Splicing out BRAF's resistance

By Kai-Jye Lou, Staff Writer

U.S. researchers have uncovered a mechanism by which melanomas expressing mutant BRAF acquire resistance to targeted drugs.¹ The findings represent the first time a splice variant has been implicated in cancer drug resistance. The findings give companies a new resistance mechanism to screen against in the design of next-generation BRAF inhibitors and also further the case for combining Zelboraf with downstream kinase inhibitors.

BRAF is part of the Ras/Raf/MEK/MAPK signaling cascade, which is collectively known as the MAPK pathway. Activating mutations in BRAF—which result in tumor dependency on the MAPK signaling cascade—are found in about half of melanoma cases. The V600E mutation is by far the most predominant and occurs in about 90% of melanomas driven by mutant BRAF.²

In August, the FDA approved Zelboraf vemurafenib to treat unresectable or metastatic melanoma with the BRAF V600E mutation. The agency concurrently approved the cobas 4800 BRAF V600 Mutation Test from **Roche** for detecting the mutation. Zelboraf is marketed by Roche's **Genentech Inc.** unit and co-promoted with **Daiichi Sankyo Co. Ltd.** Daiichi gained the small molecule when it acquired **Plexxikon Inc.** for \$805 million in April.

Unsurprisingly, there have been documented cases of resistance to Zelboraf,²⁻⁶ but none was known to be mediated by alterations in BRAF.

"Vemurafenib is the most active agent identified in melanoma patients to date, but as with many other cancer therapies, resistance to this drug invariably develops over time," said David Solit, laboratory head in the human oncology and pathogenesis program at the **Memorial Sloan-Kettering Cancer Center**. "Thus, identifying the underlying mechanisms of resistance is critical in order to improve the care of these patients."

Now, a team led by Solit has identified an acquired resistance mechanism mediated by a structural change in BRAF itself.

The researchers first generated five Zelboraf-resistant clones from a human melanoma cell line carrying the V600E mutation. In 3 of the Zelboraf-resistant lines, cells expressed a truncated 61 kDa BRAF splice variant that lacked a domain responsible for binding Ras, an upstream GTPase in the MAPK signaling cascade. This domain prevents the dimerization of BRAF when Ras activation levels are low.⁷

Compared with full-length V600E, the truncated splice variant showed an increased ability to dimerize. Importantly, the researchers

found that by introducing a mutation that prevented dimerization, the splice variant gained sensitivity to Zelboraf.

The group also looked at samples from 19 patients with V600E mutant melanomas that had acquired resistance to Zelboraf. Six of those samples expressed truncated BRAF transcripts lacking the exons encoding the Ras-binding domain. In contrast, truncated BRAF transcripts were not detected in melanomas that were vemurafenib-naïve or intrinsically resistant to BRAF inhibitors.

Results were published in *Nature*.

"Not only was the mechanism of resistance to vemurafenib identified by our group novel, but the generation of splice variants is in fact a novel paradigm of drug resistance in general and one that may be applicable in the setting of other kinase inhibitor-directed therapies," said Solit, who is corresponding author on the paper.

Martin McMahon, the Efim Guzik distinguished professor of cancer biology at the Helen Diller Family Comprehensive Cancer Center at the **University of California, San Francisco**, agreed.

"This is the first time in cancer where the mechanism of drug resistance is found to be due to a change in splicing," he told *SciBX*. "The implication is that if this resistance mechanism is the case for BRAF in melanomas, it could also be the case for other genes in other forms of cancer."

Combos and considerations

In addition to supporting the idea of combining Zelboraf with inhibitors of other members of the MAPK signaling cascade, drug developers can now consider the mechanism when designing new drugs.

"The role of Ras in mediating dimerization and the bypass of this need by alternative splice forms of oncogenic BRAF are important insights that are elegantly stressed in this manuscript," said Gideon Bollag, SVP of research at **Plexxikon**. "The alternative splice forms can be identified by diagnostic tests," and cells expressing the resistant splice form provide a model to identify combination or next-generation therapies, he added.

McMahon added that the results in the paper support going after multiple targets along the same signaling cascade.

Indeed, the Sloan-Kettering group showed that the Zelboraf-resistant cell lines were still sensitive to MEK inhibitors, which act downstream of BRAF.

Chris Bowden, VP of product development for clinical oncology at **Genentech** and the clinical lead on Zelboraf, noted that **Genentech** already is exploring combinations of Zelboraf with both biologics and other targeted therapies.

"This research further supports much of what we are already doing, as it suggests that combinations may be a potentially effective treatment strategy in the future," said Bowden. "For example, we are researching

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Genentech Inc.**

this in a Phase I study of Zelboraf and GDC-0973, an investigational MEK inhibitor, in people with newly diagnosed V600E-positive melanoma and in those whose melanoma has progressed while on treatment with Zelboraf.”

Genentech received worldwide rights to GDC-0973 from **Exelixis Inc.** in 2008.

Bollag said Plexxikon is working on next-generation inhibitors that it hopes to advance into the clinic next year. He added that the truncated BRAF splice variant and resistant cell lines described in the paper could be valuable reagents for screening and identifying such inhibitors.

Solit said his group is screening additional patients to get a better estimate of the prevalence of the truncated BRAF splice variant in melanoma.

“We are also looking to use the information to develop more effective BRAF inhibitors or combinations of inhibitors that work in patients who have developed this form of drug resistance,” he told *SciBX*.

Sloan-Kettering has a pending patent covering the work reported in *Nature*. The licensing status is undisclosed.

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e-mail: solitd@mskcc.org
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COMPANIES AND INSTITUTIONS MENTIONED

Daiichi Sankyo Co. Ltd. (Tokyo:4568; Osaka:4568), Tokyo, Japan
Exelixis Inc. (NASDAQ:EXEL), South San Francisco, Calif.
Genentech Inc., South San Francisco, Calif.
Memorial Sloan-Kettering Cancer Center, New York, N.Y.
Plexxikon Inc., Berkeley, Calif.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
University of California, San Francisco, Calif.