EZH2 moves

By Joanne Kotz, Senior Editor

Epizyme Inc. and **GlaxoSmithKline plc** have independently reported small molecule inhibitors of histone methyltransferase enhancer of zeste homolog 2 that showed efficacy in preclinical models of lymphomas with activating mutations of the target.^{1,2} Both companies plan to take inhibitors of the histone methyltransferase into the clinic in cancer.

Epizyme initially will focus on non-Hodgkin's lymphoma (NHL) patients with activating mutations in *enhancer of zeste homolog 2* (*EZH2*). GSK declined to disclose how it will select a specific patient population. Neither company disclosed timing for an IND.

EZH2 is the catalytic subunit of polycomb repressive complex 2 (PRC2) and represses gene transcription by adding methyl groups to histone H3 lysine 27 (H3K27). Amplification or overexpression of *EZH2* and other components of PRC2 occurs in a variety of cancers, including breast cancer and lymphoma.

Even more compelling are results showing that EZH2 mutational status might help select patients who would respond to EZH2 inhibition.

In 2010, researchers at Epizyme first reported that somatic point mutations in Tyr641 lead to a gain in EZH2-catalyzed trimethylation of H3K27 (H3K27me3).³ Mutations at this site and other sites that were subsequently shown to increase EZH2 activity occur in about 10%–20% of NHL cases.

Now, Epizyme and GSK have taken steps toward pharmacologically validating EZH2 as a target in this subset.

Both companies conducted high throughput screens to look for PRC2 inhibitors. Optimization of the lead hits resulted in the identification of EPZ005687 by Epizyme and GSK126 by the GSK team. The compounds inhibited enzyme activity with *K_i* values of about 25 nM and 1 nM, respectively.

Importantly, both molecules inhibited mutant and wild-type EZH2 with similar potency, which meant the researchers could study the effects of blocking EZH2 in cancers with mutant and wild-type versions of the target.

The teams looked at the effects of inhibiting EZH2 in lymphoma cell lines with mutant and wild-type *EZH2* and found mutational status is a major—but not sole—determinant of lymphoma cell sensitivity to EZH2 inhibition.

EPZ005687 decreased cell proliferation in two NHL cell lines with activating mutations but not in a cell line containing wild-type *EZH2*.

GSK126 decreased cell proliferation in six of nine cell lines tested that contained mutant *EZH2*. On the other hand, 35 of 37 lymphoma cell lines containing wild-type *EZH2* were resistant to EZH2 inhibition.

Epizyme published its results in *Nature Chemical Biology*. The GSK team reported its findings in *Nature*.

“Clearly, the most important finding is the discovery of potent and selective small molecule EZH2 inhibitors. This represents an important step in the maturation of EZH2 as a drug discovery target,” said Patrick Trojer, senior director and head of biology at epigenetics company **Constellation Pharmaceuticals Inc.**

Constellation has a preclinical program targeting EZH2. Trojer said the company has identified selective small molecule inhibitors of EZH2 and is optimizing and testing them in preclinical cancer models.

“These two landmark papers define a novel chemotype that selectively inhibits mutant and wild-type EZH2 and provide pharmacologic target validation of the enzymatic function of somatically altered EZH2 in non-Hodgkin's lymphoma,” added James Bradner.

Bradner is an investigator in the Department of Medical Oncology at **Dana-Farber Cancer Institute** and assistant professor in the Department of Medicine at **Harvard Medical School**. He also is a founder of epigenetics companies **Acetylon Pharmaceuticals Inc.** and **Tensha Therapeutics Inc.**

A path to the clinic

Epizyme and partner **Eisai Co. Ltd.** have identified the molecule they expect to take into the clinic and hope to submit an IND “in the very near future,” according to Epizyme EVP and CSO Robert Copeland. Epizyme's lead molecule comes from a distinct chemical series from EPZ005687 and “as an initial indication we'll be targeting *EZH2* mutant-bearing non-Hodgkin's lymphomas,” he noted.

GSK continues to have an active EZH2 inhibitor program, said Caretha Creasy, director of biology and translational medicine at GSK. She did not specify GSK's patient selection strategy or the company's timing for entering the clinic, but she did say that in NHL, “*EZH2* mutation plus high levels of H3K27me3 predicted sensitivity very well, which would be an obvious selection criterion for the clinic.”

Both companies also are exploring other cancer subtypes in which EZH2 inhibitors might work, although GSK is not disclosing which cancers.

Epizyme is looking at cancers in which PRC2 subunits are known to be amplified, including prostate, breast and esophageal cancers and myeloma, said Copeland.

Looking for markers

There are other oncogenic alterations besides mutant *EZH2* that could result in tumor sensitivity to EZH2 inhibition, and these genetic alterations could be used to define patient populations.

One potential marker for EZH2 efficacy is genetic alternations of lysine-specific demethylase 6A (KDM6A; UTX), a histone demethylase that removes methyl groups from H3K27. Inactivation of *UTX* leads

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Dana-Farber Cancer Institute

to higher levels of H3K27me3, similarly to activating mutations in *EZH2*.

“An exploration of these *EZH2* inhibitors more broadly in cancer, specifically in *UTX* mutated or deleted tumor models, is an obvious and important next step for this research,” said Bradner.

In addition to *UTX* mutations as a potential marker, Trojer noted that “prostate cancers frequently carry a heterozygous deletion of miR-101, a microRNA that attenuates *EZH2* expression. Thus, prostate cancer cases with miR-101 deletions have elevated levels of *EZH2* and might be dependent on *EZH2* activity.”

Epizyme has patents and patent applications covering composition of matter, methods of screening and methods of clinical use around *EZH2*. The program is partnered with Eisai. GSK declined to disclose the patent and licensing status of its work.

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