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NOW, SMALL IS BIG | BY ROBIN MEJIA

# WHEN IT COMES TO CANCER, WHAT'S RATIONAL?

**N**ot too long ago, doctors could basically describe a cancer by what they could see, albeit with the help of a microscope. In fact, cancers are named by the tissue in which they occur, not by the mechanism that makes cells proliferate out of control. Advances, especially in DNA technologies, have let researchers start to describe cancers at a molecular level. What they are finding is that existing classification schemes are inadequate. Tumors that appear identical by traditional measures may very well be caused by different molecular problems. The conventional classification system—identifying cancers by tissues—is sort of like describing car problems by make or model. If you were to tell your mechanic, “I’ve got a Toyota Camry that won’t start,” that’s helpful; Camrys are different from Chevy Suburbans. But your mechanic needs more information to get you back on the road.

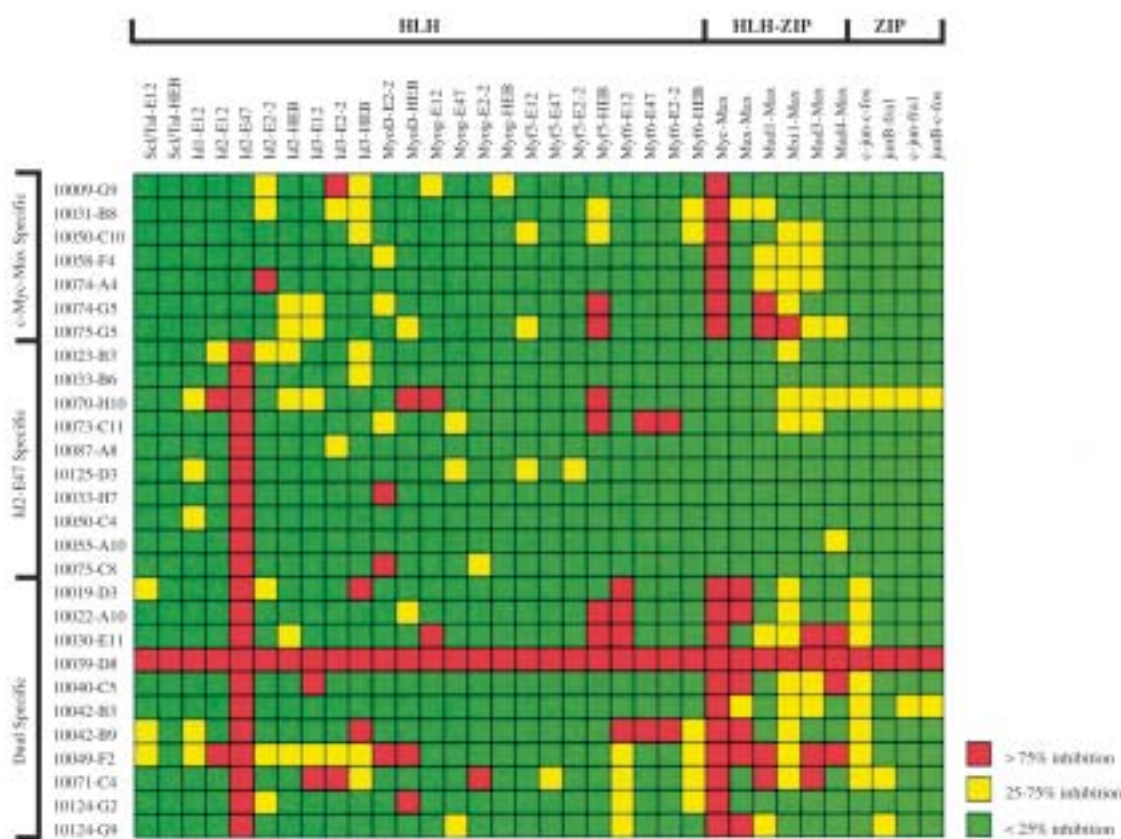
The smartest approach may be to, in essence, look under the hood at cancer. A better understanding of the molecular problems that cause cancer promises to explain why some tumors respond well to traditional therapy, yet other similar-looking cancers, treated with the same drugs, still prove fatal. Molecular information is helping researchers design drugs to target specific molecular actions. The research community has become so taken with this approach, they refer to the process as “rational drug design.”

The cancer drug Gleevec is the poster child for this kind of research. By targeting a specific protein mutation seen in patients with chronic myelogenous leukemia, Gleevec has offered rapid relief to patients. The drug kills tumors but doesn't go on a rampage, destroying lots of noncancerous cells, so there are few side effects. Its success has been remarkable and has spurred more research into targeted cancer treatments.

Last March, Peter Wipf, a professor in the Department of Chemistry at the University of Pittsburgh, contacted Edward Prochownik, the Paul C. Gaffney Professor at Children's Hospital and a professor of molecular genetics and biochemistry as well as pediatrics for the School of Medicine. Wipf asked Prochownik, an MD/PhD molecular biologist, to present his most current cancer research to a group of University chemists. In itself, the invitation might not seem like a big deal. After all, "interdisciplinary" has become a buzzword. But this meeting was the kind that reminds us why there's buzz in the first place: The collaboration it led to could have big payoffs. Wipf heads a program to build a University of Pittsburgh chemical library for drug development. It's one of only four such university programs in the country and funded by a \$9.6 million grant from the National Institutes of Health. Prochownik is pursuing new therapies to fight many common cancers. He is part of a class of cancer researchers poised to capitalize on the wealth of newly available information offered by genetics that may lead to rational designs. Both men are exploring the possibility of creating cancer-fighting compounds that are made from small molecules, like Gleevec is.

To backtrack: Gleevec can best be viewed as a harbinger of change. It zeroes in on a single mutation. Not all cancers are so easily targeted; so researchers study the genes and proteins involved in different cancers in order to determine the best points of attack. Prochownik has his sights set on a protein called "myc." Myc is involved in many cancers, including some common lymphomas and colon, breast, and prostate cancers. One scientist noted the ubiquity of the protein's role in cancers in the title of a recent *Cancer Cell* paper: "Myc—Is this the oncogene from Hell?"

Myc regulates more than 600 genes. And,



**The top of the chart lists protein pairs that normally interact; the red squares indicate a disrupted interaction. The compounds Edward Prochownik and colleagues are studying stopped the interaction of myc and max (which is key to many cancers) but did not affect many other proteins. That's a good thing when it comes to drug development.**

like so many genes that can promote cancer, many of these myc-targeted genes are essential to normal cell functions. In fact, so many of these genes are key to development that researchers cannot make a myc knockout mouse—that is, a mouse with the myc gene "turned off." When they try, the embryo dies. In adults, cells turn on myc production during differentiation and proliferation. These, of course, are the very activities that, in overdrive, can cause normal cells to go cancerous.

For a while, Prochownik had focused on strategies that could affect myc's target genes. Eventually, he realized that he needed to take a step back. Not only does myc regulate hundreds of genes, many of those genes seem to code for redundant functions. Even if he could inhibit one or a few of the targets he knew promoted tumor growth, other genes regulated by myc would still keep pumping out proteins. He started pondering how scientists could inhibit myc itself.

It would have been easier, or at least a more straightforward problem, if one single failure sent myc into overdrive. But that's not the case. Some breast cancer patients have 10 copies of the gene for myc. In other cancers, the gene gets transposed onto a section of the

chromosome that's heavily expressed. Other times, a protein that normally keeps myc production under control fails.

It became difficult to imagine a single drug that could manipulate myc under all of these circumstances. So Prochownik sought out another point of attack.

"When you look at a picture long enough, you start to see things," he says.

In order for myc to act on target genes, it interacts with another protein, one that scientists call "max." Without max, myc simply can't do its jobs.

"It became apparent that this interaction of myc and max might be its Achilles' heel," Prochownik says.

At first, Prochownik wanted to study potential drugs in mammalian cells. These cells are a complex environment. Many compounds that seem to work elsewhere fail in mammals. Any number of things can go wrong in the mammalian cell—drugs can't get across the cell membrane, they aren't soluble in the cell environment, they break down too quickly to have much effect. So Prochownik put together a team to develop a mammalian cell-based screening technique. The team of four worked on the project for a year and a half. They put

in a lot of hours. It amounted to, says Prochownik, a complete failure. By the end, he was nearly ready to throw in the towel.

However, the team had never tried using a simpler organism for an initial screening. That would still bring up the possibility of false positives—they might find chemicals that looked promising initially but couldn't hack it in a cell. But it seemed better than nothing.

"We'd put in so much time and effort, we decided it was worth a try," Prochownik says. After several months of effort, they could afford the days it would take to plate out a modified yeast strain and try to screen for myc-max activity. The first test took five days—and, to their pleased surprise, it worked.

The assay they developed offered a streamlined peek at the proteins' interactions. The assay had been engineered so that if myc and max interacted, the yeast made beta-galactosidase (a sugar). That sugar makes the plate turn brown. So if a compound disrupted the interaction, Prochownik's team knew, because the plate wouldn't turn brown.

With a test in hand, the researchers were ready to screen potential drugs. They just needed something to screen. Small molecules, or, more specifically, low-molecular-weight compounds, are attractive to researchers, as they can often be turned into pills fairly easily. Nucleic acids and proteins often disrupt interactions as well as small molecules do, but the human digestive tract is designed to break them down—nucleic acids and proteins are, after all, part of a well-balanced diet. (That doesn't mean they can never be used as drugs, but their administration tends to be trickier.)

John Lazo, chair of the Department of Pharmacology and a collaborator of Prochownik's, had a library of low-molecular-weight compounds from a chemistry supply company. The library was created for automated screens. During the testing, a robot takes a sample of one of the chemicals and adds it to the yeast plates. The researchers let the yeast grow for a bit, then check to see if the plate turns brown. If it does, they know myc and max are still interacting.

At its heart, the automated screening technique lets scientists facilitate serendipity. The researchers picked the interaction they cared about, but they didn't select the compounds that they thought were likely to affect myc-max interaction; they simply tested every small molecule in the chemical company's library—all 10,000 of them.

This kind of experimentation has been a part of cancer discoveries for years. Researchers

found many of today's chemotherapy drugs by simply testing whatever chemicals they happened to have on their shelves. Two things have changed, however. One is processing power. "Technology makes it possible for us to screen thousands of these compounds at a time instead of five or six," notes Prochownik. The other is specificity. Using their improved understanding of cancers, researchers are able to screen for specific properties—like whether a compound inhibits protein interaction—instead of simply throwing drugs at a tumor and watching to see if it shrinks.

In this case, the numbers game seems to be panning out. Of the 10,000 chemicals Prochownik's team screened, seven disrupted myc-max interaction.

**L**ike any single cancer drug, Gleevec has its limits. Already doctors are seeing cases of resistance in patients who've come out of remission.

"What you really need are very specific attacks targeted at multiple levels," says Prochownik. He envisions therapy regimens that involve multiple small molecule treatments, possibly in combination with other targeted approaches or traditional chemotherapy.

Another group looking at myc-max interaction has had some initial success targeting RNA to stop the interaction. If that group's research evolves into a therapy, Prochownik imagines it could be used alongside small-molecule drugs to attack a common enemy on different fronts. (The RNA studies are also in early stages, but Prochownik finds them encouraging. They show that inhibiting myc seems to make some traditional chemotherapy treatments more effective. And it appears that such treatments only need to be administered for short times to have these effects, an important consideration, given that myc is needed by healthy cells.)

Prochownik won't make predictions about how well the compounds he's discovering will fare as drugs. That's not to say he isn't optimistic—his laptop is loaded with myc-max slides, and he's writing a review article on the viability of myc as a target. But at 53, he has been in this game long enough to know the vast majority of compounds that look promising in early lab tests don't actually make it to pharmacists' shelves. Yet his work is moving ahead quickly. He's already studying his seven compounds in mice to see which, if any, the animals can tolerate. If the compounds stop myc in mice—without harmful side effects—he'll move on to Phase 1 clinical trials, the

first step in testing drugs in humans.

If you want to see Prochownik truly excited, ask about the value of the screen he has validated—the modified yeast assay he was so reluctant to pursue initially. Remember his (very legitimate) fear that what worked in a simple system might not work in mammals? It turns out he has found a simple system that is also a great proxy. Every molecule that affected myc and max in yeast has done the same thing in mammalian cells. This is no small accomplishment. It means that he can confidently use it to screen more compounds, which he'll be doing shortly. It also means other researchers looking at other protein interactions have a new tool in their box. The assay can be modified to screen for a variety of different protein interactions.

In addition to being good drug candidates, small molecules like those Prochownik's team have identified are particularly attractive for development. They are easy, and cheap, to make into drugs. And cost becomes important even in trials, long before drugs get to market. In addition, small molecules are easy for chemists to modify. That's where Peter Wipf and the Combinatorial Chemistry Center come in.

The chemical library Wipf is building is similar in concept to the commercial one Lazo purchased. But because it's being developed at Pitt, it will give researchers here an edge in drug development. The library will be available to all University researchers.

Wipf is now working with Prochownik and pharmacologists Lazo and Julie Eiseman, who is a research associate professor of pharmacology, to create derivatives of the compounds that have already been shown to disrupt myc-max interactions. Prochownik's team will then screen these likely candidates, looking for compounds that act even more strongly or specifically against myc and max. They'll be increasing their odds of creating a drug that will work in people. And, by studying which modifications make the cut, the team will be able to tease out more about how the molecules actually work.

"It's very promising," says Wipf, in terms of the fundamental knowledge about cell biology to be had.

"I think the future is one where we're going to be combining these kinds of targeted therapies with a molecular knowledge of the tumor," says Prochownik.

In this future, a breast cancer won't just be a breast cancer, it will be a cancer with a molecular profile, and treatments to match. ■