Short-circuiting metastases

By Michael J. Haas, Senior Writer

A U.S.–Dutch research team has shown that Src inhibitors in development to treat primary cancers could also prevent metastatic bone tumors after surgical removal of a breast tumor.¹ The question is whether Src inhibitors will prove safe in the setting of long-term prophylaxis.

Cancer cells can migrate from a primary tumor to distant sites and remain latent for many years before developing into metastatic tumors. The problem is that it's impossible to tell whether tumor cells have disseminated until a metastatic tumor arises. In addition, such tumors are invasive and aggressive. Indeed, more than 90% of cancer patients die from metastatic, not primary, tumors.

Breast cancer metastasizes most frequently to the bone, but the mechanisms by which disseminated cells survive in a latent state and later develop into metastatic tumors have been poorly understood.

To elucidate the mechanisms of breast cancer metastasis, the research group performed genomic analyses on 615 primary breast tumors and found that activity of the tyrosine kinase Src in the tumors correlated with the development of bone metastases.

Mice injected with Src-expressing breast cancer cells had a higher incidence of bone metastases and shorter survival than controls injected with Src-knockout cancer cells or mice that were injected with Src-expressing cells and then treated with the Src inhibitor dasatinib. The compound is marketed as Sprycel by **Bristol-Myers Squibb Co.**

Microarray analyses on bone metastases from breast cancer patients revealed two molecules that contributed to the survival of Src-expressing cancer cells: tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and chemokine C-X-C motif ligand 12 (CXCL12; SDF-1). Both targets are highly expressed in bone marrow compared with their abundance in other tissues.

Additional studies in Src-expressing and Src-knockout breast cancer cells suggested the kinase is required for “cell survival in response to CXCL12, and for resistance to the pro-apoptotic effect of TRAIL,” the team wrote in their report in *Cancer Cell*¹ (see **Figure 1, “Src: survival in the bones”**).

Xiang Zhang, lead author on the study and a research fellow at **Memorial Sloan-Kettering Cancer Center**, told *SciBX* that all three molecules—Src, TRAIL and CXCL12—are potential targets to treat bone metastases of breast cancer.

“These findings scream for the development of a selective Src inhibitor.”

—Irwin Gelman,
Roswell Park Cancer Institute

“This study should encourage clinicians to study the available Src inhibitors in breast cancer patients after the primary tumor has been removed, to see whether the therapy can prevent” bone metastases, he said.

The team was led by Joan Massagué, chairman of cancer biology and genetics at Memorial Sloan-Kettering and an investigator at the **Howard Hughes Medical Institute**. The team included scientists from **Erasmus University Medical Center**.

Primary sources

Irwin Gelman, interim chairman of cancer genetics at **Roswell Park Cancer Institute**, said Src has not received as much attention as other cancer targets because research has focused mainly on primary tumors. Although Src is activated in many primary tumors, it does not seem critical to their survival, he said.

“The Massagué team’s study is very interesting because it draws attention to the specific role Src plays in cancer metastases and should bring researchers back to Src as an attractive target,” especially since there are not many selective Src inhibitors available, he said. “These findings scream for the development of a selective Src inhibitor.”

Gelman did note that Src inhibitors have had toxicity issues related to off-target effects—a hazard that arises when targeting the highly conserved ATP-binding site of a tyrosine kinase.

Some companies have been working around this problem by targeting interactions between kinases and their respective protein substrates. But that strategy is not without its own challenges, in part because the generally broad and shallow substrate binding sites can make them difficult to target with small molecule inhibitors.

Another strategy involves allosteric binding².

Gelman also noted that the Massagué team’s study showed that two other Src family kinases—FYN (FYN oncogene related to SRC FGR YES) and YES1 (v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1)—are not involved in bone metastases. This indicates that the development of bone metastases is highly specific to Src and thus should be targetable with selective Src inhibition, he said.

Bristol-Myers Squibb’s Sprycel dasatinib is the one Src inhibitor on the market. The compound, which also inhibits Bcr-Abl tyrosine kinases, is approved to treat acute lymphoblastic leukemia (ALL) and chronic myelogenous leukemia (CML). Sprycel also is in Phase II and Phase III testing to treat castration-sensitive and castration-resistant prostate cancer, respectively.

The two disclosed Src inhibitors in the clinic are AZD0530 from **AstraZeneca plc** and KX2-391 from **Kinex Pharmaceuticals LLC**. The former is in Phase II testing to treat breast and prostate cancer with metastatic bone disease, advanced ovarian cancer, and recurrent osteosarcoma localized to the lung. The latter is in Phase I testing to treat refractory solid tumors.

Figure 1. Src: survival in the bones. Researchers found that the survival of breast cancer cells in bone marrow is mediated by Src.

[a] In particular, they showed that Src is required for the prosurvival effect of chemokine C-X-C motif ligand 12 (CXCL12; SDF-1), a factor that is expressed in the bone metastasis microenvironment. CXCL12, which binds to CXC chemokine receptor 4 (CXCR4; NPY3R), is known to lead to the activation of protein kinase B (PKB, Akt), promoting cancer cell survival. Inhibiting Src should disable this prosurvival mechanism.

[b] Src was also found to be required for resistance of breast cancer cells to the proapoptotic effects of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL). TRAIL mediates cell death signals through so-called death receptors like TNF receptor superfamily member 10a (DR4; TNFRSF10A; TRAILR1; CD261) and TNF receptor superfamily member 10b (DR5; TNFRSF10B; TRAILR2; CD262). Inhibiting Src should make cancer cells more sensitive to TRAIL-induced cell death.

Selected compounds on the market or in clinical development to treat cancer that target proteins along this pathway are displayed in the figure.

The three companies declined to comment on the new findings.

Getting testy

While the paper in *Cancer Cell* taps Src as a good target for treating bone metastases in breast cancer patients, the findings also suggest that monitoring Src activity would be good way to predict a primary tumor's metastatic potential and thus help guide treatment following surgery.

"Right out of the box, the ability to test primary tumors for a predictor of bone metastasis is a major advantage," said Gelman. "If you take out the primary tumor and find the Src activation signature, then you would know that the patient is statistically more likely to get bone metastases."

He said Src-based prediction of the risk of bone metastases would have at least two applications. First, it would allow the postsurgical treatment regimen to be tailored to the individual patient, depending on whether the cancer was Src-active or not, he said.

Second, Gelman said, "you could use the Src-activity signature to enroll patients in a Phase II trial" of a Src inhibitor to prevent bone metastases, he said. This would overcome a major obstacle in con-

ducting a metastasis trial by ensuring that the trial population has a high risk of developing metastases, he said. Without some method for detecting and enrolling at-risk patients, it is hard to show a treatment effect on metastatic disease.³

Kristiina Vuori, EVP for scientific affairs and director of the NCI-designated Cancer Center at the **Burnham Institute for Medical Research**, had a different take. "I don't think Src activity is that good of a predictor" of bone metastases, she said.

Of the 615 primary breast tumors examined by the Massagué team, 384 showed Src activity. But only about 40% (155) of the latter group involved bone metastases. Vuori noted it is not clear why the other 60% of Src-activated cancers did not progress to metastasis.

"The results suggest that something else is needed—some other factor is involved," she said. The identification of additional factors, in combination with Src, could lead to a more predictive test, Vuori told *SciBX*.

Vuori also would like to see safety studies of long-term Src inhibition and research on other factors that are predictive of bone metastasis.

Indeed, another study by Massagué and researchers from Memorial Sloan-Kettering, **Princeton University** and the **Cancer Institute of New Jersey** showed a correlation between the expression of two metalloproteinases—ADAM metalloproteinase with thrombospondin type 1 motif 1 (ADAMTS1) and matrix metalloproteinase 1 (MMP1)—in primary breast tumors and bone metastases.⁴ That team also showed that serum levels of MMP1 were correlated with metastases, and that MMP1 and ADAMTS1 are potential targets to treat overt bone metastases that have developed from latent, disseminated breast cancer cells.

Yibin Kang, assistant professor of molecular biology at Princeton and leader of the study, said it was too early to know whether Src plus MMP1 and/or ADAMTS1 would predict bone metastases better than any single marker. But “since these markers are linked to two different processes for the late-onset recurrence of bone metastases, it will be of interest to test this hypothesis,” he said.

Gelman thinks the specificity of Src’s role in bone metastases outweighs potential safety issues associated with long-term Src inhibition.

“In normal human cells Src is involved only in very transient events,” and Src-deficient mice are fairly normal, except for mild increases in bone density, he said. “So a selective Src inhibitor shouldn’t be very toxic,” even over a long period of time.

In addition to follow-on therapy after breast cancer surgery, selective Src inhibition also could be useful in earlier stages of breast cancer treatment because the dissemination of primary tumor cells isn’t necessarily a late-stage event in cancer, noted Gelman.

“There is a lot of experimental evidence to support the idea that distal seeding occurs early in the disease process—at least in some can-

cers,” he said. Incorporating a Src inhibitor into the treatment regimen for the primary breast tumor would target any disseminated cells well before they have the chance to develop into overt metastases, he said.

“The good news is that metastatic breast cancer can be treated with a good Src inhibitor,” Gelman said. “The bad news is that there aren’t many selective Src inhibitors in clinical trials.”

Zhang said the team was not disclosing the IP status of the findings reported in *Cancer Cell* or details about their ongoing work.

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COMPANIES AND INSTITUTIONS MENTIONED

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