The path of Iressa resistance

By Michael J. Haas, Senior Writer

A report in the *Journal of Clinical Investigation* provides evidence that cancer cells develop resistance to Iressa gefitinib, an epidermal growth factor receptor inhibitor, by upregulating insulin-like growth factor-1.¹ Although the paper showed that the resistance can be overcome by inhibiting both receptors, companies working in the space had mixed views on whether the resistance mechanism is common to other epidermal growth factor receptor inhibitors and whether the findings would be widely applicable to human cancers.

There was agreement that the data bolster the rationale for using multiple growth factor inhibitors to treat cancer, and that it could provide markers to predict patient response.

The JCI team included researchers from Harvard Medical School's Massachusetts General Hospital Cancer Center, Vanderbilt University School of Medicine and the University of Minnesota Cancer Center. They were led by Jeffrey Engelman, instructor of medicine at Massachusetts General, and Carlos Arteaga, professor of medicine and cancer biology at Vanderbilt.

Vive la résistance

Gefitinib is marketed as Iressa by AstraZeneca plc to treat metastatic or locally advanced non-small cell lung cancer (NSCLC).

Epidermal growth factor receptor (EGFR) inhibitors block a signaling cascade responsible for activating the phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt) pathway for cellular growth and survival. Iressa and the other approved small molecule EGFR inhibitor—Tarceva erlotinib from OSI Pharmaceuticals Inc., Genentech Inc. and Roche—are effective in about 10–20% of lung cancer cases, but their effectiveness is limited or lost as resistance develops. A number of studies have linked about half of these resistant cancers to a key point mutation in the EGFR gene itself, but those reports did not account for why or how resistance occurs in the other half expressing the wild-type gene.^{2,3}

At least three recent studies—two of them involving Engelman—found that Iressa-resistant cancers can upregulate c-Met receptor tyrosine kinase, thereby providing an alternative to EGFR signaling for PI3K and Akt activation. This alternative pathway was found in cancer cells with the mutant EGFR as well as in those with the wild-type gene.^{4–6}

The current JCI study looked for potential mechanisms of resistance

in Iressa-resistant cancer cells having only the wild-type gene.

First, the Engelman-Arteaga team developed Iressa-resistant cells by growing squamous epithelial cells in increasing concentrations of Iressa over a period of several months.

They found that insulin receptor substrate-1 (IRS-1)—a substrate of the insulin-like growth factor-1 (IGF-1) receptor that activates the PI3K and Akt pathway—was upregulated in these cells. This led the team to hypothesize that the resistant cells had lost sensitivity to Iressa because of activation of the IGF-1 receptor and IRS-1 signaling cascade, which Iressa does not target.

The team confirmed this hypothesis by showing that treatment of the resistant cells with a combination of Iressa and an IGF-1 receptor inhibitor restored sensitivity to Iressa.

Subsequent gene profiling and small hairpin RNA experiments indicated that upregulation of the IGF-1 receptor resulted primarily from decreased expression of its negative regulator, insulin-like growth factor binding protein-3 (IGFBP3). Indeed, Iressa plus recombinant IGFBP3 restored Iressa sensitivity.

In addition, head and neck cancer cells sensitive to Iressa did not develop resistance when treated with a combination of Iressa and a small molecule IGF-1 receptor inhibitor.

The team obtained comparable results *in vitro* with Erbitux cetuximab, an anti-EGFR antibody marketed by ImClone Systems Inc., Bristol-Myers Squibb Co. and Merck KGaA. Cetuximab-resistant squamous epithelial cells developed by the team also downregulated

IGFBP3 and were sensitive to the combination of cetuximab and a small molecule IGF-1 receptor inhibitor.

Lastly, seven mice bearing xenograft Iressa-resistant tumors were treated with a combination of Iressa and a small molecule IGF-1 receptor inhibitor. All of the mice responded to therapy, and only one had a tumor recur—two months after treatment was stopped. By contrast, mice treated with Iressa or the IGF-1 receptor inhibitor alone experienced either tumor progression or tumor recurrence.

Collectively, the results indicate that cancer cells can develop resistance to Iressa by activating an alternative growth factor signaling cascade—the IGF-1 receptor and IRS-1 pathway—and that combining Iressa with an IGF-1 receptor inhibitor can overcome this resistance.

Neither Engelman nor Arteaga was available for comment. But Geoffrey Allan, president and CEO of Insmmed Inc., told SciBX: “This paper is the first to provide an in-depth look at the mechanism of resistance to epidermal growth factor receptor inhibitors. It provides concrete, detailed evidence for something that was postulated on the basis of earlier—but limited—studies.”

Insmmed's rhIGFBP3 (recombinant IGFBP3), is in preclinical development for breast, liver, colon, prostate and ovarian cancers. The company recently completed a Phase I trial of INMS-18, a small molecule inhibitor of IGF-1 receptor and hairy-related 2 (HER2), and plans to take that compound into a Phase II trial to treat prostate cancer in the near future.

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David Kirn, president and CEO of **Jennerex Biotherapeutics Inc.**, agreed with Allan. "Previous studies have shown resistance to result from specific mutations in the EGF receptor gene itself," he said. "This is the first clear evidence of a downstream compensatory mechanism of resistance."

Jennerex's JX-594, a recombinant vaccinia virus that targets EGFR, is in Phase II testing to treat liver cancer and melanoma, and Phase I/II testing to treat lung cancer.

Rational combination

Company and institution representatives contacted by **SciBX** agreed that the *JCI* paper supports the use of a combination of growth factor inhibitors to treat cancer. Opinions differed, however, on which cancers and compounds might trigger the resistance described in the paper.

"There is widespread understanding that monotherapy is not the optimum pathway because of the complexity of cancer," said Elwyn Loh, executive director for global development and global development leader for growth regulation products at **Amgen Inc.** "In that context, this paper provides a real mechanistic framework to think about this combination of agents."

But Loh said it was not yet clear whether the *JCI* results were relevant in any other cancers. "The paper offers suggestive evidence that one can apply the findings to a spectrum of human cancers," he said. "But we need to do experiments in people to confirm this."

He also wanted to know whether the mechanism of resistance described in the *JCI* article is the primary pathway of Iressa resistance. "Is this mechanism dominant across many different cell lines—or just one of multiple pathways of resistance?" he asked.

Loh said the answer would indicate whether a combination of EGFR and IGF-1 receptor inhibitors would be widely useful in overcoming Iressa resistance.

Amgen's Vectibix panitumumab anti-EGFR antibody is marketed for metastatic colorectal cancer. The company's AMG479 anti-IGF-1 receptor antibody is in Phase II trials to treat breast cancer, pancreatic cancer and Ewing's sarcoma. Amgen plans to start additional Phase II studies of AMG479 in other cancers, including NSCLC, but has not announced timelines.

"As far as we're concerned, this mechanism of resistance is one of many," said Iain Hutcheson, senior research associate at **Cardiff University**. He added that cancers can resist Iressa by using multiple signaling routes to maintain PI3K and Akt activation, and that targeting PI3K and Akt, instead of molecules further upstream, might be a better strategy.

By contrast, Insmed's Allan thought the mechanism would be generally applicable to tumors resistant to EGFR inhibitors. "Insmed did an *in vitro* study in breast cancer cells of its recombinant IGFBP3 in combination with Herceptin, and the combination seemed to give better tumor control," he said.⁷

That Insmed research collaboration—which included scientists from **McGill University**, the **University of California, Los Angeles's** David Geffen School of Medicine and **Xanthus Pharmaceuticals Inc.** (formerly Xanthus Life Sciences)—found that the IGF-1 receptor was highly expressed in breast cancer cells that had developed resistance to Herceptin. But the combination of Herceptin and recombinant IGFBP3 restored the cancer cells' sensitivity to Herceptin, both *in vitro* and in xenograft mouse tumors.

Genentech markets its Herceptin trastuzumab anti-HER2 antibody in the U.S. to treat breast cancer. Roche markets the drug elsewhere.

Allan noted that the *JCI* report is also consistent with epidemiological data showing that levels of IGFBP3 are inversely related to the incidence of breast cancer.^{8,9}

Clinical strategy

David Epstein, SVP of oncology research at OSI, said therapeutic application of the *JCI* findings would take time to assess.

"The finding that concurrent inhibition of IGF-1 receptor abrogates the emergence of resistance to EGFR tyrosine kinase inhibitors is a new observation," Epstein said. But he added that it remains to be seen whether cancers would develop this mechanism of resistance in response to other small molecules and antibodies against EGFR and whether it would occur in a broad range of tumors.

In addition to Tarceva, OSI's OSI-906, an IGF-1 receptor inhibitor, is in Phase I trials to treat advanced solid tumors. Epstein said OSI is planning Phase II trials that will combine the compound with Tarceva and other signal transduction inhibitors.

Although the prevalence of this resistance mechanism will have to be established, Epstein suggested the *JCI* findings might yield useful biomarkers.

Jennerex's Kirn agreed. "With a screen based on IGFBP3 levels, you could predict which patients are likely to develop, or are developing, resistance" to treatment with Iressa, he said. Studies to examine this potential correlation could begin right away, he added.

Kirn noted more data on the safety of the inhibitor combination would be needed. A key question to answer, he said, is whether normal cells are "as dependent, or less dependent, than cancer on the EGF receptor and IGF receptor pathways." If normal cells depend on both, the combination of inhibitors might have increased toxicity, he said.

Uma Sundaram, senior licensing associate at Massachusetts General, said the findings reported in *JCI* are patented and available for licensing.

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COMPANIES AND INSTITUTIONS MENTIONED

Amgen Inc. (NASDAQ:AMGN), Thousand Oaks, Calif.
AstraZeneca plc (LSE:AZN; NYSE:AZN), London, U.K.
Bristol-Myers Squibb Co. (NYSE:BMJ), New York, N.Y.
Cardiff University, Cardiff, U.K.
Genentech Inc. (NYSE:DNA), South San Francisco, Calif.
Harvard Medical School, Boston, Mass.

ImClone Systems Inc. (NASDAQ:IMCL), New York, N.Y.
Insmmed Inc. (NASDAQ:INSM), Richmond, Va.
Jennerex Biotherapeutics Inc., San Francisco, Calif.
Massachusetts General Hospital Cancer Center, Charlestown, Mass.
McGill University, Montreal, Quebec, Canada
Merck KGaA (Xetra:MRK), Darmstadt, Germany

OSI Pharmaceuticals Inc. (NASDAQ:OSIP), Melville, N.Y.
Roche (SWX:ROG), Basel, Switzerland
University of California, Los Angeles, Calif.
University of Minnesota Cancer Center, Minneapolis, Minn.
Vanderbilt University School of Medicine, Nashville, Tenn.
Xanthus Pharmaceuticals Inc., Cambridge, Mass.