New players in melanoma

By Lauren Martz, Staff Writer

Although mutant BRAF dominates any discussion about melanoma targets, activating mutations in the kinase occur only in 50% of patients, implying that other factors are at work. Researchers at **Children's Hospital Boston** and colleagues have used zebrafish models to identify two such factors: DHODH, an enzyme already being targeted for autoimmune diseases, and a histone methyltransferase called SETDB1.^{1,2}

In one *Nature* paper, the team reported that inhibitors of dihydroorotate dehydrogenase (DHODH) increased therapeutic responses in melanoma cells and mouse models in combination with PLX4720, a small molecule inhibitor of the oncogenic BRAF V600E mutation from **Plexxikon Inc.** PLX4032, a related BRAF inhibitor with better pharmacokinetics from Plexxikon and **Roche**, is in Phase III testing for melanoma.

A second *Nature* paper from the same group identified SET domain bifurcated 1 (SETDB1) as a melanoma oncogene that is overexpressed in the majority of melanomas and causes the disease to be more aggressive.

Repurposing leflunomide

DHODH catalyzes a step in the synthesis of pyrimidine nucleotides. There are at least five disclosed DHODH inhibitors in development for autoimmune indications, including Arava leflunomide, which is marketed by **sanofi-aventis Group** to treat rheumatoid arthritis (RA).

The target's role in melanoma was uncovered in a search for molecules that suppress neural crest progenitors. Neural crest cells are multipotent progenitors that give rise to diverse cell types including melanocytes and are the cells from which melanomas originate.

The team found markers of neural crest progenitors in 52 of 70 human melanoma samples, suggesting that impaired differentiation of the progenitors into melanocytes is a feature of the disease.

The group screened 2,000 molecules in zebrafish and found that one of the most potent inhibitors of neural crest progenitors was NSC210627, a research tool with an unknown mechanism. Subsequent *in vitro* studies revealed that NSC210627 inhibited DHODH.

To expand on the findings, the researchers picked up the more readily available DHODH inhibitor leflunomide and tested it in combination with PLX4720. In human melanoma cell lines, the combination suppressed cell growth better than either compound alone. In nude mice with human

melanoma xenografts, leflunomide plus PLX4720 caused almost complete regression in about 40% of tumors, which was more effective than either treatment alone or vehicle controls.

"We are in the process of planning a clinical trial" of leflunomide plus PLX4032, said Leonard Zon, director of the Stem Cell Research Program at Children's Hospital Boston, professor of pediatrics at **Harvard Medical School** and a **Howard Hughes Medical Institute** investigator.

Zon expects the combination to offer better efficacy than BRAF inhibition alone. "Leflunomide is altering the fate of the cell, driving it away from being a neural crest cell, and the Plexxikon drug is attacking cell proliferation. The two together therefore would alter different aspects of the melanoma," he said.

The two-pronged attack on melanoma also could decrease the emergence of resistance to PLX4032—cases of which were seen in Phase II testing of the compound.^{3,4}

"Discovering new ways to block the onset of BRAF inhibitor resistance in melanoma patients is currently one of the hottest topics in cancer research," said Keiran Smalley, assistant professor in the Comprehensive Melanoma Research Center & Program in Molecular Oncology at the **H. Lee Moffitt Cancer Center & Research Institute**.

Smalley said it is not yet clear whether leflunomide can help accomplish this goal. "In the experiments shown, animals were only treated with the drugs for relatively short periods of time—up to 12 days *in vivo*—and this is probably too early for resistance to have emerged," he said. "It is also known that melanoma cells have many mechanisms of escape from BRAF inhibition, so even if leflunomide is effective at abrogating resistance, it may not work for every cell line in every tumor."

Previous studies have shown that BRAF inhibition can induce survival mechanisms

that can cause resistance to the treatment. These include mutation of neuroblastoma Ras viral oncogene (NRAS), overexpression of platelet derived growth factor B (PDGFB; PDGF2) and overexpression of MAP kinase kinase kinase 8 (MAP3K8; COT).⁵

Smalley's lab is seeking to develop therapeutics for the 50% of patients who do not benefit from BRAF inhibition and is also studying the mechanisms by which melanoma cells evade BRAF inhibitors.

Gideon Bollag, SVP of research at Plexxikon, said that to enable clinical testing, the team should determine the appropriate dosing of leflunomide in animal studies for melanoma both alone and in combination with BRAF inhibitors, and they will need to gather toxicology data.

Smalley noted that toxicology studies will be particularly important because "leflunomide is known to have immunosuppressive activity—this is the basis of its anti-inflammatory activity in arthritis—and immunosuppression is associated with an increased risk of cancer development. The development of squamous cell carcinomas is already a known side effect of BRAF inhibitors. If we start combining BRAF inhibitors with immunosuppressive agents, the effect of this drug combination upon development of squamous cell carcinoma is hard to predict."

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H. Lee Moffitt Cancer Center &
Research Institute**

Bollag did say Plexxikon is evaluating the DHODH findings that were published in *Nature*. “It is always of great interest to find combinations with our compounds. We are grateful that people are doing this work to challenge our thinking about how to best treat the condition,” he said.

GSK2118436, a BRAF inhibitor from **GlaxoSmithKline plc**, is also in Phase III testing for melanoma.

Ready, SETDB1, go

Whereas the DHODH study used information about the initiation of melanoma from the zebrafish model to find a new way to get at the cancer, the SETDB1 study started with a search for amplified oncogenes that might promote melanoma.

In a genetic analysis of 101 human melanoma cell lines, the team found a copy number amplification on chromosome 1q21 that was associated with the disease.

To determine which amplified genes within the region contributed to melanoma, the team overexpressed those genes in the zebrafish melanoma model and found that *SETDB1* was a culprit. *SETDB1* overexpression increased melanoma aggressiveness and invasiveness in the fish compared with overexpression of a control protein. In human tissue samples, high levels of *SETDB1* expression occurred in 70% of melanoma patients.

In human melanoma cell lines, *SETDB1* overexpression downregulated the methyltransferase's target genes, including transcriptional regulators and *homeobox (HOX)* genes.

“From the studies shown, SETDB1 seems to be important at an early stage of melanoma development,” said Smalley. “If increased *SETDB1* expression proves to be an important event in early melanoma development, this could be a future target for melanoma prevention or early intervention.”

One catch is that expression of an enzymatically inactive mutant of the protein did not inhibit cell growth, which hints that other methyltransferases in complex with SETDB1 might compensate for the loss in SETDB1's activity.

“One may need to inhibit the formation of this complex by small molecule inhibitors,” said Gerd Pfeifer, professor and chair of cancer biology at the **Beckman Research Institute at City of Hope**.

“The team knocked out the target in the zebrafish, and the loss was compensated for by other methyltransferases,” noted Bollag. “The team needs to determine the right enzymatic activity to eliminate and then to determine how to target it safely.”

Bollag told *SciBX* that in principle, use of a general methyltransferase inhibitor could be a good approach to overcome the compensatory mech-

anisms for SETDB1 loss, but “the challenge comes from the expectation that a more promiscuous methyltransferase inhibitor could bring with it safety liabilities.”

Zon said that the next step for this work would be to develop a compound that blocks SETDB1's methyltransferase activity.

Zon told *SciBX* that patent applications for the use of combination drugs for each melanoma strategy have been independently filed and that the IP is available for licensing through Children's Hospital Boston.

The SETDB1 paper also included researchers from **Whitehead Institute for Biomedical Research**, **Paris Diderot University** and **Sapienza University of Rome**.

The DHODH paper included researchers from **Massachusetts Institute of Technology**, **Whitehead Institute for Biomedical Research**, **University of East Anglia**, **University of Michigan**, **University of Cambridge** and **Genzyme Corp.**, which ran an assay used in an experiment.

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COMPANIES AND INSTITUTIONS MENTIONED

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