NEWS

New Checkpoint Blockers Begin Human Trials

If a tumor cell were envisioned as a car with defective brakes and a stuck accelerator, will cutting the emergency brake force it to crash? Or, in biological terms, will using a drug to eliminate cell cycle checkpoints after giving DNA-damaging chemotherapy or radiation kill tumors more effectively than chemotherapy or radiation alone?

The question is not new. In a 1994 paper, Lee Hartwell, Ph.D., then at the University of Washington in Seattle, and Michael Kastan, M.D., Ph.D., of Johns Hopkins Hospital in Baltimore, speculated that drugs that target the checkpoint at G2—the gap between DNA replication and mitosis—might someday be used against human tumors. Such checkpoint genes, they wrote, “if and when they are identified, will be logical targets for inhibition in order to increase cancer cell kill after exposure to certain antineoplastic therapies.”

Now the theory is being tested in humans. In May 2005, the biotech company CanBas, based in Numazu, Japan, launched the first human trial of a drug—a peptide—specifically designed to abrogate cell cycle checkpoints. South San Francisco biotech company Exelixis expected to follow CanBas into the clinic in June with a potent, orally bioavailable small-molecule inhibitor of the chk1 and chk2 checkpoint kinases. Several other companies are developing checkpoint blockers. (See table, p. 1027.)

The concept remains simple: prevent cancer cells from stopping to repair their DNA after chemotherapy or radiation, thus killing them. The tumor cell “wants to arrest, repair the DNA, and then live,” said Gary Schwartz, M.D., a medical oncologist at Memorial Sloan-Kettering Cancer Center in New York. “If it can’t repair the DNA ... it will die.”

A Cell Cycle Breakthrough

The new drugs are the fruit of more than two decades of basic research into cell cycle checkpoints. In 1982, Arthur Pardee, Ph.D., of the Dana-Farber Cancer Institute in Boston, showed that caffeine could sensitize mammalian cells to DNA-damaging agents by eliminating cell cycle arrest at G2. But because of caffeine’s multiplicity of biochemical effects, no one knew how the chemical overrode cell cycle controls.

The checkpoint breakthrough resulted from the yeast experiments by Hartwell and postdoc Ted Weinert, Ph.D., now at the University of Arizona in Tucson. (Hartwell won the 2001 Nobel Prize in Physiology or Medicine for his cell cycle work.) They found yeast mutants that, when irradiated, did not arrest but proceeded through mitosis before dying. Artificially arresting the cell cycle at G2 could rescue these mutants from cell death, thus demonstrating that the yeast mutants were not defective in DNA repair but only in their ability to stop the cell cycle. Therefore, specific genes apparently existed to halt the cell cycle at ‘checkpoints,’ allowing DNA repair to take place. Hartwell and Weinert’s 1989 paper in Science formalized the checkpoint concept, and the field took off.

Over the next decade, several key mammalian checkpoint genes were identified and cloned. A group at Tel Aviv University in Israel cloned ATM, for “ataxia telangiectasia (mutated),” in 1995. The following year a closely related checkpoint kinase, ATR, was cloned by Stuart Schreiber, Ph.D., at Harvard University in Cambridge, Mass. Finally Stephen Elledge, Ph.D., then at Baylor College of Medicine in Houston, cloned human chk1 in 1997 and showed that it phosphorylates and inhibits cdc25, thus preventing activation of the cdk2–cyclin B complex. This complex, originally known as MPF, or maturation promoting factor, is the key effector for cells entering mitosis.

Eventually chk1, working downstream of ATR, emerged as the major kinase that regulates the G2 checkpoint. (The ATM–chk2 pathway is now seen as secondary.) As such, chk1 presented an obvious drug target. That’s because almost all tumor cells are defective at G1—the cell cycle gap preceding DNA replication—due to mutations in tumor suppressor genes, including p53, Rb, p21, or p16. So, in the absence of the G1 checkpoint, tumor cells rely heavily on the G2 checkpoint, and thus chk1, to correct otherwise fatal genetic errors.

“The big gatekeeper’s gone,” Schwartz explained. Giving a chk1 inhibitor eliminates the backup gatekeepers—the S and G2 checkpoints. “The cells are in G2; they’re going to move to M [mitosis],” he said. “The cells have abnormal DNA content, they haven’t repaired their DNA, they’re now in M, and they have one thing to do: death.”

CanBas CEO and cofounder Takumi Kawabe, M.D., Ph.D., added, “That’s why we are trying to target the G2 checkpoint and trying to disrupt the G2 checkpoint—to directly sensitize cancer cells to DNA-damaging agents.”

Ready for Prime Time

Meanwhile, normal cells with intact p53, and thus an intact G1 checkpoint,
don’t rely on the G\textsubscript{2} checkpoint for DNA repair, so abrogating that checkpoint shouldn’t affect them. “Normal cells do have the G\textsubscript{1}/S, other checkpoints, to protect the cell from replication and division in the presence of DNA damage,” explained Bin-Bing Zhou, Ph.D., a molecular oncologist at Incyte Corporation in Wilmington, Del.

“In essence, the tumor cell might be defenseless—it has no checkpoint remaining,” said Larry Karnitz, Ph.D., of the Mayo Clinic in Rochester, Minn., “whereas the surrounding normal tissue has some checkpoints remaining. So that’s where you hope to get your therapeutic index.” It’s an attractive theory but remains unproven. “There’s some [tissue culture] evidence that that might be true,” said Karnitz. “And so that’s what everybody’s going with.”

Cell culture data showing tumor cell chemosensitization is very strong. “We can clearly do this in a cell culture dish and make cells much more sensitive,” said Karnitz. “The question becomes: Is there some reason the tumor would be more sensitive than normal tissue?”

Some evidence exists for tumor selectivity. In 1999, Kawabe’s group at the Nagoya City University School of Medicine in Japan used a peptide to block the kinase activity of chk1 and achieved selective cancer cell killing in culture. “We believe that was the first data that showed … G\textsubscript{2} checkpoint abrogators killed cancer cells without affecting normal cells,” Kawabe said of the research.

At the American Association for Cancer Research (AACR) annual meeting in April, Pfizer researchers presented data showing that the company’s chk1 inhibitor in combination with gemcitabine, irinotecan, or cisplatin did have increased antitumor activity in colon and prostate cancer xenograft models, while apparently adding little toxicity. Chiron Corporation presented similar data for a breast cancer xenograft model at last year’s AACR meeting. And Exelixis’ lead compound greatly increases the survival of mice in models of human leukemia, according to Michael Morrissey, Ph.D., Exelixis’ senior vice president for discovery. It also more potently blocks pancreatic tumor growth in combination with chemotherapy than chemotherapy alone.

So hopes are high as the new checkpoint inhibitors enter clinical trials. In the past, the nonselective chk1 inhibitors UCN-01 and 17-AAG have shown antitumor activity in human trials, although checkpoint inhibition may not be their main mechanism of action. Several chemosensitization trials of these drugs are under way or planned.

**Double-edged Sword?**

One big worry casts a shadow over trials of chk1 inhibitors: secondary tumors. Will knocking out chk1 lead to DNA damage and other replication defects in normal dividing cells that could cause cancer years later? Accumulating evidence over the last decade shows that chk1 and other checkpoint kinases are not “nonessential” genes with no effect on unstressed cells, as once thought. Instead, they play a direct role in routine DNA repair. Besides stopping the cell cycle in response to stress, “there are a whole bunch of [other] things that happen, including the phosphorylation of a bunch of repair enzymes, and the activation of different types of repair, and induction of hundreds of different genes,” said Baylor’s Elledge.

Knocking out the chk1 gene in mice, first reported in 2000, was lethal early in embryogenesis, also suggesting a far broader role for the gene, at least in development. In 2004, Jeffrey Rosen, Ph.D., and colleagues at the Baylor College of Medicine created conditional chk1 adult mice with only 50% gene function. These mice suffered extensive DNA damage during replication along with other cell cycle defects. “So this isn’t something you want to do for a long period of time, because you could probably cause cancer,” said Elledge.

Giving the drugs for short periods might be safe. “For normal cells … genetic instability in most cases can be tolerated for a short time,” said Zhou. “But if you treated them for too long, then that genetic instability will cause tumors.”

Kawabe pointed out that many chemotherapy drugs also cause DNA damage and put patients at risk for secondary tumors. “We have to balance the chance between the therapeutic effect versus potential oncogenesis,” he said. “And … the DNA damage by

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**Some Companies With Products That Target Cell Cycle Checkpoints in Cancer**

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**The cell cycle checkpoint at G\textsubscript{2}/M has become a target for new drugs designed to kill tumor cells defective in the G\textsubscript{1} checkpoint following DNA-damaging chemotherapy or radiation.**
itself—not the replication defects—should be repaired by [the] G₁ checkpoint in normal cells.”

Aside from concern about secondary tumors, will the drugs even make chemotherapy more effective, as they’re intended? “It may be the case that they’ll work in some tumors and not in others,” said Elledge, who points out that cancer cells might have other intact repair pathways in addition to the ones regulated by chk1. Kawabe agreed. “Our data suggest that there are multiple G₂ checkpoint signal cascades in human cells,” he said. “So you need more than one G₂ abrogator to sensitize a variety of cancer cells.” CanBas is developing such second-generation small-molecule inhibitors against novel targets in resistant tumors.

Given the uncertainties, it may take years before the final verdict on checkpoint blockers is in. An almost endless number of possible drug combinations and tumor types exist, so making a definitive judgment won’t be easy. But the drug and biotech industries are committed to this approach. “Chk1 targeting has become a focus for pharmaceutical companies,” said Schwartz. “You’re going to see a lot more. It’s hot.”

—Ken Garber