Melanoma: three ways around BRAF inhibition

By Michael J. Haas, Senior Writer

Independent research teams have identified three mechanisms by which melanoma can develop resistance to BRAF inhibitors,^{1,2} a promising class of therapeutics that include late-stage clinical compounds from **Plexxikon Inc.** and **GlaxoSmithKline plc.** The findings suggest that BRAF inhibitors will need to be combined with other types of drugs, although future studies will have to determine the relative frequency of each mechanism.

About 60% of melanomas express an activating mutation in the serine/threonine kinase BRAF that enables tumor cell growth and proliferation. The most advanced inhibitor of mutant BRAF is PLX4032 (R7204; RG7204) from Plexxikon and **Roche**. The compound is in Phase III testing. Phase II data showed that some patients whose melanoma initially responded to the compound later developed resistance.^{3,4}

To identify the mechanisms underlying that resistance, a U.S.-Australian team and a U.S.-Swiss team each conducted *in vitro* testing in BRAF inhibitor-resistant human melanoma cell lines and tumor samples from patients treated with PLX4032.

Collectively, the two teams found that BRAF inhibition induced one of three prosurvival mechanisms in melanoma (see **Figure 1**, “**Pathways of acquired resistance in melanoma**”).

The U.S.-Australian team found that expression of mutant *NRAS* (*neuroblastoma Ras viral (v-Ras) oncogene*) activated the signaling of MAP kinase kinase 1 (MAP2K1; MEK1) and MAP2K2 (MEK2) in some resistant tumors.

The group also found that overexpression of platelet derived growth factor B (PDGFB; PDGF2) enabled tumor cell survival via an undetermined pathway in a different subset of resistant tumors.¹

The U.S.-Swiss group found that overexpression of *MAP3K8* (*MAP kinase kinase kinase 8; COT*) also led to resistance by activating MEK1 and MEK2 signaling.²

Knockdown of *PDGFB*, *NRAS* or *MAP3K8* or inhibition of MEK1 and MEK2 decreased the viability of resistant tumor samples or cell lines compared with BRAF inhibition or resensitizing the cells to BRAF inhibition.

The U.S.-Australian team was led by Roger Lo, assistant professor of medicine at the David Geffen School of Medicine at the **University of California, Los Angeles** and a member of UCLA's Jonsson Comprehen-

sive Cancer Center. The group included researchers from the **Vanderbilt-Ingram Cancer Center** and the **Peter MacCallum Cancer Centre**.

The U.S.-Swiss team was led by Levi Garraway, assistant professor of medicine at **Harvard Medical School** and **Dana-Farber Cancer Institute**, and included researchers from the **Broad Institute of MIT and Harvard**, **Massachusetts General Hospital**, **University Hospital Zurich**, **Novartis Institutes for BioMedical Research** and the **Genomics Institute of the Novartis Research Foundation**.

Both groups published their results in *Nature*.

“The two studies are consistent with our understanding that there appear to be multiple mechanisms of RAF pathway regulation involved in melanoma's acquired resistance to PLX4032,” Gideon Bollag, SVP of research at Plexxikon, told *SciBX*.

He noted that although BRAF is a primary target in melanoma, “we use the more general term RAF because we really aren't sure which members of the RAF family of proteins are specifically involved in resistance.”

Fighting back

Nevertheless, the mechanistic understanding has aided Plexxikon's design of next-generation RAF inhibitors. “We predict that our new compounds will be less susceptible to the proposed resistance mechanisms,” said Bollag.

Not surprisingly, he said the findings indicate that overcoming resistance might require combining BRAF inhibitors with other agents.

Jeffrey Legos, GSK's medicine development leader for its GSK2118436 BRAF inhibitor, agreed.

He said the MEK-activating *NRAS* mutations identified by Lo's team are consistent with GSK's unpublished studies in BRAF inhibitor-resistant melanoma cell lines and “suggest that a MEK inhibitor could work sequentially or in combination with a BRAF inhibitor” to combat resistance in melanoma.

GSK is already exploring those two strategies in the clinic. The company's GSK1120212 MEK inhibitor is in a Phase II trial to treat BRAF mutation-positive melanoma patients who have been treated with a BRAF inhibitor or other therapies. The company is also testing the combination of GSK2118436 and GSK1120212 in a Phase I trial to treat BRAF mutation-positive metastatic melanoma.

GSK2118436 also is in a Phase II trial as monotherapy to treat BRAF mutation-positive metastatic melanoma. The company expects to begin separate Phase III trials of GSK2118436 and GSK1120212 as single agents to treat advanced or metastatic melanoma this month.

Legos also noted that the *PDGFB* overexpression mechanism identified by Lo's team suggests that inhibiting phosphoinositide 3-kinase (PI3K) and protein kinase B (PKB; Akt)—a major signaling pathway downstream of *PDGFB*—could help treat BRAF inhibitor-resistant melanoma. Further studies need to confirm whether *PDGFB* actually activates PI3K signaling in those tumors, he said.

Legos also cautioned that both teams had presented “preliminary data in a small subset of cells and patient samples” that require confirmation.

Figure 1. Pathways of acquired resistance in melanoma. BRAF inhibitors can activate one of several prosurvival pathways in melanoma. According to two *Nature* studies, [a] BRAF inhibitors can induce [b(1)] overexpression of MAP kinase kinase kinase 8 (MAP3K8; COT), [b(2)] mutations in *neuroblastoma Ras viral (v-Ras) oncogene (NRAS)* that activate **CRAF (RAF1)** or [b(3)] overexpression of platelet derived growth factor B (PDGFB; PDGF2).

Overexpression of MAP3K8 or activation of CRAF can lead to upregulation of [c] MAP kinase kinase 1 (MAP2K1; MEK1) and MAP2K2 (MEK2) signaling and consequent [d] tumor cell survival, growth and proliferation.

Overexpression of PDGFB can also activate an unidentified pathway [d].

^A**E6201** from **Eisai Co. Ltd.** (Tokyo:4523; Osaka:4523) is an inhibitor of **MEK1** and **MAP kinase kinase kinase 1 (MAP3K1; MEKK1)** in Phase I to treat psoriasis. **AS 703026** from **Merck KGaA** (Xetra:MRK) is a MEK inhibitor in Phase I to treat acute myelogenous leukemia (AML) and solid tumors. Three other MEK inhibitors are in Phase I to treat solid tumors: **R7420** from Roche, **R7167** from Roche and **Chugai Pharmaceutical Co. Ltd.** (Tokyo:4519) and **TAK-733** from **Takeda Pharmaceutical Co. Ltd.** (Tokyo:4502). Two other MEK inhibitors are in Phase I to treat cancer: **RDEA119** from **Ardea Bioscience Inc.** (NASDAQ:RDEA) and Bayer and **WX-555** from **UCB Group** (Euronext:UCB).

Bollag agreed, adding that future studies “would have to determine the relative frequency of the three mechanisms of resistance and identify additional pathways of acquired resistance.”

Lo said his team’s follow-on studies have elucidated the prosurvival mechanism downstream of PDGFB. He declined to disclose details but said the group is preparing a manuscript.

His team also plans to study the effects of PDGFB, NRAS and MEK blockade in animals with xenografts of PLX4032-resistant melanoma tumors.

Garraway acknowledged that *in vivo* studies were an important next step but said his team had not yet generated the appropriate

mouse models. “We have also noted that the available MEK inhibitors aren’t perfect in terms of selectivity and potency,” he said. “So the design of new inhibitors will be an exciting area of research as well.”

The University of California has filed for a patent on the findings described by Lo’s team. He said the IP is available for partnering or licensing.

Dana-Farber and the Broad Institute have filed for a patent on the Garraway team’s findings. He declined to discuss the licensing status of that IP.

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COMPANIES AND INSTITUTIONS MENTIONED

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