Targeting mTOR: Something Old, Something New

By Ken Garber

Cancer drug development is slow—a truism that holds true especially for inhibitors of the mTOR (mammalian target of rapamycin) signaling pathway. Rapamycin (sirolimus), a natural product and the original mTOR inhibitor, was first purified in 1972. Starting in 1975, National Cancer Institute investigators found that it dramatically blocked growth in most solid tumor cell lines. But antitumor work fell by the wayside and didn’t resume until the mid-1990s, using rapamycin analogues—the so-called rapalogs. Finally, in 2007, the rapalog temsirolimus won approval from the U.S. Food and Drug Administration for treating renal cell carcinoma—more than three decades after rapamycin first showed anticancer activity.

Now, activity at both pharmaceutical companies and academic labs is at a peak. Because mTOR is centrally involved in cancer cell metabolism, growth, and proliferation, “there is growing interest in targeting this pathway,” said NCI investigator Phillip Dennis, M.D., Ph.D.

Interest is high even though most clinical trials of mTOR inhibitors as single agents have been disappointing, except in renal cell carcinoma and mantle cell lymphoma. Biologists are making progress in deconstructing this failure. The surprising 2005 discovery that mTOR inhibitors block a negative feedback pathway, leading paradoxically to activation of the oncogenic kinase AKT, offered an explanation for tumor cell drug resistance.

“We were quite surprised about this paradoxical upregulation of AKT, because until then AKT had always been seen to be upstream … of mTOR,” said Khuri. Other groups published similar results. The findings point to a negative feedback loop that appears to normally put brakes on mTOR signaling via AKT inhibition; blocking mTOR in cancer cells eliminates this loop, superactivating the pathway.

Although details of the feedback mechanism remain to be worked out, most researchers believe it’s a main reason that mTOR inhibitors haven’t done better in the clinic. Another factor: Several groups recently found that mTOR inhibition leads to feedback activation of the mitogen-activated protein kinase signaling pathway. “That may also lead to rapamycin resistance,” said Khuri.

More mechanisms are likely to emerge. For example, mTOR exists in two separate multimolecular complexes, known as TORC1 (or raptor) and TORC2 (rictor). Rapamycin and its analogues inhibit only TORC1. “Are there mutations or amplifications in components of TORC1 so that rapamycin no longer is effective?” asked Dennis. “There’s another layer of complexity waiting to be unraveled.”

Exploiting Addiction

Faced with this complexity, researchers have turned to new drugs to overcome resistance. For example, mTOR exists in two separate multimolecular complexes, known as TORC1 (or raptor) and TORC2 (rictor). Rapamycin and its analogues inhibit only TORC1. “Are there mutations or amplifications in components of TORC1 so that rapamycin no longer is effective?” asked Dennis. “There’s another layer of complexity waiting to be unraveled.”

### Multitargeting with mTOR: Selected Strategies

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insulin-like growth factor receptor (IGF-1R). That may work because the positive feedback loop to AKT is thought to depend on the activity of an IGF-1R substrate.

This strategy also hits the mTOR pathway both upstream and downstream of AKT, an approach others are taking (see table). For example, drug companies Novartis and Exelixis are both testing dual PI3K–mTOR inhibitors in the clinic. These drugs block both TORC1 and TORC2 complexes, unlike rapamycin and the rapalogs (temsirolimus, everolimus, and deforolimus), which hit only TORC1. Dual inhibition “will actually negate a recognized feedback mechanism of resistance,” said Dennis, because TORC2 can activate AKT, and AKT is also downstream of PI3K. OSI Pharmaceuticals and Astra-Zeneca, meanwhile, have dual TORC1–TORC2 inhibitors in early clinical development.

These drugs and drug combinations look promising. That’s because AKT activation by mTOR inhibitors may lead to a critical dependence on the PI3K–AKT–mTOR signaling pathway, sensitizing tumors to AKT-directed treatment. Khuri calls this phenomenon “pharmacologically enhanced oncogene addiction.” It’s an appealing, if clinically unproven, hypothesis. “If you can establish dependence on a pathway, you can pharmacologically enhance that addiction,” said Khuri. “You overdrive the pathway, and then you give that second hit.” The logical result: growth arrest or tumor cell death. Some preclinical work and clinical results support this hypothesis, according to Khuri.

Whether it’s better to double-hit the pathway simultaneously or sequentially is a big question, though. In theory, if mTOR inhibitors make cancer cells addicted to the pathway, then using them first (and inhibiting AKT or PI3K later) makes the most sense. Clinical trials could settle this question.

In the meantime, there are hints that pharmacologically enhanced oncogene addiction may be real. At the San Antonio Breast Cancer Symposium in December, Ruth O’Regan, M.D., of Emory’s Winship Cancer Institute, reported phase I results of a multicenter combination trial of the mTOR inhibitor everolimus with trastuzumab and paclitaxel for trastuzumab-resistant breast cancer. About half of the heavily pretreated and highly treatment-refractory patients had a partial response. “You would not expect to see this kind of response rate in particular in this patient population,” said O’Regan. Peter Houghton, Ph.D., a cancer researcher at St. Jude Children’s Research Hospital in Memphis, Tenn., called the clinical trial results “remarkable.”

The trial was designed on the premise that trastuzumab resistance is caused by loss of the tumor suppressor PTEN, which activates the mTOR pathway. But O’Regan speculated that trastuzumab, which is upstream of AKT in the pathway, regains its effectiveness because the tumor becomes dependent on the pathway when mTOR is inhibited (see graphic). “What that potentially can do is resensitize a cell that perhaps was resistant to a drug like trastuzumab [by] hyperactivating the pathway, the AKT pathway, to trastuzumab,” said O’Regan—in other words, pharmacologically enhanced oncogene addiction. The combination is now in a phase II trial.

Concerns and Complications

There are pitfalls to all these approaches. One is toxicity. Although blocking mTOR does not cause general immunosuppression—one early concern—it could, in theory, cause hyperglycemia because mTOR inhibition, in vitro, blocks glucose uptake. Drugs inhibiting IGF-1R can also have this effect because most also hit the insulin receptor, at least to some extent. So the combination might, in theory, cause diabetes symptoms. Drugs that target PI3K and mTOR together have similar issues. Dual TORC1–TORC2 inhibitors, because they powerfully shut down AKT signaling, could also be toxic. “It’s an approach worth trying,” said Houghton. “The question would be whether there’s a therapeutic window.”

Simultaneously targeting the mTOR and mitogen-activated protein kinase pathways also carries a toxicity risk. “Preclinically, it works quite well. The question is, will it be tolerable?” said Dennis. “If one effectively shuts down both pathways, I would argue that there would
be very little cellular proliferation anywhere and potentially a lot of toxicity.”

Another pitfall is new forms of resistance. For example, a phase I rapamycin trial enrolled patients with recurrent glioblastoma whose tumors lacked PTEN (as determined from the original resected tumor). Charles Sawyers, M.D., and his group at the University of California, Los Angeles, reported that rapamycin concentrations within tumors reached levels sufficient to inhibit mTOR in vitro, as measured in a second tumor sample removed after rapamycin treatment. But several patients did not show mTOR pathway inhibition as measured by an antibody to an activated pathway component. The Sawyers group concluded that something impaired delivery of the drug to tumor cells, despite high levels of the drug within the tumor—an unknown resistance mechanism.

In this trial, researchers selected in advance the subset of patients, those with PTEN-null tumors, likely to respond to an mTOR inhibitor. PTEN status was an imperfect predictor of response in this trial and in others, but patient selection remains a goal. “We should enrich for patients up front,” said Dennis. “If we know that a tumor does not have mTOR activation, why would we use an mTOR inhibitor?” In a phase II lung cancer treatment trial, Dennis’s group will use biopsy samples to determine pathway activation (using an antibody to an activated pathway protein) and select patients accordingly.

At Emory, Khuri’s group also is planning to select patients likely to respond and is exploring the use of positron emission tomography imaging for this purpose. As positron emission tomography techniques advance, Khuri said, “we may be able to say, for example, this person’s dependence on signaling through the PI3K–AKT–mTOR axis is suddenly increased—it’s an ideal time to treat with an mTOR inhibitor, or a combination.” But for now, Khuri added, “we’re dependent on biopsy sampling.”

With or without patient selection, many drugs and drug combinations targeting mTOR pathway components are moving forward into the clinic, and results are eagerly awaited. Houghton is optimistic. “Rapamycin in combination is definitely going to work, both with cytotoxic drugs and other signaling inhibitors,” he said. Dual inhibitors of the TORC complexes and of PI3K and mTOR should succeed too, he added, but the toxicity question must be answered. “The next couple of years, we’re going to find out,” Houghton said. “I think it’s a very exciting time.”

Rapamycin Versus Rapalogs

Rapamycin, the original mTOR inhibitor, is already one of the most productive small molecules in biology. Researchers at Ayerst Pharmaceuticals in Montreal first isolated the compound in 1972 from the bacterium Streptomyces hygroscopicus, which was present in a soil sample from Rapa Nui, better known as Easter Island. In 1994 the drug was used to discover its target, mammalian target of rapamycin (mTOR). This was one of the early triumphs of chemical biology, the use of small molecules to probe biological function. Rapamycin is now widely used as an immunosuppressant to prevent organ rejection in kidney transplant patients. It’s also used in drug-eluting coronary stents.

Rapamycin’s potent anticancer effects have long been known (see article). When rapamycin patents for cancer use expired, Wyeth (Ayerst’s successor company) developed a patent-protected rapamycin analogue, temsirolimus, which was approved in 2007 for treating renal cell carcinoma. Novartis and Ariad Pharmaceuticals have similar “rapalogs” in advanced development for many tumor types. But temsirolimus is expensive—about $5,000 a month, according to University of Chicago cancer researcher Ezra Cohen, M.D. Rapamycin, which currently costs about one-fourth that amount, could theoretically be marketed for even less because rapamycin for cancer is in the public domain.

Cohen and colleague Mark Ratain, M.D., at the University of Chicago, among others, are pushing to develop rapamycin as an inexpensive targeted anticancer drug. They have contacted nonprofit drug development organizations, as well as pharmaceutical companies that could, in theory, create new intellectual property around rapamycin drug combinations. And they hope the National Cancer Institute will sponsor drug trials that would lead to rapamycin approval in cancer. Cohen argues that government will end up, through Medicare, paying for mTOR inhibitors, so developing rapamycin will be cost effective. “One way or another, public funds are going to have to pay for these drugs,” he said. “In the long run, it’s much cheaper to develop rapamycin.”

James Zwiebel, M.D., chief of the Investigational Drug Branch in NCI’s extramural Cancer Therapy Evaluation Program (CTEP), said CTEP has no current plans to sponsor rapamycin development. “NCI drug development resources are limited, and we want to be able to explore newer, promising agents that hit this pathway,” Zwiebel wrote in an e-mail.

Rapamycin, although just as effective as the rapalogs in vitro, is poorly absorbed in vivo; the drug is only 10%–15% bioavailable. Raising the dose could get around that problem but runs the risk of toxicity. Cohen’s group is giving frozen grapefruit juice to patients to enhance the bioavailability of rapamycin. (Grapefruit juice inhibits the enzymes that metabolize the drug.) A phase I trial, published last year, showed fourfold improvement in bioavailability. The rapalogs have somewhat better “pharmaceutical properties”—bioavailability, half-life, solubility—than rapamycin. But these differences may not be crucial, according to Phillip Dennis of the NCI’s intramural research program. Dennis’s group has launched rapamycin prevention clinical trials in lung cancer and in Cowden syndrome, a rare disorder caused by germline mutations in the tumor suppressor PTEN. He would like to see the drug somehow brought to market: “Wouldn’t it be great to have an effective anticancer drug that was oral, generic, and affordable?”

But rapamycin faces an uphill battle, partly because new ways to target mTOR are arriving (see article.) “There may emerge fairly rapidly a better way to inhibit mTOR than with the rapamycins,” wrote Janet Dancey, M.D., a former CTEP investigator now at the Ontario Institute for Cancer Research, in an e-mail. —Ken Garber