Finding the perfect combination

By Tracey Baas, Senior Editor

Two independent research teams have used cell culture methods to show that high hepatocyte growth factor/scatter factor levels drive resistance to BRAF inhibitors in melanoma.^{1,2} The findings could provide a rationale for combining hepatocyte growth factor/scatter factor or c-Met proto-oncogene inhibitors with BRAF inhibitors to overcome treatment resistance in the disease.

A team at **Genentech Inc.** focused on growth factor-driven resistance mechanisms in tumor cells, whereas a group from the **Broad Institute of MIT and Harvard** studied the contribution of the tumor microenvironment to resistance.

The Genentech team hypothesized that drug resistance in tumor cells may result from the engagement of cell signaling pathways that can compensate for the loss of activity in the inhibited pathway, thus allowing cancer cells to survive even when treated with a kinase inhibitor.

To test that idea, the group treated a panel of 41 cancer cell lines that were highly sensitive to kinase inhibition with a kinase inhibitor plus one of six different growth factors.

Indeed, most of the cell lines lost sensitivity to the kinase inhibitor in the presence of one or more of the tested growth factors, suggesting activation of alternative pathways could promote resistance.

The factors that most frequently promoted resistance to kinase inhibitors were hepatocyte growth factor/scatter factor (HGF/SF), fibroblast growth factor (FGF) and neuregulin 1 (NRG1).

The researchers next looked at the effects of these growth factors in BRAF mutant melanoma.

In about half of BRAF mutant melanoma cell lines, HGF/SF induced expression of its receptor, c-Met proto-oncogene (MET; HGFR), and triggered resistance to the BRAF kinase inhibitor Zelboraf vemurafenib.

However, in those same cells, the resistance could be reversed by treating the cells with a combination of Zelboraf and the MET inhibitor Xalkori crizotinib.

The findings translated to the *in vivo* setting. Zelboraf plus a MET inhibitor blocked tumor growth better than Zelboraf alone in two xenograft mouse models of BRAF mutant melanoma in which MET was artificially activated. Moreover, increased plasma HGF/SF levels in patients with BRAF mutant melanoma correlated with poorer survival.

Zelboraf is marketed by **Daiichi Sankyo Co. Ltd.**, **Chugai Pharmaceutical Co. Ltd.** and **Roche** to treat melanoma in patients with BRAF V600E mutations.

Xalkori is marketed by **Pfizer Inc.** to treat non-small cell lung cancer (NSCLC) and is in Phase I testing to treat other solid tumors.

In the second study, the team from the Broad Institute used a coculture system to study how 23 stromal cell types influenced the drug resistance of 45 cancer cell lines. They concluded that stromal-mediated resistance was common in the cells.

Cocultures of BRAF mutant melanoma cell lines and stromal fibroblasts led to MET activation and resistance to BRAF inhibition in the melanoma cells. Fibroblast-conditioned growth medium also led to resistance in the BRAF mutant melanoma cell lines, suggesting the stromal fibroblasts were secreting a factor that caused the resistance.

An antibody array-based analysis of 567 secreted factors showed that increased BRAF resistance correlated with increased levels of HGF/SF. Moreover, HGF/SF-neutralizing antibodies or Xalkori reversed resistance in cocultures of BRAF mutant melanoma cells and fibroblasts.

Finally, the researchers used immunohistochemistry to examine HGF/SF expression in biopsy samples from patients with BRAF mutant melanoma. The team detected HGF/SF in the tumor-associated stromal cells in 68% of the patients, and HGF/SF secretion from stromal cells was associated with poorer response to BRAF inhibitors ($p < 0.05$).

Both studies were published in *Nature*.

At least two HGF/SF mAb antagonists are in the clinic. **Amgen Inc.**'s rilotumumab is in Phase II testing for brain cancer and gastric cancer and Phase I/II trials for colorectal cancer. **Aveo Pharmaceuticals Inc.**'s ficlatuzumab is in Phase II testing for NSCLC and Phase I trials for head and neck cancer.

A **Novartis AG** group published a similar approach this month using a cell-based assay to screen the potential of 3,482 different secreted proteins to activate alternative kinase pathways and cause resistance to kinase inhibitors in tumor cells. A number of secreted proteins induced resistance, with ligands of epidermal growth factor receptor 1 (EGFR1; HER1; ErbB1), fibroblast growth factor receptor (FGFR) and MET families showing a broad ability to compensate for inhibition of the original targeted kinase.³

Novartis declined requests for an interview.

Providing the relevance

"Both studies provide new information that can be quickly applied in the clinic. The immediate next step is to use stromal HGF/SF levels

"Being able to subgroup cases by overexpression of particular RTK ligands could provide an important opportunity to select appropriate combination therapies. Peripheral blood or circulating tumor cells from patients undergoing targeted anticancer therapies could be interrogated for the presence of RTK ligands and other growth factors before and during treatment."

—Jeffrey Settleman,
Genentech Inc.

or tumor MET overexpression as potential predictive biomarkers of response to combined BRAF inhibition plus HGF/MET antagonists for clinical trials in *BRAF* mutant melanoma,” said Roger Lo. “And in the long run, the screening strategy will help to guide strategic selection of combination therapies to treat a variety of cancers.”

Lo is assistant professor of medicine at the **University of California, Los Angeles David Geffen School of Medicine** and a member of UCLA’s Jonsson Comprehensive Cancer Center. He was an author on one of the papers.

Gideon Bollag, SVP of research at Daiichi’s Plexxikon unit, agreed. However, he noted that “it is unclear if the elevated HGF levels occur in response to the inhibitor or rather if activation of the HGF/MET pathway predicts poor outcome independent of therapy. Nonetheless, appropriate agents should be selected—perhaps crizotinib and vemurafenib—protocols written and trials commenced.”

“More and more literature points to the protumorigenic role of stromal cells. Perhaps these cells initially attack the cancer cells, but often tumors are able to circumvent these cells and eventually hijack the cells to support tumor growth, invasion and metastasis,” Bollag said. “We predict that future treatment decisions will include characterization of the stromal cells in tumor biopsies or using direct imaging techniques, so that appropriate agents can be selected on a case-by-case basis.”

Both teams also plan to expand their screening studies to find the most clinically relevant mechanisms of acquired drug resistance in cancer cells.

“Our study has already provided a number of leads regarding mechanisms of resistance and secreted factors that induce that resistance,” said Todd Golub, leader of the Broad Institute team. “We are going to further pursue these leads in *BRAF* mutant melanoma and also in non-*BRAF*, nonmelanoma cancers.”

Golub is professor of pediatrics at **Harvard Medical School**, an investigator at **Dana-Farber Cancer Institute** and director of the cancer program at the Broad Institute.

“The Genentech team is currently investigating several hundred growth factors on larger panels of patient-derived tumor cell lines. In order to predict which RTK [receptor tyrosine kinase] ligand-dependent mechanisms of resistance are potentially clinically relevant, the prevalence of the various growth factors that can drive RTK-mediated drug resistance will have to be assessed; therefore, the more patient-derived cancer cell lines and tumor samples we can examine, the better,” said team leader Jeffrey Settleman, who is also senior director of discovery oncology at Genentech.

Once the company identifies the most clinically relevant mechanisms of acquired resistance, the next steps will be selecting the most appropriate patient subgroups for clinical trials and using the best combination of therapies for each subgroup.

“Being able to subgroup cases by overexpression of particular RTK ligands could provide an important opportunity to select appropriate combination therapies,” Settleman said. “Peripheral blood or circulating tumor cells from patients undergoing targeted anticancer therapies could be interrogated for the presence of RTK ligands and other growth factors before and during treatment.”

“Resistance to single therapy is common, and when it does

occur in the clinic, it’s really too late to start thinking about rational combinations,” Golub added. “We want to use our coculture system to identify the important mechanisms underlying drug resistance with the ultimate goal of providing rational combination therapies.”

“The tumor most likely uses multiple mechanisms to induce resistance and escape drug efficacy,” continued Golub. “My gut feeling is that a therapeutic cocktail—like those for HIV—might be more effective at treating an individual’s cancer than sequential therapeutic delivery.”

A different kind of animal

The findings of the two papers also will change how researchers use animal studies to model *in vivo* relevance.

“The important take-home message is that the tumor microenvironment may be just as important as the tumor cells themselves,” said Settleman. “This means we’re going to have to be creative with our animal models. Simple xenografts may not be sufficient. For example, murine stromal factors may not affect human cancer cells, and consequently we might miss that important interaction between stromal and cancer cells.”

“In order to make the best possible use of these new insights to help drive drug development and provide strategic combination therapies, we will undoubtedly need to explore such mechanisms more deeply,” he said.

Genentech did not disclose the patent or licensing status of its findings.

The Broad Institute also did not disclose patenting or licensing status. The general screening approach is not patented.

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