small-molecule RTK inhibitor)	Metastatic squamous cell carcinoma and gastric cancer	Phase 2
Facet Biotech	$\alpha 5\beta 1$ integrin	Volociximab (a chimeric IgG4 mAb)	Various solid tumors	Phase 2
MethylGene	c-MET, VEGFR 1,2,3, Tie 2 and Ron RTKs	MGCD265 (small-molecule RTK inhibitor)	NSCLC	Phase 2
Merck	IGF-1	Dalotuzumab (MK-0646, a humanized mAb)	Metastatic colon cancer and others	Phase 2
	Cathepsin K	Odanacatib, a [2,2,2-trifluoro-1-(biphenyl-4-yl)ethyl]-4-fluoroleucine derivative of 4-fluoroleucine	Breast and bone metastases	Phase 2
Willex	uPA	WX-UK1 (serine protease inhibitor)	Breast cancer and other solid tumors	Phase 2b
	uPA and other serine proteases	Mesupron (pro-drug of WX-UK-1)	Pancreatic, breast, and head and neck cancer	Phase 2

surfaces is known to be a survival factor for melanoma cells and doubtless other tumor cells as well⁶. Indeed, with N-cadherin expression, epithelial tumor cells acquire a mesenchymal cell phenotype, more like fibroblasts, with an ability to migrate and navigate across basement membranes, pass between endothelial cells and enter into vascular lumina.

Recent work by cancer biologist Robert Weinberg, director of the Ludwig Center for Molecular Oncology at MIT (Cambridge, MA, USA), shows that the entire EMT program can be triggered by any one of at least seven early embryonic transcription factors (e.g., Snail1/2, survival of motor neuron protein interacting protein (SIP1), ZEB1, ZEB2, Slug, KLF8, E12/E47A-E2A). Several other proteins also repress E-cadherin indirectly, including Twist, Goosecoid, E2.2 and Foxc2. "It seems

increasingly clear that human cancers can turn on these long-silent genes, and in so doing the cells acquire in one fell swoop most all of the attributes that they need in order to execute the steps of the invasion metastasis cascade," says Weinberg.

So far, only one company has attempted to clinically exploit EMT by specifically targeting N-cadherin. Adherex Technologies (Durham, NC, USA) has been conducting a phase 2 trial for melanoma patients with ADH-1, a cyclic pentapeptide containing the N-cadherin extracellular domain cell adhesion recognition motif (histidine-alanine-valine). Cadherins normally bind to each other from cell to cell, but if any one of those three amino acids is disrupted, intermolecular binding is prevented. ADH-1 blocks the adhesion sites and hence the binding. Surgical oncologist Douglas Tyler

of Duke Medical Center (Durham, NC, USA), principal investigator in the ADH-1 trials, had previously demonstrated marked improvements in tumor responses in a rat model of melanoma using systemic ADH-1 plus locally administered melphalan (L-phenylalanine mustard) compared with melphalan alone. These preclinical studies led the way for initiation of clinical trials with systemic ADH-1 plus melphalan for patients with advanced extremity melanoma. After a positive phase 1 trial, phase 2 results with overall response rates of 58% versus 40% with chemotherapy alone were presented at the American Society for Clinical Oncology (ASCO) meeting in late May 2009 in Orlando, Florida. Ultimately, Adherex would like to evaluate the durability of these results beyond 3 months, but the product is currently on hold due to lack of resources and

the switching of priorities to monetize more mature projects. Tyler says, “We would like to learn more about what ADH-1 is doing in the tumor that makes it more sensitive to the chemotherapy, but we’re actually very excited. This is the only drug that has really targeted the N-cadherin molecule, which is important in melanoma and some other malignancies.”

One other cadherin-like molecule currently under investigation is receptor tyrosine kinase (RTK) anaplastic lymphoma kinase (ALK). BerGenBio (Bergen, Norway), founded in 2007 by researchers from the University of Bergen, is evaluating small-molecule and mAb inhibitors of RTK AXL, which has an extracellular domain comprising fibronectin III and immunoglobulin motifs similar to cadherin-type adhesion molecules. Preclinical studies carried out by BerGenBio in three-dimensional cell culture and animal models of angiogenesis, metastasis and survival have shown that RTK AXL is essential for breast cancer metastasis but not for angiogenesis. Another protein associated with EMT is autocrine motility factor (AMF), a C-X-X-C motif cytokine also known as neuroleukin. As yet, no companies have taken any therapeutic agents into the clinic that specifically target AMF, although it is known that Genentech/Roche’s (S. San Francisco, CA, USA) humanized mAb Herceptin (trastuzumab), which primarily targets the epidermal growth factor (EGF) family receptor (EGFR) HER2, inhibits AMF expression and augments the activity of specific simple sugar inhibitors of AMF, such as erythrose 4-phosphate and D-mannose 6-phosphate⁷.

Developmental signaling pathways

An important function of E-cadherin is interaction (by means of its cytoplasmic domain) with β -catenin, which together with α -catenin, anchors the cadherin complex to the cell’s actin cytoskeleton. β -catenin comprises a multiprotein complex (including AXIN, adenomatous polyposis coli (APC) and glycogen synthase kinase-3 β) that is part of the Wnt signal transduction pathway. When Wnt binds to its receptor (frizzled), disheveled (Dsh/Dvl) protein then stabilizes the β -catenin complex, which then travels to the nucleus where it triggers the expression of a range of mitogenic and EMT-related genes; in Wnt’s absence, excess cytoplasmic β -catenin is targeted for degradation in the proteasome.

In malignancies, such as colon adenocarcinoma and squamous cell carcinomas, however, the Wnt pathway is upregulated. Mutations in certain components (e.g., APC or AXIN) of the β -catenin multiprotein complex become constitutively active, allowing β -catenin to

localize to the nucleus and begin transcription of proliferative and/or cell cycle genes, such as *c-Myc* (also known as *MYC*) and cyclin D1. Apart from a few nonsteroidal anti-inflammatory drugs (NSAIDs; e.g., aspirin, Indocin (indomethacin) and OSI Pharmaceuticals’ (Melville, NY, USA) Aptosyn (exisulind)) that have shown limited activity in suppressing β -catenin transcription, few compounds specifically targeting the Wnt pathway have been developed—partly because β -catenin regulation involves protein-protein interactions, which are hard to block using small molecules. Even so, in September, a team at Novartis identified two new enzymes that can regulate β -catenin stability: tankyrase TRF1-interacting ankyrin-related ADP-ribose poly-

merase (TNKS) and TNKS2 (ref. 8). These kinases help break up the multiprotein complex, facilitating ubiquitin-mediated degradation of β -catenin. According to Novartis, small-molecule inhibitors of TNKS and TNKS2 are currently under development.

Other developmental signaling pathways linked with EMT are the Hedgehog/Smoothed pathway and the Notch pathway. Three companies currently have compounds targeting the Hedgehog cascade in active development. In September, Genentech reported that its orally active small-molecule GDC-0449 showed potent antitumor activity in 8 out of 18 patients in a phase 1 trial for metastatic skin cancer (basal cell carcinoma), with 1 unconfirmed partial response,

Box 4 Noncoding RNAs and metastasis

One area of research yet to be fully exploited by industry is the vast group of ‘noncoding’ or regulatory RNAs that include microRNAs (miRNAs), nucleolar RNAs, piwi-interacting RNAs, medium RNAs that range from 30–300 nt and the very large RNAs that can extend up to 100 kb. Although some commercial efforts are currently targeting miRNAs, as yet very little work has been done on how to translate knowledge concerning other noncoding RNAs into therapeutic programs.

For noncoding RNAs, “It will take time and resources to evaluate and find which transcripts in these groups are really functional and which ones are important in the pathogenesis of diseases, such as cancer,” says cancer biologist Fabricio Costa of Children’s Memorial Research Center at Northwestern University’s Feinberg School of Medicine. “In some cases, expression of certain noncoding RNAs can predict how aggressive and prone to metastasis a cancer might be,” he says. Examples include the MALAT-1 (metastasis-associated lung adenocarcinoma transcript-1) gene—a large (8 kb) noncoding RNA that is overexpressed in lung cancers and which has been associated with lung cancer progression²³—and DD3, a large (2–4 kb depending on splice variants) noncoding RNA overexpressed in prostate cancers²⁴. “I would love to see pharma and biotech take a look at these two in particular and this new field of research in general,” says Costa, who has been looking for possible therapeutic candidates based on noncoding RNAs. “This group could play a huge role in the management of cancer progression and metastasis,” he says.

In the area of miRNAs, several candidates have already been associated with metastasis. For example, miR-200 and miR-205 inhibit the EMT-inducing transcription factors ZEB1 and ZEB2. In breast carcinoma, loss of miR-200 correlates with a decrease in E-cadherin. Acting in the opposite direction, miR-21 is upregulated in many cancers and facilitates TGF β -induced EMT. Other miRNAs, such as miR-10b, miR-373 and miR-520c, have also been associated with progression in breast carcinoma. And in lung adenocarcinomas, miR-NLET7a2 precursor miRNA expression also correlates with poor survival.

Several companies are investigating either the reconstitution of tumor-suppressive miRNAs or sequence-specific knockdown of oncogenic miRNAs by antagomirs. Regulus Therapeutics (Carlsbad, CA, USA) has described preclinical data showing that miR-296 induces angiogenesis in glioblastoma, whereas miR-451 increases the efficacy of Gleevec in the same indication. At a Keystone Symposium in January, the company also presented data that it had successfully downregulated miR-34a using an antagomir strategy in human hepatocellular carcinoma cells. The company has also studied the activity of three prometastatic miRNAs—miR-21, miR-10b and miR-122—in glioma, breast cancer and liver carcinoma, respectively. Elsewhere, Asuragen (Austin, TX, USA) subsidiary Mirna Therapeutics has reported preclinical data on miR-Rx34, which suppresses metastasis in nude mice xenograft models of NSCLC, lung cancer, prostate cancer and pancreatic cancer. The company is also developing antagomirs against miR-NLET7. *GM and AM*

7 patients achieving stable disease and 2 experiencing disease progression. The company is also currently testing the drug in a phase 2 trial of metastatic colon cancer together with chemotherapy plus the company's anti-VEGF mAb Avastin (bevacizumab). Elsewhere, Bristol-Myers Squibb (Princeton, NJ, USA)/Exelixis (S. San Francisco, CA, USA) and Infinity Pharmaceuticals (Cambridge, MA, USA)/Mundipharma (Göteborg, Sweden) are carrying out phase 1 testing of small-molecule inhibitors of the Smoothened receptor in individuals with metastatic solid tumors.

Aberrant Notch pathway activation is associated with brain, breast and lung cancers. Although drug development programs targeting Notch and its ligand delta-like ligand 4 (DLL4) are even less mature than those for Hedgehog, some compounds have already reached the clinic. For example, Merck (Whitehouse Station, NJ, USA) is developing MK-0752, a small molecule that targets the protease γ -secretase—an enzyme that cleaves Notch, enabling it to translocate to the nucleus and activate transcription of neoplastic and pro-angiogenic factors. Since 2005, the pharma company has initiated several phase 1 trials of MK-0752 (either singly or in combination with chemotherapy) in patients with metastatic breast cancer or refractory pediatric, central nervous system cancer. Drawbacks to inhibiting γ -secretase are the possible toxic effect on normal stem cells in the body (potentially narrowing MK-0752's therapeutic window) and the observation that mutations in signal transduction downstream of Notch (e.g., in the phosphatase and tensin homolog (PTEN)—phosphoinositide 3 kinase—Akt pathway) may give rise to drug-resistant cells. One way around the narrow therapeutic window might be to target the Notch ligand DLL4 instead. OncoMed Pharmaceuticals (Mountain View, CA, USA), which in December 2007 struck a worldwide strategic alliance with GlaxoSmithKline for four mAbs aimed at cancer stem cells, is targeting DLL4 with a humanized mAb (OMP-21M18) that is currently in a phase 1 trial for solid tumors; if the antibody proceeds through to phase 2 trials, GlaxoSmithKline can exercise its option to take over development. Last year, in a partnership with Sanofi-aventis (Paris), Regeneron (Tarrytown, NY, USA) also initiated testing a fully human anti-DLL4 mAb (REGN421) in solid tumors.

Targeting IGF and TGF β

If the Notch pathway has only recently risen on to the radar of drug developers, enthusiasm for the association of the IGF pathway with cancer signaling has waxed and waned several

times over the past two decades. Again, IGF is known to induce rapid internalization of E-cadherin from the membrane to the cytoplasm, inducing EMT through the nuclear factor κ B (NF- κ B)-Snail axis in mammary epithelial cells or by upregulating the transcription factor Zeb in prostate carcinoma cells. Pfizer and OSI Pharmaceuticals currently have the most advanced IGF-targeting programs. Pfizer's fully human IgG2 mAb, which binds IGF-1 receptor's (IGF-1R) extracellular domain, is currently in phase 3 trials for non-small cell lung cancer; OSI has initiated phase 3 testing of OSI-906, a small-molecule inhibitor of the RTK domain of IGF-1R, for patients with metastatic adrenocortical carcinoma. The same receptor is also being targeted by Merck, which has a humanized IgG1 antibody (MK-4606) currently in phase 2 trials for metastatic colon cancer, and by Amgen (Thousand Oaks, CA, USA), which is combining a fully human anti-IGF-1R mAb (AMG479) with its anti-EGFR human mAb Vectibix (panitumumab) or chemotherapy in a phase 2 trial for patients with advanced solid tumors. Two other companies, ImClone (New York, a wholly owned subsidiary of Eli Lilly) and Sanofi-aventis, also have anti-IGF-1R mAbs in early clinical development. One company, Silence Therapeutics (London), is even developing a small interfering RNA (siRNA) that inhibits IGF-1R for the potential treatment of prostate cancer.

Another growth factor that could provide opportunities for therapeutic targeting is TGF β , which has contrasting roles in tumor initiation and metastasis. During the early phase of tumorigenesis, TGF β inhibits tumor epithelial cell growth by inducing cell cycle arrest and apoptosis. Paradoxically, in later stages of tumor progression—as mutations in cell cycle regulators and constitutive activation of Ras circumvent its inhibitory activity—the secreted cytokine confers a pro-metastatic effect on tumor cells and the surrounding stromal cells, impairing immune surveillance and promoting invasion (e.g., via angiopoietin-like 4 induction), angiogenesis (via VEGF induction) and migration to distant sites. When overexpressed on breast, colon, liver, lung, prostate and gastric cancers, TGF β has been shown to promote EMT via the Smad pathway (inducing such transcription factors as Snail and Slug) and elevates the expression of many of the same proliferative/cell cycle genes as Wnt.

One of the most advanced therapies against TGF β in cancer is Antisense Pharma's (Regensburg, Germany) trabedersen, a phosphorothioate oligodeoxynucleotide (oligo) specific for the 5'-cgcatgtcta-ttttga-3' sequence of the mRNA encoding the

TGF β 2 isoform. As TGF β 2 is the most highly expressed isoform in astrocytoma cells, the company is developing the drug for the treatment of malignant brain cancers, where it has to be delivered intracranially by pump. A pivotal phase 3, multicenter clinical trial for recurrent or refractory grade III anaplastic astrocytoma is currently underway to compare the 2-year survival rates and tumor responses of patients receiving trabedersen with those receiving standard chemotherapy.

An even more ingenious route to the inhibition of TGF β is being taken by NovaRx (San Diego). The company currently has brought Lucanix (belagenpumatucel-L), a therapeutic cancer vaccine (Box 5), into phase 3 trial for patients with advanced and metastatic NSCLC. Other companies less advanced in the clinic have also been pursuing TGF β as a target, albeit with more conventional approaches. For example, Genzyme (Cambridge, MA, USA) is developing GC-1008, a human mAb against TGF β . In August 2008, the company began a phase 1/2 dose-escalation study in individuals with advanced metastatic melanoma or renal cell carcinoma, the results of which are awaited this year. Other companies, such as Johnson & Johnson subsidiary Scios (Fremont, CA, USA) and Eli Lilly (Indianapolis) were developing small-molecule inhibitors of the TGF β type I receptor kinase, but news of either compound has not been forthcoming since 2007, suggesting that development of these inhibitors is not a priority.

Heparin-binding growth factors

Heparin-binding growth factors (HBGFs), such as HGF and heparin-binding EGF-like growth factor, are another group of proteins widely implicated in metastases. One lesser-known HBGF, pleiotrophin, is known to be elevated in aggressive solid tumors (e.g., glioblastoma, melanoma and pancreatic cancer) and pleiotrophin serum levels often can be seen to drop precipitously after tumor resection. In the late 1990s, Washington, DC-based Georgetown University investigator Anton Wellstein found a receptor for pleiotrophin by screening a human cDNA phage display library against immobilized pleiotrophin. A short protein fragment was isolated that was part of the extracellular domain of the RTK ALK. "We discovered the soft spot on the ALK receptor where it binds the ligand," says Wellstein. "And I thought hitting directly at the ligand-receptor interaction would be the best target for future drug development."

In his recent work, Wellstein has developed a high-affinity human single-chain variable fragment (scFv) antibody to target the

Box 5 Treatments of last resort?

The cancer field is littered with failures of cancer vaccines in advanced, metastatic cancer. Much of the reason for the high attrition rate is the fact that these treatments are often tested in patients that have undergone multiple rounds of treatment and are consequently very sick, in the end stages of disease. That said, many cancer vaccines applied to late-stage cancers have not specifically targeted antigens associated with metastatic events. There are, however, some notable exceptions.

The poster child for cancer vaccines is Dendreon's Provenge (sipuleucel-T), which is indicated for individuals with metastatic hormone-resistant prostate cancer and awaits a final decision from the FDA on May 1. This patient-specific vaccine is produced by incubating a patient's own blood, enriched for dendritic cells and other antigen-presenting cells, with a recombinant fusion protein composed of prostatic acid phosphatase and granulocyte-macrophage colony-stimulating factor. Prostatic acid phosphatase is normally present only in vanishing amounts in the blood, but often (though not exclusively) it is elevated in people with metastatic prostate cancer. Another antigen that is highly expressed in metastatic disease is melanoma-associated antigen (MAGE A3). By packaging MAGE-A3 in liposomes, GlaxoSmithKline Biologicals (Brussels) has created astuprotimut-r, which is now in phase 3 testing for patients with metastatic MAGE-A3-positive melanoma. Elsewhere, NovaRx (San Diego), is developing Lucanix (belagenpumatucel-L), a therapeutic cancer vaccine comprising DNA tumor cell lines genetically modified to express antisense DNA specific for TGF β . Here the idea is to suppress TGF β -mediated immune tolerance of the tumor cells in the vaccine and stimulate a more efficacious T-cell response to the cancer. In August 2008, NovaRx initiated a multicenter, randomized phase 3 trial in patients with advanced and metastatic NSCLC.

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Two other ATP-competitive inhibitors of c-MET currently under development are MethylGene's (Montreal) MGCD-265 and GlaxoSmithKline's foretinib (XL-880). MethylGene's compound is an orally delivered N-(3-fluoro-4-(2-arylthieno[3,2-b]pyridin-7-ylloxy)phenyl)-2-oxo-3-phenylimidazolidine-1-carboxamide that binds not only c-MET, but also VEGF receptors (VEGFR)-1, VEGFR-2 and VEGFR-3, as well as Tie 2 and Ron RTKs. Last September, MethylGene initiated a phase 2 trial for MGCD-265 in combination with Tarceva (erlotinib) or the chemotherapy Taxotere (docetaxel) in advanced metastatic NSCLC. The GlaxoSmithKline drug, which inhibits the c-MET, VEGFR2 and AXL RTKs, is also currently in a phase 2 trial for recurrent or metastatic squamous cell cancer of the head and neck and a phase 2 trial for metastatic gastric cancer.

Finally, ArQule (Woburn, MA, USA; formerly Cyclis Pharmaceuticals), partner Daiichi Sankyo (Tokyo) and Asian licensee Kyowa Hakko Kirin (formerly Kyowa Hakko Kogyo) are developing ARQ-197, an orally administered small molecule that inhibits c-MET by binding outside the ATP-binding pocket of the kinase. In October 2007, the program entered phase 2 trials for microphthalmia transcription factor tumors and pancreatic cancer in the United States and Eastern Europe, respectively; and last October, a phase 2 trial in hepatocellular carcinoma was also initiated. In a recent announcement, ArQule said it expected to have data from a phase 1/2 NSCLC trial initiated in March 2008 in the first half of this year.

Yet more companies are attempting a different tact: targeting the extracellular portion of the c-MET receptor rather than inhibiting its intracellular RTK activity. For example, Genentech/Roche is developing MetMab (RG-3638), a humanized, monovalent 5D5 Fab antibody that binds the extracellular domain of c-MET, preventing HGF binding and subsequent receptor activation. In April 2009, Genentech initiated a phase 2 trial evaluating MetMab in combination with OSI Pharmaceutical's Tarceva for second- and third-line metastatic non-small cell lung cancer. And Amgen's rilotumumab (AMG-102), a fully human IgG2 class anti-c-MET mAb, is also under development for the potential treatment of various types of advanced-stage cancers. Although results from a phase 2 trial in glioblastoma multiforme indicated that rilotumumab has only limited efficacy when administered as a monotherapy, the company is continuing a phase 2 study combining AMG-102 with platinum-based chemotherapy for metastatic colorectal cancer.

ligand-binding domain in ALK that blocks binding of pleiotrophin and at the same time does not appear to agonize the signal cascade that pleiotrophin clearly stimulates in tumor and stromal cells. *In vivo* murine studies have shown that this scFv antibody will inhibit invasion of aggressive human glioblastoma cells (U87MG) into an endothelial monolayer, which is one of the initial steps in the metastatic cascade. "It blocks the pleiotrophin effect on invasion and thus can inhibit metastasis," says Wellstein. He hopes human trials can begin within the next 12 to 18 months.

Another HBGF that induces and supports tumor progression is HGF, the natural ligand for the c-MET RTK. HGF binding causes dimerization of c-MET, leading to increases not only in the expression and/or secretion of MMP2, MMP7, MMP9 and uPA, but also in the phosphorylation and activation of multiple downstream pathways, including E-cadherin (which is ubiquitinated and targeted to lysosomes), Erk1/2 (cell proliferation/differentiation), Akt (cell survival/apoptosis), protein kinase C, paxillin/focal adhesion kinase (FAK) (cytoskeletal functions involved in migration and adhesion) and retinoblastoma protein (Rb/Rb1; cell cycle). The c-MET receptor is inappropriately activated in many cancers through diverse mechanisms, including overexpression and a variety of mutations. For example, somatic mutations of c-MET are selected for during the metastatic spread of head and neck squamous carcinoma; and

although MET gene amplifications are relatively infrequent in primary tumors, they are often found in advanced solid tumors in gastric, lung or esophageal cancer.

Early work in the laboratory of Toshikazu Nakamura at Osaka University identified a fragment of the HGF α -chain that contains an N-terminal hairpin domain (N-domain) and four kringle domains that inhibit HGF signaling. Several years ago, the fragment, termed NK4, was licensed by Kringle Pharma (Osaka, Japan). Since that time, however, no progress has been reported, and the Japanese biotech is currently seeking to outlicense commercialization rights to the peptide.

Several other drugs targeting the HGF/c-MET axis are making further headway in the clinic. For example, Exelixis' small-molecule pan-RTK inhibitor (XL-184), which blocks the ATP binding site of c-MET, is currently in late-stage trials. Preliminary results from a pivotal phase 3 test of the drug in individuals with metastatic medullary thyroid cancer were reported at January's JP Morgan Healthcare Conference in San Francisco. According to the company, antitumor activity was observed, with tumor shrinkage (30% or more) observed in 90% of the subjects and 29% of patients experiencing a partial response. If these initial results pan out, Exelixis plans to file a new drug application for the second half of 2011. The company is currently co-developing the molecule with Bristol-Myers Squibb, which licensed exclusive worldwide rights to XL-184 in December 2008.

One other HBGF co-receptor, glypican-1, is being pursued as a drug target. Cancer researcher Murray Korc and his group at Dartmouth Medical School (Hanover, NH, USA) have demonstrated that glypican-1 is expressed on both epithelial tumor cells and stromal cells together and is critical for well-organized proliferation, angiogenesis, invasion and metastasis of human pancreatic cancers. Korc has isolated endothelial cells from glypican-1 knockout mice and added VEGF *in vitro*. A greatly diminished mitogenic effect is observed on the endothelial cells of the glypican-1 knockouts versus the proliferative endothelial cells isolated from wild-type mice⁹. So, in addition to expression of glypican-1 from tumor epithelium, its presence in the tumor stromal/endothelial cell microenvironment appears to be important in making the process work. “We think it actually all fits,” says Korc. “You have overexpression of multiple ligands that are heparin binding and that activate multiple types of receptors, and you have overexpression of glypican-1, which seems to be very important.”

Korc is now seeking how best to downregulate glypican-1. “We need to come up with a mouse trap that prevents glypican-1 from doing its thing,” he says. Because the glypican-1 is a large molecule and resides on cell surfaces and in the ECM as well as in connective tissues, Korc is considering monoclonal or polyclonal antibodies as potential therapies.

Hitting hypoxia

Because tumor masses grow faster than their blood supply, and because tumor-associated vasculature is leaky and chaotic in configuration, a low oxygen tension environment (hypoxia) is the rule rather than the exception in solid tumors. In recent years, evidence has also been accumulating that hypoxic tumor cells have an increased invasive and metastatic potential. Hypoxia stabilizes hypoxia-inducible factor (HIF1- α), which, by targeting the transcription factors Twist and Snail, induces EMT as well as changes in tumor cell metabolism (upregulating hexokinase), angiogenesis, invasion and apoptotic potential. HIF1- α also boosts levels of an enzyme, lysyl oxidase (LOX), which cross-links collagen in the ECM. High levels of LOX have been correlated with shorter metastasis-free survival and poor prognosis in head and neck cancer and estrogen receptor-negative breast cancer.

In 2004, work by Amato Giaccia and postdoctoral fellow Janine Erler, both of Stanford University School of Medicine (Stanford, CA, USA), revealed that tumor cell-secreted LOX is required for hypoxia-induced invasion through increased cell-matrix interactions

and FAK activation, thus enabling metastatic dissemination. Continuing her research at the Institute of Cancer Research in London, Erler went on to show that LOX secreted by hypoxic tumor cells plays an important role in generating a pre-metastatic niche through recruitment of bone marrow-derived cells, which prepare tissue for the incoming metastasizing tumor cells¹⁰. The latter phenomenon could be due to LOX-mediated ECM modifications, which Erler is currently in the process of characterizing in her laboratory.

Giaccia has co-founded Arresto BioSciences (Palo Alto, CA, USA), where a humanized mAb is now in preclinical development to inhibit LOX. “Our data show that if extracellular LOX is inhibited, we can inhibit the metastatic process,” says Giaccia, who prefers an antibody rather than a small molecule for targeting LOX. “If you are going after the metastatic process, you really want to go after a target that’s involved in metastasis and not a target that’s necessarily involved with primary tumor growth,” he adds. “When we target LOX, we’re seeing very little effect in the primary tumor, but instead we are seeing our effect on metastasis.”

Other companies investigating the potential of hypoxia-related targets are Threshold Pharmaceuticals (Redwood City, CA, USA), Enzon Pharmaceuticals (Bridgewater, NJ, USA) and Oncothyreon (Seattle WA, USA). Researchers at Threshold have been developing the pro-drug TH302, a 2-nitroimidazole-triggered bromo analog of ifosfamide that is converted into its DNA-alkylating active form, dibromo isophosphoramidate mustard, under hypoxic conditions. In January, the company presented data at the JP Morgan Healthcare Conference indicating efficacy in a phase 1/2 trial for soft tissue sarcoma: of 20 evaluable patients, 5 (25%) had partial responses, 12 (60%) had stable disease and 3 (15%) had progressive disease. The drug has also shown activity in a preclinical model of metastatic prostate cancer. Elsewhere, Enzon (in collaboration with Santaris Pharma of Horsholm, Denmark) has a locked nucleic acid antisense oligonucleotide (EZN-2968) targeting HIF1- α for the treatment of advanced cancers, such as renal cell or colorectal cancers, in phase 1 testing. And finally, Oncothyreon is carrying out phase 1 safety testing of PX-478 (the *N*-oxide of melphalan), a small-molecule inhibitor of HIF1- α , for advanced solid cancers.

Moving out of the neighborhood

Another way in which tumor epithelial cells maintain their contacts with ECM components, such as fibronectin, collagen and laminin, is through integrins. By interacting in the

cytoplasm in a complex with FAK and SRC family kinases, integrins mediate attachment to the actin cytoskeleton. In turn, calcium-dependent GTPases, such as RhoC in breast cancer, are involved in the induction of cytoplasmic extensions (filopodia) in tumor cells, enabling them to migrate out of the primary cancer and into distal sites.

Several companies have attempted to target integrins as a means of controlling advanced cancers. At last May’s ASCO meeting, Johnson & Johnson subsidiary Centocor Ortho Biotech (Malvern, PA, USA) announced results of a randomized, phase 2 trial of its anti- α v β 3/ α v β 5 integrin fully human mAb intetumumab (CNTO-95) in 129 individuals with metastatic melanoma with or without chemotherapy. The trial showed a trend toward prolongation of progression-free survival, overall survival and disease control and comparable activity to decarbazine chemotherapy. Two other integrin-targeted molecules, Facet Biotech’s (Redwood City, CA, USA) volociximab (chimeric IgG4 mAb targeting the AAB1 component of α 5 β 1 integrin) and MedImmune/AstraZeneca’s (Gaithersburg, MD, USA) Abegrin (etaracizumab; a humanized anti- α v β 3 integrin) have gotten as far as phase 2 testing but have shown less impressive efficacy in single agent/chemotherapy combination trials for various metastatic solid tumors. Andrew Mazur, now of Northwestern University, was also involved in an α 5 β 1 integrin antagonist program when at Attenuon. The drug, ATN-161, is a small peptide derived from the synergy region of fibronectin that blocks α 5 β 1 integrin binding. Phase 2 trials in which ATN-161 was administered by intravenous infusion in renal cell carcinoma demonstrated preliminary hints of beneficial activity at the highest dose before funding was terminated. Mazur says the peptide is being licensed out along with his former company’s antibody huATN-657 to a still undisclosed pharma as an anti-metastatic agent or a therapy for Crohn’s disease.

As yet, industry has not announced any clinical programs specifically targeting RhoC. Complegen (Seattle) disclosed in 2008 that it has identified selective inhibitors with nanomolar affinity for RhoC using its high-throughput screen in yeast; initial testing of the compounds has also been carried out in xenograft transplantation studies for metastasis. But until now, most interest has focused on compounds that inhibit farnesylation/prenylation of RhoC and other RAS family members, either by intervening in the mevalonate pathway involved in the biosynthesis of farnesyl diphosphate (using approved HMG-CoA reductase inhibitors, such as statins,

or bisphosphonates and isoprenoids) or by the development of specific prenylation inhibitors. This line of therapeutic attack, however, has also fallen out of favor. As of January, Janssen Pharmaceutica (a Johnson & Johnson subsidiary in Beerse, Belgium) and Merck/Schering-Plough, the two companies most actively pursuing prenylation inhibitors, had discontinued development of their prenylation inhibitors in advanced cancers owing to lack of efficacy and toxicity concerns.

Commercial interest has also centered on targeting components further down the signal transduction pathway. Last year, Bristol-Myers Squibb received FDA approval for its Src inhibitor Sprycel (dasatinib). Sprycel, which also inhibits the BCR-ABL tyrosine kinase that interacts with FAK, is currently approved for the treatment of the hematological malignancies acute lymphoblastic leukemia and chronic myelogenous leukemia. Bristol-Myers Squibb has also been testing the compound in late-stage trials for castration-resistant prostate cancer and late-stage (androgen-resistant) breast cancer that has spread to the bone. Two other companies, AstraZeneca and Kinex Pharmaceuticals (Buffalo, NY, USA), have also been developing small-molecule inhibitors of Src; as yet, however, compelling evidence of efficacy in terms of extending survival or delaying progression of advanced solid tumors remains to be shown for the latter programs.

Into the circulation

Once tumor cells have undergone EMT and then migrated from the primary site, they often aggregate with platelets and fibrin in the circulation, embolize in capillaries or directly attach to endothelial cells by means of integrins, P-selectin and the epithelial cell adhesion molecule (EpCAM), which through its intracellular domain associates with β -catenin, FHL2 and Lef-1, leading to the initiation of cellular proliferation programs¹¹.

One molecule present in the bloodstream that has been shown to interfere with this process is heparin. It is thought to act in two distinct ways: first, heparin inhibits endoglycosidase heparanase, an enzyme that is often secreted from cells, such as stimulated platelets and leukocytes, aiding the degradation of ECM heparin sulfate and facilitating extravasation across the vascular endothelium to sites of inflammation; and second, heparin binds to P-selectin on endothelial cells, which is used by metastasizing tumor cells to anchor on to the vascular wall.

No commercial programs have yet focused on heparin's ability to block P-selectin, but reports have appeared describing the ability of 2,6-*O*-disulfated dermatan sulfate to inhibit

platelet-tumor cell association *in vivo* and suppress metastatic growth in mouse models¹².

One company seeking to take advantage of heparin's inhibitory effect on heparanase is Global TransBiotech (Monterey Park, CA, USA). Last year, the company in-licensed PI-88 (phosphomannopentaose), an oligosaccharide inhibitor of heparanase from Progen (Toowong, Australia), which had previously been developing the drug for hepatocellular carcinoma. Global TransBiotech is focusing on further development and registration of the PI-88 in Taiwan, China, Hong Kong and Singapore; last September, it announced that it had completed a phase 2 trial of the drug in metastatic melanoma, with registration trials expected this year.

Another heparin mimetic is the small-molecule suramin, a polysulfated naphthylurea currently under investigation by Optimum Therapeutics (Columbus, OH, USA). Suramin has been found to enhance the activity of chemotherapeutic Taxotere more than tenfold in human NSCLC xenograft models. The company is currently taking suramin into phase 1/2 trial in patients with second- or third-line NSCLC tumors.

Unlike the heparin-mimetics, therapeutic approaches to address EpCAM have proceeded considerably further in the clinic. In 1995, Centocor (in partnership with GlaxoSmithKline) developed and launched in Germany the anti-EpCAM murine mAb Adjuqual (edrecolomab) for the treatment of residual colorectal cancer after surgery. Although this drug was withdrawn in 2001, last year, Fresenius Biotech (Hamburg, Germany, in collaboration with TRION Pharma of Munich) received marketing approval from the European Medicines Agency (EMA; London) for another anti-EpCAM molecule. Removab (catumaxomab) is a bispecific rat/mouse hybrid antibody comprising a Fab fragment of a mouse IgG2a antibody specific to human EpCAM and a Fab fragment of a rat IgG2b antibody specific to human CD3 on T cells, indicated for intraperitoneal treatment of malignant ascites (excess fluid in the peritoneal cavity) in EpCAM-positive carcinoma patients. The mAb recognizes EpCAM-positive tumor cells and CD3⁺ T-cells by means of its bispecific binding arms and also binds Fc γ receptor I/III-positive immune cells through its Fc domain. Three other anti-EpCAM agents are also in clinical development, including a fully human IgG1 mAb (adecatumumab), a recombinant fusion protein comprising human anti-EpCAM mAb linked to interleukin (IL)-2 (tucotuzumab celmoleukin) and a humanized anti-EpCAM scFv fused to recombinant *Pseudomonas* exotoxin (VB4-845) sponsored by

MicroMet (Bethesda, MD, USA), Merck Serono (Darmstadt, Germany) and Viventia Biotech (Mississauga, ON, Canada), respectively.

Elsewhere, Galapagos (Mechelen, Belgium) has also been working on a small-molecule RGD integrin receptor modulator, now designated GLPG0187, which it acquired from ProSkelia (Paris, a spinout from Sanofi-aventis' skeletal disease group) in 2006. In January (2010), Galapagos announced that a phase 1 trial with GLPG0187 had been completed in healthy volunteers and that the proposed disease indication would be expanded from bone metastasis to a broader range of metastatic cancers. The company says the product demonstrated biological activity as indicated by certain biomarkers in healthy subjects as well as a good safety profile. A new phase 1 trial will begin later this year in cancer patients. According to Galapagos senior vice president for drug discovery Graham Dixon, the company's data from murine models suggest that GLPG0187 may not only prevent tumor cells from gaining ingress into bone tissue but also prevent osteoclasts from attaching to bone matrices, where they would ordinarily begin osteolytic activity to set up a metastatic niche. Dixon says the drug has been engineered to bind to a range of integrins in the RGD family, which may account for other opportune properties, such as an antiangiogenic effect in primary and distal sites as well as apoptosis of osteoclasts.

As yet, the molecular components that mediate initial engraftment and by which tumor cells extravasate from the circulation remain an area of active investigation. What is clear is that of the millions of cancer cells that enter the circulation, only a few successfully engraft, and even fewer proceed to proliferate at secondary sites. In some cases, cancer cells divide within the occluded lumen of vessels until the tumor mass becomes so large it obliterates the vessel, pushing aside endothelial cells, pericytes and smooth muscle cells and invading the surrounding tissue.

Countering vessel growth

To proceed from a micrometastasis comprising a few tumor cells to a larger, clinically significant macrometastasis, the incipient tumor must recruit a blood supply. Macrophages and other bone marrow-derived cells recruited to the tumor by inflammatory mediators are thought to potentiate the angiogenic stimulus by expression of VEGF-A, fibroblast growth factor 2, platelet-derived growth factor (PDGF) and angiopoietins. These factors accelerate the recruitment of other immune cell effectors and proteases (e.g., MMPs and uPA) to facilitate ECM remodeling.

One means of interfering with this process is to downregulate pro-inflammatory signals, thereby blocking the influx of cells that ramp up the immune response. Inhibition of prostaglandin synthesis by inactivation of cyclooxygenases 1 and 2 (COX-1 and COX-2) by means of NSAIDs, such as aspirin and indomethacin, has been shown to slow the progression of colorectal cancer in animal models as well as upregulate expression of the metastasis repressor NM23 (see 'Colonization' below). Indeed, a phase 2 trial of Pfizer's Celebrex (celecoxib) plus interferon- α in 20 individuals with renal cell carcinoma showed two partial responses and a minor benefit for time to progression. It has also been shown that COX-2 expression in the stroma of intestinal tumors can induce VEGF production and angiogenesis through elevated prostaglandin E2 levels.

A wealth of evidence has now accrued for the pivotal role of VEGF-A (together with angiopoietins, PDGF and other factors) in pathological blood vessel growth. The prototypical VEGF inhibitor is Avastin, which is now approved for use in a host of cancers—colon, lung, metastatic breast, glioblastoma and renal cell carcinoma, mostly in conjunction with chemotherapy. As well as biologics, such as Regeneron's Aflibercept (a recombinant decoy receptor comprising portions of VEGFR-1 and VEGFR-2), tens of small-molecule VEGF RTK inhibitors, as well as pan-RTK inhibitors, are in clinical development or registered for marketing in advanced cancers. Many of these angiogenesis inhibitors are now being tested in combination trials with chemotherapies in patients with metastasizing tumors.

As it has been shown that resistance to Avastin often emerges as a result of pericyte recruitment by the tumor vasculature via PDGF receptor (PDGFR) signaling, several recently approved angiogenesis inhibitors also inhibit this pathway. For example, Bayer (Leverkusen, Germany) and Onyx (S. San Francisco, CA, USA) have launched Nexavar (sorafenib), an oral, small-molecule inhibitor of VEGFR, PDGFR and Raf for the treatment of advanced renal cell carcinoma and unresectable hepatocellular carcinoma. And Pfizer has launched Sutent (sunitinib; SU-11248), an oral small-molecule inhibitor of VEGFR2, PDGFR-beta, c-Kit, Flt3 and DDR1 (discoidin domain receptor tyrosine kinase 1) for the treatment of gastrointestinal stromal tumors after disease progression or intolerance to Novartis' Gleevec (imatinib mesylate) and of advanced renal cell carcinoma.

Programs that are in earlier stages of development include Amgen and Takeda's project to develop an inhibitor (AMG-386) of the proangiogenic factors angiopoietin-1 (Tie-2) and

angiopoietin-2. A related protein, angiopoietin-like 4, has also been shown to be important for mediating extravasation of DTCs into lung tissue. Currently in phase 2 testing in recurrent and metastatic breast cancer, ovarian, peritoneal or fallopian tube tumor and renal cell carcinoma, AMG-386 is a 'peptibody' Fc fragment linked to a peptide that the companies plan to take into a multicenter phase 3 trial in ovarian cancer this year. Elsewhere, Pfizer subsidiary CovX (San Diego) is developing a fusion protein comprising an angiopoietin-2 binding peptide with an antibody scaffold (which is used to confer favorable pharmacokinetics to the molecule). Last September, the company initiated a phase 1/2 trial in patients with renal cell carcinoma.

Yet another mode of attack—developing a drug that mimics the antiangiogenic molecule thrombospondin-1—is under development by Abbott (Deerfield, IL, USA). The company is testing a thrombospondin-1 mimetic peptide (ABT-510) that causes endothelial cells to apoptose through activation of Fas/Fas ligand (FasL) and the Src-related kinase p59 Fyn. The mimetic is currently in a phase 1 trial for solid tumors. Vascular biologist Randolph Watnick of Children's Hospital Boston has recently identified another factor, the highly conserved glycoprotein prosaposin, which upregulates thrombospondin-1. Secretion of prosaposin into the ECM initiates p53-dependent activity of thrombospondin-1. According to Watnick, injecting prosaposin into mice with highly metastatic human cell lines results in 80% fewer metastases to the lung and none at all to lymph nodes, with overall survival increased by 30% over controls. On the other hand, when prosaposin expression is inhibited in tumor cells, much greater metastasis formation is observed¹³. The fact that prosaposin activates expression of p53 gives Watnick confidence that there must certainly be other antimetastatic factors in the process beyond just inhibition of new vascular formation. "Thrombospondin-1 is one of the major players," he says, "but a lot of other things are going on and contributing to antimetastatic activity." Indeed, Watnick's work has established that oncogenes RAS (also known as HRAS) and c-MYC are suppressors of thrombospondin-1.

The future of prosaposin as an antimetastatic therapy is unclear, but the Children's Hospital Boston technology transfer team is actively looking to license the idea to commercial interests. Watnick envisions prosaposin or a similar agent being used for prophylaxis after tumor resections. "Now the next step is obviously to show whether or not it has any activity on existing metastases. We're going down those roads now," he says.

Colonization

Assuming that EMT is a key process in metastasis, it follows that DTCs on arrival at a distant organ must in turn shed their mesenchymal phenotype to establish macrometastases containing rapidly proliferating epithelial tumor cells—the mesenchymal-epithelial transition. It is possible that such sites lack the EMT-inducing factors present at the primary tumor; however, currently the molecular mechanisms and stromal factors involved in signaling the mesenchymal-epithelial transition remain an area of active investigation. One set of targets where there has been slow progress is the metastasis suppressor genes (not mentioned above). These are factors that have been shown to have activity in inhibiting metastatic colonization. Suppressor gene research has been notoriously difficult to translate to the clinic, partly because of a lack of understanding as to how these genes mediate their antimetastatic effects. Of several genes that have been identified to date, two have been the focus for drug development.

KiSS-1, expression of which is significantly downregulated in secondary tumors, is thought to mediate its activity by enhancing the activity of I- κ B, which inhibits the binding of NF- κ B to promoters of genes encoding pro-inflammatory and pro-metastatic factors. It encodes a 54 amino acid peptide, metastin, that acts as a ligand for the human placental orphan G protein-coupled receptor OT7T175. Researchers led by Tetsuya Ohtaki at Takeda Pharmaceuticals (Tokyo) reported 9 years ago that metastin attenuates pulmonary metastasis in a mouse xenograft model using a melanoma cell line, with no effect on the primary tumor, even after cancer at secondary sites has been established¹⁴. Importantly, although KiSS-1 expression becomes downregulated in certain cancers, OT7T175 continues to be expressed, suggesting that exogenous application of the peptide would have activity. Although Takeda was considering metastin as an antimetastatic drug and had commenced studies on sustained-release formulations of the peptide and screening of small-molecule compounds that mimic it, the company does not appear to have undertaken further development of the drug.

More recently, Patricia Steeg and her colleagues at the National Cancer Institute (Bethesda, MD, USA) have been pursuing translational approaches involving the histidine kinase Nm23-H1. The gene encoding Nm23-H1 was the first metastasis suppressor gene discovered and its expression in human tumors often is associated with poor patient survival. As well as binding many proteins, Nm23-H1 is thought to partly mediate its

Box 6 Cancer stem cell controversy

One particularly troublesome property of metastatic cancers is resistance to chemotherapy, which has been attributed to drug resistance mechanisms in DTCs (cancer stem cells). Along these lines, Markus Frank and George Murphy at the Children's Hospital and Brigham and Women's Hospital in Boston have characterized a subgroup of melanoma cells that express the P-glycoprotein family member ABCB5 (ATP-binding cassette, sub-family B (MDR/TAP), member 5), which is a chemoresistance mediator. First cloned in the Frank laboratory, ABCB5 operates, at least in part, as an Adriamycin efflux transporter, and based on that discovery Frank shifted his research into the area of tumor stem cell biology²⁵. His interest in ABCB5 was tweaked by its close association with CD166 or ALCAM (activated leukocyte cell adhesion molecule), a known biomarker correlated with progression of primary melanoma, which is also expressed in mesenchymal progenitor skin cells of humans and mice²⁶. It was with these underpinnings that Frank and his colleagues proposed that ABCB5 might be a marker identifying melanoma and perhaps other DTCs.

In a xenograft-transplanted mouse model of human melanoma, Frank and Murphy established that the relative intensity of ABCB5 expression was threefold greater in lymph node metastases, compared to thin primary melanomas. But in benign melanocytic nevi, ABCB5 showed close to zero expression. When they targeted ABCB5-positive cells with an anti-ABCB5 mAb, they found that tumor formation and growth were significantly inhibited, versus controls where tumor formation and growth occurred in 100% of the subjects. Although these presumed stem cells account for a distinct minority in tumor tissue, they appear to drive cancer progression. "What's really astonishing is that by targeting 10% of the cells in an established melanoma xenograft, the tumor growth is halted," says Frank. However, the group has demonstrated that not every ABCB5-positive cell is a melanoma initiator, but the result of their work in targeting the antigen suggests to them that it is much more than a biomarker. "I would say that ABCB5 is quite intriguing as a chemoresistance mediator which identifies the melanoma stem cells," says Frank, adding, "They could be the cells left behind after chemotherapy in patients."

The work has not been without controversy. In a packed hotel conference room during the 2009 International Melanoma Congress in November, organized by Massachusetts General Hospital chief

of dermatology David Fisher, a session was specifically dedicated to the issue of ABCB5-positive cells and their potential as cancer stem cells. On the program were Frank and University of Michigan (Ann Arbor) researcher Sean Morrison, who has conducted research with Frank's antibody and believes his data show that there is no significant difference between ABCB5-positive and ABCB5-negative cells in their ability to initiate tumor formation in mice. Moreover, Morrison finds that cells with tumor-initiating capabilities are rather common—as many as one in four cells taken from human primary and metastatic melanoma tumors compared to Frank's observation that as few as 1 in 1,090,000 human metastatic melanoma cells formed tumors within 8 weeks of transplantation into mice²⁷.

But specific technical differences exist between the work done by the two researchers. Frank used nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice, whereas Morrison used a much more severely immunocompromised mouse (NOD/SCID interleukin-2 receptor gamma chain null (Il2rg2/2)). Frank argues that, of course, it is easier to initiate tumors in mice with virtually no immune system. However, because cancer patients typically have some immune capabilities, it may not be valid to use a mouse that is so profoundly immunosuppressed. Morrison counters that the nature of the immunity in partially suppressed immune mice that are receiving a cross-species graft is completely different from tumor-specific immunity in a human patient who is recognizing and mounting a response to his own tumor. Furthermore, Frank takes issue with the Morrison method of injecting the human tumor cells mixed with a gel medium containing growth factors and nutrients (Matrigel) that could reinforce the tumor cells' vitality. Morrison responds that no matter what is done to the cells, if they do not have the genome of a tumor-initiating cell, they will not engraft and form a tumor mass.

Fisher, who is not working with ABCB5, says "I would say the pendulum was swinging much more in somewhat of a skeptical direction about the question of whether there is a stem cell at all and secondly, whether ABCB5 would be the marker of that stem cell if it exists." But Fisher has raised another issue that could have far-reaching ramifications in translational research. "I think that people are finding some reason to be concerned that a mouse with some intact immunity is going to be informing responses in patients better than a mouse with less intact immunity," he says. *GM*

the clinical problem of metastases. Thus, whereas breast patients are now living longer on Herceptin, many more of those patients are relapsing with drug-resistant brain metastases. To make matters worse, certain mechanisms of drug resistance might also render tumors more competent for metastasis. For example, lung adenocarcinomas resistant to EGF RTK inhibitors often acquire amplifications of c-MET, with prometastatic consequences.

The current vogue in oncology is to combine targeted agents with each other or with cytotoxic chemotherapies, such as Taxol (paclitaxel) and Adriamycin (doxorubicin), for the treatment of advanced and metastatic cancers. This approach is predicated on accumulating evidence that redundancy in cancer signaling

pathways (e.g., between EGF receptor and KRAS or Notch and Akt) often enables cancer cells to circumvent inhibition and become resistant to single, targeted agents. And yet, all too often, even combinations of drugs are proving unsuccessful or merely capable of extending survival by a matter of months.

One part of the problem is the inherent drug resistance of many advanced cancers. Many tumor cells that have undergone EMT to acquire progenitor-like qualities very often become resistant to targeted therapies that inhibit mitogenic activity or evolve such mechanisms as chemoresistance regulators (Box 6). And as anyone with cancer likely has a large number of DTCs, only a small number of which are required to regenerate a tumor,

to be effective any drug must eliminate nearly all these cells to avoid relapse. Thus, even if DTCs are as susceptible to therapy as epithelial tumor cells in the primary tumor, the survival of just a few cells might still lead to relapse.

A second problem is that cells in secondary cancers are a moving target (and a different moving target from cells in the primary tumor epithelium). Genomic instability in DTCs, together with the selective pressure mounted by a different set of signals in the surrounding stroma of a secondary organ, results in tumor cells that accumulate genetic lesions that diverge from those present in tumor cells at the primary site. According to Paul Workman of the UK's Institute of Cancer Research, "the full benefits of the [combination] approach

will only come to fruition when we can really apply genetic stratification and pathway-activation profiling,” together with a comprehensive systems biology perspective. To account for cancer dissemination, targeted treatments will have to take into account not only the primary tumor cell profiling but also the profiling of DTCs in remote sites. This is an area that is still in its infancy, and compared with primary tumors, much more effort is required to collect and characterize material from clinical biopsies of metastatic lesions—not a straightforward task in itself as such lesions can be hard to locate and access in the body.

If researchers and clinicians ultimately hope to treat or prevent metastasis, several issues will have to be addressed. One significant dilemma in the development of metastasis inhibitors is that investigators are currently handicapped from the very beginning because safety-conscious regulators have by and large required potential cancer drugs, especially new chemical entities, to be tested in patients only during late-stage disease as a last resort after several therapies have failed and where safety is not the all-consuming issue anymore. “That’s really a difficult problem to tackle,” says Reuven Reich. “The moment you have metastases, a metastasis inhibitor would not help at all. You would have to do clinical trials at the very early stages, not at the end.”

Another issue is the continuing emphasis in clinical trial design of complete and partial response (of the primary tumor) to cancer drugs as the main endpoints for human testing. As evident from clinical experience, the relationship between degree of shrinkage of a primary tumor and permanence of effect and cancer patient survival is weak at best. Clearly, the endpoints of greater relevance for metastasis treatments are progression-free survival (time from patient randomization until objective tumor progression or death) and time to progression (time from when patient was randomized to tumor progression).

A key question in designing treatments is where in the metastatic process—EMT, migration from tumor, intravasation, dissemination through the circulation, embolism and adhesion, extravasation into a remote organ as a micrometastasis or colonization as a macrometastasis—are the most effective points for therapeutic intervention. The fact that cancers in most patients have already metastasized at the time of initial diagnosis suggests that the

early stages of the process might not be effectively treated (unless biomarkers can be identified that are accessible to diagnosis; see Box 3). And as many DTCs appear to disseminate but never blossom into a full-blown macrometastasis, agents that target colonization at a distal site might represent a good starting point for drug development. In addition, therapies that interfere with the local autocrine and paracrine signaling mechanisms in organs that keep dormant micrometastases alive could also prove useful.

Because of the nature of DTCs and the ability of a very few cells to go on and seed a metastasis, administration would probably have to be carried out over a lifetime after initial cancer diagnosis, much in the same way as those at risk for cardiovascular disease take statins and antihypertensive drugs. The necessity of early drug use and potential lifetime use combine to make long-term drug safety trials in some ways pivotal for potential metastasis inhibitors. “Unfortunately, that’s the kind of clinical trial companies will never do,” says Northwestern University entrepreneur-in-residence Mazar, who spent more than 7 years at Abbott Laboratories, where he was involved in the preclinical development of two biologic compounds that would ultimately complete phase 3 trials. “It would be a long trial. Phase 3 could be many years, and without having some indication that this thing’s really going to work, they would be reluctant to go into it,” he says.

On the other hand, one might reasonably think that if metastatic cancer is lethal, then industry would be attracted to the field because first-to-market players are typically the big winners. One might also think that like statins, everyday use of antimetastatic agents would amount to huge annuities for pharma. “Pharma has not been as interested in the process of dissemination as they have the primary tumor field,” says cancer biologist Bruce Zetter of Children’s Hospital Boston, who has been founder of two cancer-related companies (Box 3). “Half of cancer patients already have metastases in place at the time of diagnosis, which makes the solution to the problem very difficult,” he says. “And the other half is cured by surgery. So you don’t have to treat them.”

For all these reasons, it seems that the agents against metastatic cancer most likely to be developed by industry are those with at least some efficacy in treating epithelial tumor cells

in the primary cancer. There is no doubt that cancer biologists are unearthing targets that do have overlapping roles in tumor initiation and/or proliferation as well as metastasis. And there is also little doubt that the study of metastasis as a process is becoming more manageable. “If you had asked me that 10 or 20 years ago, I would have said it’s impossibly complex and well beyond our capabilities to attack and to understand the process in a conceptually simple fashion,” says Weinberg of MIT. “But it’s not complex beyond measure anymore. One can begin to understand it in terms of a finite number of signaling pathways. It’s something that’s within reach of current experimental techniques, and I think that within the next 5 to 10 years, we should have a very clear and detailed understanding of how metastasis occurs.”

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