Getting Around PLX4032: Studies Turn Up Unusual Mechanisms of Resistance to Melanoma Drug

By Rabiya S. Tuma

About half of all melanomas are driven by a mutation in the B-RAF gene, called V600E. So in 2009, when early small trials showed many patients with the mutation responding to a drug called PLX4032, researchers were jubilant. The drug is now in a pivotal phase III trial for metastatic melanoma; there is talk about accelerated approval; and its developers, Plexxikon and Roche, have already opened an expanded access program.

The only bad news about PLX4032 so far is the duration of response: Patients are developing resistance to the small-molecule inhibitor in 2–18 months. Phase II trial results, announced in November, showed that 52% of patients with the mutation responded to PLX4032, but the median duration of response was 6.2 months.

Now, two research groups exploring this acquired resistance report that melanoma tumors use multiple distinct routes to get around PLX4032. The drug works by inhibiting the mutated B-RAF, which otherwise drives tumor growth by activating the mitogen-activated protein (MAP) kinase pathway. Acquired resistance, however, does not seem to be secondary mutations in the B-RAF target gene itself. Rather, the melanoma tumors and cell lines analyzed to date use other kinase proteins to sidestep B-RAF and reactivate the pathway. Or they compensate for the loss of MAP kinase pathway activity by upregulating other, unrelated growth factor receptor pathways.

Investigators interviewed for this article emphasized that more mechanisms of resistance are likely to turn up as researchers analyze more patient samples. However, the current data already suggest combination therapies that might mitigate the problem.

“We know from other targeted therapies—other kinases in other tumor types—this is the way it goes when you start with a single drug,” said Charles Sawyers, M.D., chair of the human oncology and pathogenesis program at Memorial Sloan-Kettering Cancer Center in New York and a Howard Hughes Medical Institute investigator. “We need to move quickly either to sequential or combination drugs that block the most common roads of resistance from the get-go. I think these two papers are the first attack on the problem, but I don’t know they are going to be the final answer.”

Sawyers, who wrote an editorial that accompanies the papers in the Dec. 16 issue of Nature and has been involved in developing other targeted agents, said that the most surprising aspect of the two studies is the lack of secondary mutations in the B-RAF gene. “So far, there is no evidence that the B-RAF gene itself becomes mutated as a way to escape the inhibitor. In every single other case of a targeted kinase inhibitor, the main mechanism of resistance is a new mutation in the target kinase. It is pretty surprising that they didn’t find that.”

Multiple Paths to Resistance

In one of the studies, Levi Garraway, M.D., Ph.D., assistant professor of medicine at the Dana-Farber Cancer Institute and Harvard Medical School in Boston, and colleagues, expressed nearly 600 different kinase proteins in PLX4032-sensitive cells to find proteins that allowed the cells to grow in the presence of the drug. Two kinases did so: C-RAF and COT—which, like B-RAF, stimulate the MAP kinase pathway. C-RAF and COT induce resistance to PLX4032 by bypassing B-RAF altogether and activating downstream proteins directly, much as a detour in a road goes around an obstacle but ultimately rejoins the main route. C-RAF expression was expected on the basis of previous work showing that it could form a complex with wild-type B-RAF protein that activates the MAP kinase pathway. When Garraway and colleagues examined biopsy samples from three patients on PLX4032, they found that COT expression increased in two cases compared with the matched pretreatment biopsy specimens.

Meanwhile, Roger Lo, M.D., Ph.D., assistant clinical professor of medicine at the University of California, Los Angeles, and colleagues used a combination of in vitro assays and patient biopsy samples to look for mechanisms of resistance. They identified two mutually exclusive mechanisms in cell lines and confirmed their findings in 12 patient samples. In one patient, a mutation in N-RAS reactivated the MAP kinase pathway, obviating the need for B-RAF activity—the same way COT worked in Garraway’s experiments. In five patient samples, Lo and colleagues found that the platelet-derived growth factor receptor (PDGFR) was overex-
pressed and that the overexpression drove resistance through MAP kinase-independent pathways. (They could not uncover the mechanism of resistance in six patient samples.)

Sawyers said that whereas the C-RAF, COT, and N-RAS mechanisms make sense, it is less clear how to interpret the PDGFR overexpression data. On one hand, when Lo and colleagues blocked PDGFR expression with small-interfering RNAs, cell proliferation stopped in the presence of PLX4032, which supports the idea that the growth factor pathway drives resistance. On the other hand, treatment of the cells with imatinib—an effective PDGFR inhibitor used to treat some cancers driven by PDGFR—had no effect.

However, Michael Davies, M.D., Ph.D., assistant professor in the departments of melanoma medical oncology and systems biology at the M. D. Anderson Cancer Center in Houston, thinks the PDGFR results make sense in light of data published in the Dec. 14 issue of Cancer Cell. In that report, Meenhard Herlyn, D.V.M., D.Sc., of the Wistar Institute in Philadelphia and colleagues reported that increased activity of the insulin-like growth factor receptor (IGF-1R) conferred resistance to a different V600→E inhibitor called SB-590885. Activation of IGF-1R stimulated the PI3K–AKT pathway, which has already been implicated in melanoma cell survival. And, Davies points out, continued on page 177
PDGFR also activates the PI3K–AKT pathway.

“So a common mechanism is coming through,” Davies said. “Growth factor receptors are known to activate a variety of different pathways, but clearly one of the pathways that would be a logical target is the PI3K–AKT pathway.”

**Translation to Clinic**

Everyone interviewed said the new data point to some promising combinations. For example, several drug companies have PI3K or AKT inhibitors in development, and combining one of those with a B-RAF V600→E inhibitor might prolong patient responses. Alternatively, researchers could try to block reactivation of the MAP kinase pathway by combining PLX4032 with a drug that inhibits a downstream step in the pathway, such as MEK or ERK. Theoretically the double blockade of the MAP kinase pathway would preclude resistance through the N-RAS, C-RAF, and COT mechanisms.

“Probably the most obvious next step is to do combination trials with a MEK inhibitor,” Sawyers said. “People had thought of that already. This provides a much more clear rationale for it.”

In fact, GlaxoSmithKline is already testing the company’s B-RAF V600→E and MEK inhibitors in a phase I trial.

With the number of distinct mechanisms the cells are using to get around PLX4032, a key question is whether a two-drug combination would be adequate to preclude or slow resistance. Although only clinical trials can answer that for sure, Garraway is optimistic. “You may be able to solve much of the problem by giving two different drugs,” he said. “You might get resistance to one, but the other keeps the cells at bay. A combination may just shift what plays out dramatically.”

He warned, however, that resistance is still likely to develop over time, and clinicians will need multiple regimens that can be used sequentially. “Hopefully we will end up at an equilibrium where there are a certain number of drugs we can give up front or there are salvage mechanisms that you can give to get lasting benefit,” Garraway said. “I don’t know if that will amount to cure or to disease stabilization for many years; both would be fine compared to where we are now.”

Davies though said that he thinks a combination of PLX4032 and the other up-and-coming melanoma drug, ipilimumab, might be advantageous. “We might be able to build on the durable responses from ipilimumab and the objective responses from PLX4032,” he said. According to investigators involved in ipilimumab development, the trial has already been proposed and approved by the makers of ipilimumab but is still under discussion with Roche.

Of course, all the discussions about PLX4032 resistance and combinations are based on preclinical models and few patient samples. Therefore, a systematic analysis of more patients who have developed resistance to PLX4032 is paramount.

“Investigators need to be really careful to get these tumor samples at treatment start and at relapse in a much more comprehensive way,” Sawyers said. “Now that there are some hints as to what is going on, it needs to be addressed much more aggressively and comprehensively with 50–100 cases, carefully amassed, to see if these initial findings are reproduced and see what the larger landscape looks like.”

Garraway said that researchers are already working in that direction. He said more study results, including more patient samples, would be published in the coming months.

**Dr. Lo reported no conflicts of interest, but two of his co-authors received honoraria from Roche Pharmaceuticals. Dr. Garraway serves as a consultant for Novartis Pharmaceuticals, Inc., which is developing small molecule inhibitors of pathways mentioned, and several of his co-authors are employed by Novartis. Dr. Davies reported research support from GlaxoSmithKline.**

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