

When Breast Cancer Spreads

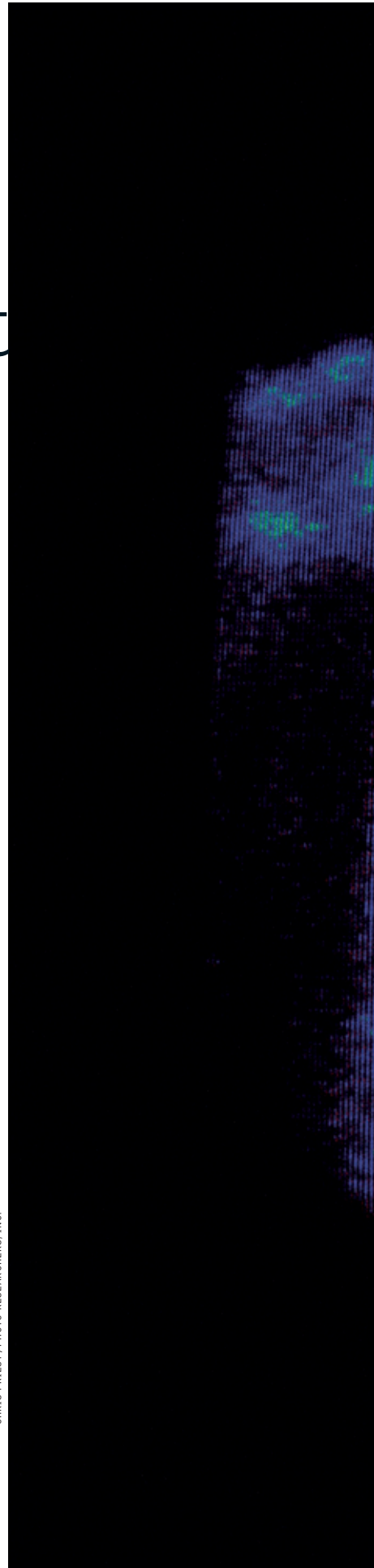
Investigating how cancer reaches from breast to bone, researchers find insights on the mysteries of metastasis.

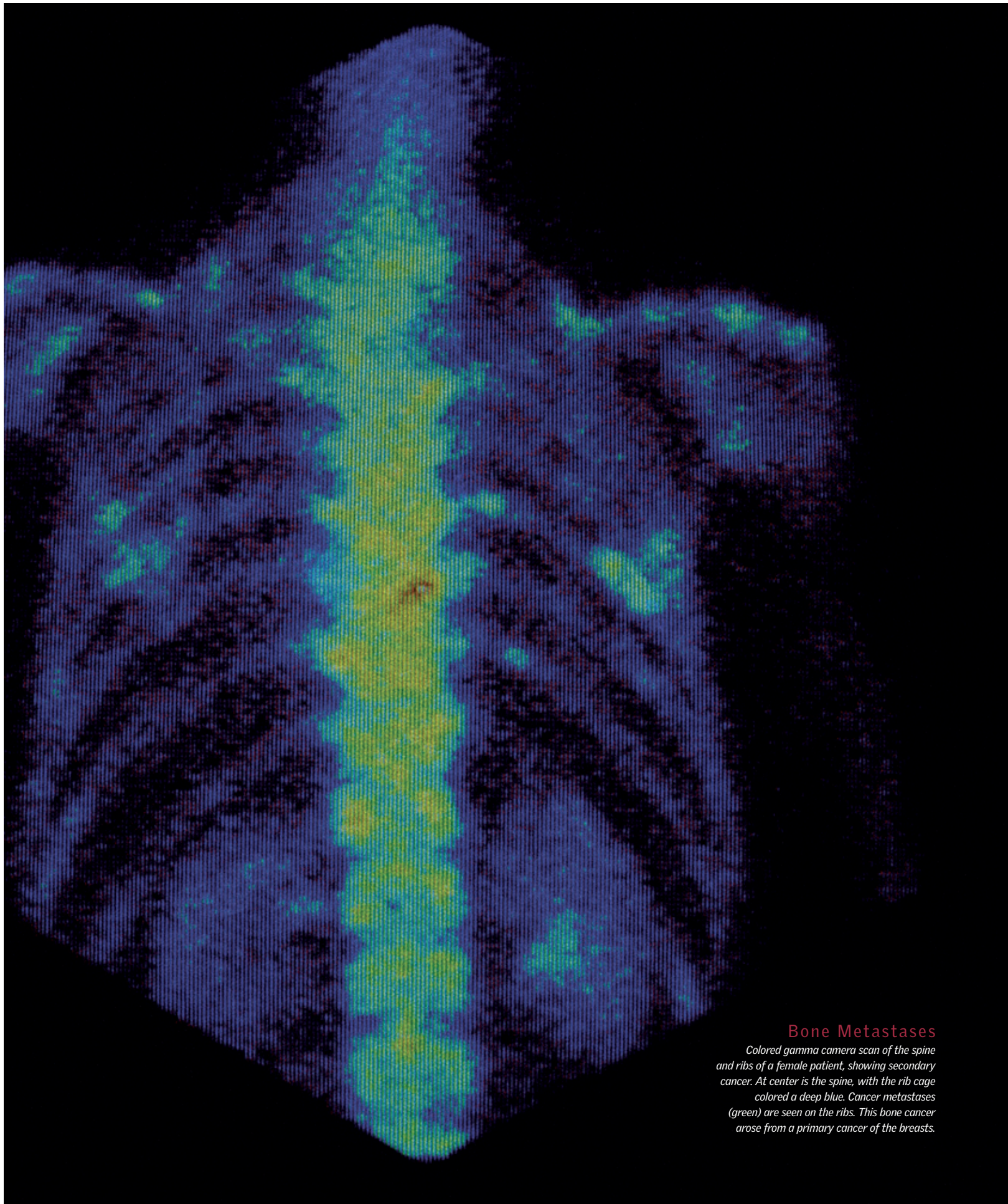
By MARGARET A. WOODBURY

In many settings—in business, for example, or in a family or on a farm—the word “growth” usually has positive connotations. But coming from a doctor’s lips, that single word provokes nothing in a patient but fear. Nikki Harrison heard it just days after her 36th birthday, as did Rhonda Olenick when she was 55, and Joan Antonucci at 59. These three women are battling an insidious form of growth that has caused cells from primary tumors in their breasts to invade and grow in other organs and tissues in their bodies.

Many primary cancers can be successfully treated by surgery or radiation as long as they haven’t spread to other parts of the body. But once cancer cells break free from the primary tumor and form secondary tumors at new locations—that is, when metastasis occurs—the prognosis is poor. Some 90 percent of cancer deaths are caused by metastatic growths—in other words, most cancer victims die from secondary tumors. “Once a breast cancer patient has metastatic disease, we do not know how to eradicate that disease. Treatment then is palliative,” says Catherine H. Van Poznak, a medical oncologist at Memorial Sloan-Kettering Cancer Center in New York City.

Given breast cancer’s toll—more than 40,000 deaths a year in the United States alone—researchers are feverishly working to understand the mechanisms that provoke local cancer cells to spread. A new study reported in the June 2003 issue of *Cancer Cell*, led by HHMI investigator Joan Massagué at Sloan-Kettering, is the first one to identify specific genes that drive cells from a solid primary tumor to a specific location elsewhere in the body—in this case, the bone, the most common site of secondary lesions (bone metastases) associated with breast cancer. And because these genes differ from those responsible for the primary breast tumor, the study helps to illuminate the general process of





Bone Metastases

Colored gamma camera scan of the spine and ribs of a female patient, showing secondary cancer. At center is the spine, with the rib cage colored a deep blue. Cancer metastases (green) are seen on the ribs. This bone cancer arose from a primary cancer of the breasts.

how cancer spreads, as opposed to how it first forms. This research, no small feat in itself, may also serve as a bridge between differing schools of thought on what triggers metastasis in the body.

Low Society

Massagué refers to certain cancer cells as ones that form a “violent society” that spawns a variety of criminals, or “rotten eggs.” To draw out that analogy, the cells of a primary tumor could be compared to a local thug who commits petty crimes only in his neighborhood, just as cells in a primary tumor affect only the tissue in its vicinity. But Massagué’s “violent” offenders are more in the fashion of a Bonnie and Clyde–type criminal who takes a crime rampage on the road. Once such cancer cells move beyond the primary tumor, researchers know that metastasis has occurred, but no one is certain exactly how this happens.

For some time, researchers have thought that a few rotten-egg types of cells have undergone mutations over time that render them more and more capable of metastasizing. Through such cumulative changes in their genomes, these cells are thought to be better able to spread violence, so to speak, than the majority of the primary tumor’s other cells.

But a new hypothesis has emerged that suggests that most cells that make up the primary tumor already possess a set of active genes predictive of whether or not the tumor will metastasize. Supporting this theory are studies that found sets of genes in the primary tumor that express a “poor-prognosis” signature—or unique gene-expression pattern—that correlates very well with how patients will eventually fare—that is, whether or not they will develop metastatic tumors in other areas of their bodies.

The crux of the debate, says Richard O. Hynes, an HHMI investigator at the Massachusetts Institute of Technology who wrote a review of the Massagué paper in the June 27, 2003, issue of *Cell*, is “whether any cell in a tumor can make a metastatic lesion, or if there are certain highly metastatic cells in a tumor that give a higher probability of making metastatic tumors.” Massagué’s work, says Hynes, “is a synthesis of both theories.”

Two Gene Signatures

Cancer researchers agree that primary tumors are a constellation of cells that differ from one another. In an attempt to determine which cells in the constellation might be better at metastasizing, Massagué and his colleagues started their experiment with a heterogeneous mixture of metastatic tumor cells taken from a woman who died of breast cancer at the age of 51. They injected this cell line into immunodeficient mice to see whether bone tumors would grow. Indeed they did. Furthermore, after removing cells from the mouse bone tumors and reinjecting them into other mice, the researchers discovered that bone tumors now developed in just half the time. “The [first] mouse had served as a cell sorter, in effect, selecting those cells from the original cell population that are better at making bone metastases,” says Massagué.

Armed with this subpopulation of tumor cells that are so well equipped to go to bone, the next obvious step, says Massagué, was to determine whether they had the same signature as that of the poor-prognosis signature other researchers had previously found in breast cancer primary tumors.

The answer was yes. But Massagué and his colleagues also found a separate and nonoverlapping set of 102 genes that spelled what they call

a metastatic signature. This meant that “the subpopulations of cells must still go and get something else to make them highly metastatic to bone,” says Massagué, meaning a population of cells must acquire both the poor-prognosis genetic signature as well as the metastatic genetic signature to move breast cancer to bone.

Patricia S. Steeg, chief of the Women’s Cancers Section of the Center for Cancer Research at the National Cancer Institute (NCI), believes that the reasons behind metastasis could be as varied as the nature of breast cancer itself, with the process being driven differently in different women, possibly as a layered system needing various components for a distant tumor to take hold and grow.

One simple version of a layered system might require a primary breast tumor to be endowed with the poor-prognosis genes before those cells can go on to acquire the additional genetic mutations found in the

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rarer metastatic signature. Massagué says proof of this is needed, but he suspects it is true.

“Having the poor-prognosis genes most likely allows cells to survive conditions that they normally would not, such as with unstable genomes caused by mutations or poorly oxygenated conditions,” he suggests. In essence, this asset allows cancer cells to handle the abuse heaped on them so they can live on to gain the second set of mutations that later drive the cells to metastasize to a specific site.

Genes Go Gangbusters

To show that their metastatic genes really did operate in high gear to send breast cancer to bone, Massagué and his colleagues took four of the most overexpressed genes in their metastatic signature and placed them back into the original cell population, which had a lesser ability to form bone metastases in mice. They did this singly and in combination. “When they put only one gene back in, little effect was seen, but when combinations of these genes were put in, they went like gangbusters!” says Danny R. Welch, professor of pathology at the University of Alabama at Birmingham, whose review of Massagué’s study was published October 30, 2003, in the journal *Breast Cancer Research*.

All this shows that “there is no such thing as ‘the’ metastatic gene,” says Massagué. Genes must work together in a 1+1- or 1+2-type fashion if they are to make a breast cancer cell able to travel to bone and grow there. And, in fact, when Massagué looked at the known functions of the most overexpressed genes in the metastatic signature, he found that most have a specific “talent” that makes them friendly to the process of forming new tumors in bone.

Two of the genes (*CTGF* and *FGF5*) are angiogenic, or capable of assisting with new blood vessel formation to supply a tumor with nutrients; another (*CXCR4*) is effective at homing cells to bone marrow; *IL11* induces cells called osteoclasts to do their work of dissolving bone; and *MMP1* digests bone collagen, assisting in invasion.

Welch says this all fits nicely with 19th-century physician-scientist Steven Paget’s seminal “seed and soil” hypothesis, which posits that

tumor cells (the seeds) must be favorably disposed to the tissue (the soil) they are to take root in if overt metastases are to form. Massagué's finding also provides evidence that the genes responsible for forming bone metastases are distinct from those that will make secondary tumors in the adrenal medulla (glands that sit atop each kidney). Different seeds, in other words, for different soil.

In his review in *Breast Cancer Research*, Welch writes that the work by Massagué and colleagues not only supports Paget's theory but "supports assertions that there are genes that specifically contribute to metastasis." This is in stark contrast to an opinion paper (*Nature*, August 22, 2002) by prominent cancer researchers René Bernards at the Netherlands Cancer Institute and Robert A. Weinberg at the Whitehead Institute for Biomedical Research in which they say that "genes and genetic changes specifically and exclusively involved in orchestrating the process of metastasis do not exist."

Their argument, in part, is that a progression model in which primary-tumor cells acquire genetic changes that make certain cells better able to grow at other sites would not serve the purpose of the primary tumor (which is to get bigger itself). In essence, endowing certain cells with a special ability to escape the original mass would seem to go against a Darwinian process that selects what is best for the tumor. Instead, Bernards and Weinberg argue that the tendency to metastasize is determined by mutations occurring early in the formation of the primary tumor, and colonization becomes manifest only when a critical mass of mutations in tumor cells is reached.

Room for Different Models

Another important consideration, which may help to reconcile competing theories, is the unique genetic makeup of the person in whom

the cancer develops, says NCI researcher Kent W. Hunter. His experiments in mice showed that when the very same cancer-inducing mechanism was used in different breeds of mice, some got metastatic disease and others did not.

A recent paper in the *Proceedings of the National Academy of Sciences* (PNAS, June 24, 2003) by Oleg Schmidt-Kittler and colleagues at several German institutions puts forth yet another theory—and one that may explain why some cancers can remain so small as to be undetected at their site of origin but loom large at distant metastatic sites. The researchers isolated single cancer cells that had disseminated to the bone marrow of women with breast cancer. They used comparative genomic hybridization—a technique that compares the genetic aberrations of one cell to another—and found that the cells in the bone marrow had fewer chromosomal aberrations, or mutations, than those in the primary tumor. They postulated that the cells had left the tumor before gaining the mutations necessary to form metastatic lesions and that only over lengthy periods would these cells truly become metastatic—able to form overt tumors.

"There is room for different models, and this one would explain long latencies," says Peter M. Siegel, a colleague of Massagué's and a coauthor on the *Cancer Cell* study, "but they haven't proven that the cells they looked at are actually predecessors of malignant disease."

In a finding published in *Science* in November 2003, HHMI investigator Tian Xu and colleague Raymond A. Pagliarini at Yale University School of Medicine reported a new method for using the fruit fly *Drosophila* as a model to understand the genes that drive the spread of cancer. Their screening test is already proving beneficial in identifying

Peering inside breast cancer's toolbox, Joan Massagué identified a set of rogue genes that accelerate the spread of cancer to bone marrow.



novel combinations of genetic malfunctions that contribute to metastatic cancer.

Also on the metastatic radar screen is work by Muhammad Al-Hajj and colleagues at the University of Michigan Medical School (*PNAS*, April 1, 2003) that found a rare subset of cells in human breast cancers that are tumorigenic—capable of forming new growths. Sean J. Morrison, an HHMI investigator at Michigan, was a coauthor of the paper. These cells have a “stem cell-like” quality, say the authors, which allows for continued self-renewal, perpetually deferring the differentiation that normally slows cell replication.

“The Massagué paper works out, mechanistically, how tumors spread, while our paper says this subset of [stem-cell-like] cells are the cells that, when they metastasize, are capable of forming tumors that keep growing and killing people,” says Michael F. Clarke, senior author of the Michigan paper.

Search for Treatments

Clarke cautions that his team’s work is preliminary but could be very useful in finding new therapies; he believes that mutations are responsible for endowing the subset of cells with their stem cell-like self-renewing quality, and that these cells would make excellent targets for new anti-cancer treatments.

For his part, Massagué finds the stem cell work “beautiful,” and says it’s even possible that the rare “rotten-egg” cells in the classical model of metastasis may be these very stem cell-like cells. “If you find the strategies these cells have developed to maintain their ‘stemness’ and target that process so they will be forced to differentiate,” he suggests, “you might deprive the tumor of the ability to go on.”

Certainly, caution is the buzzword when talking about treating

metastatic breast cancer. Just ask Nikki Harrison, who is on a host of drugs, some of which sent her into menopause at 36 and others that robbed her of body hair right up to her eyebrows and lashes.

Still, researchers toil with the ultimate expectation of better breast cancer treatments, or even a much-hoped-for cure, for Nikki, Rhonda, Joan, and thousands like them. “There are things in our study that are not good news for patients,” says Massagué. For instance, “the toolbox

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of genes will be different from patient to patient, and that complicates matters. But there is also good news: We are no longer in a state of vague proposal. We have specific genes whose products we can go look for in their serum.”

That is what excites medical oncologist Van Poznak as well. “If we find the products of these genes in a patient’s blood or even urine,” she says, “the potential is there to identify those who are at increased risk and the site in their bodies where relapse is likely to occur.” In that way, overtreatment of breast cancer patients at low risk for recurrence, and the corresponding toxicities, could be avoided. The identification of biomarkers that can accurately predict patients at risk for site-specific metastases will lead to improvements in targeted therapeutic interventions.

Researchers hope to find one or two gene-product targets and block them from their deadly metastatic cascade. One possible example is the cytokine TGFβ, which drew Massagué into breast cancer research in the

first place. Bone is chock-full of TGFβ, which activates two of the genes in the metastatic signature (*IL11* and *CTGF*). Massagué says that efforts are already under way to find ways to block this ubiquitous cytokine.

Another approach could be to target the metastatic gene *CXCR4*, says Massagué, because blockers for it are already under development, owing to its role as a portal of entry for the HIV virus.

Even so, roadblocks abound, and “one dumb tumor is worth 10 smart oncologists,” says George W. Sledge, professor of medicine, pathology and oncology at the Indiana University School of Medicine. “Metastatic tumors learn from their interactions with us [oncologists], and they change over time, becoming resistant to therapies.” Yet, he is determined not to give up on his patients. “Everyone has fallen in love,” says Sledge, “with the chronic-disease paradigm of metastatic cancer” in which patients manage to live with cancer in lieu of defeating it altogether. “But I want to *kill* the cells!”

The goal, of course, is to stanch breast cancer’s insidious advance—whether it is via one approach or a dozen—and in so doing, to remove some of the negative connotations from that deceptively simple word, growth. **11**

