AML takes LSD1

By Chris Cain, Staff Writer

Two teams have independently provided preclinical proof of concept for using lysine-specific histone demethylase 1 inhibitors to treat acute myelogenous leukemia.^{1,2} One of the teams is now running a clinical trial of a generic monoamine oxidase inhibitor that counts the demethylase among its many targets, whereas at least four biotech companies are advancing other more potent and selective inhibitors through preclinical development.

In 2004, researchers at **Harvard Medical School** and **The Johns Hopkins University School of Medicine** identified the monoamine oxidase lysine-specific histone demethylase 1 (KDM1A; LSD1) as being able to demethylate histones, thus constituting a key step in the regulation of chromatin structure and gene expression.³ Subsequent studies found LSD1 overexpression in several types of cancer, and small interfering RNA experiments showed LSD1 knockdown suppressed cancer cell growth.^{4,5}

In 2010, Italian researchers synthesized inhibitors of LSD1 with activity in acute promyelocytic leukemia (APL) cell lines.⁶ It remained to be seen, however, whether such treatment would be effective *in vivo* against a broad range of acute myelogenous leukemia (AML) subtypes.

Epigenetic regulation of gene expression is often altered in blood cancers including AML. Indeed, many epigenetic regulators—including enhancer of zeste homolog 2 (EZH2), histone methyltransferase DOT1L (DOT1L) and bromodomain containing 4 (BRD4)—have been nominated as promising targets in the last few years.⁷

Now two U.K. teams have produced independent lines of *in vivo* evidence that collectively suggest LSD1 should be added to the list of epigenetic targets.

Mice on LSD1

A team led by Tim Somervaille, group leader at **Cancer Research UK's** Leukaemia Biology Laboratory at the **Paterson Institute for Cancer Research**, set out to identify histone demethylases that regulate the function of leukemia stem cells (LSCs). His group undertook a whole-genome microarray data analysis of AML samples and found LSD1 expression was most strongly correlated with LSC potential.

To test whether LSD1 had a functional role in maintaining the LSC state, the team first used small hairpin RNA in mixed-lineage leukemia (MLL), a subtype of AML. In mouse MLL cells, shRNA against LSD1 decreased leukemia cell colony formation compared with vehicle control.

The researchers next wanted to test the effect of pharmacologically inhibiting LSD1 in additional AML cell lines. However, the only available inhibitor of LSD1 was tranylcypromine, a generic monoamine oxidase

inhibitor (MAOI) marketed to treat mood and anxiety disorders.

Tranylcypromine inhibits LSD1 at a low, double-digit micromolar IC₅₀ value, which is far above the nanomolar plasma concentrations achieved in clinical use. To obtain a more potent inhibitor, the group turned to the patent literature and found that **Oryzon Genomics** had filed for a patent in 2010 covering the composition of tranylcypromine derivatives with nanomolar IC₅₀ values against LSD1.

Rather than contact the company, Somervaille told *SciBX* his team relied on drug synthesis resources available at the Paterson Institute to produce the derivatives.

In a panel of AML cell lines and patient samples, one of the tranylcypromine derivatives lowered leukemia cell colony formation compared with vehicle. In a mouse model of MLL, the inhibitor decreased leukemic cell counts compared with vehicle control.

Results were published in *Cancer Cell*.

Somervaille noted that the effects of LSD1 inhibition were most pronounced in AML cells driven by translocations in the *myeloid-lymphoid or mixed-lineage leukemia (MLL; HRX)* oncogene. He hypothesized that this could be because LSD1 directly interacts with the regulatory complex.

Thus, his team's next steps include experiments in additional AML cell lines and patient samples driven by other mutations or translocations to characterize the mechanisms underlying the efficacy of LSD1 inhibition.

The derivative did not cause obvious toxicity to normal bone marrow stem and progenitor cells, although it did impact the production of red blood cells and platelets. Although treatment of mice significantly impacted leukemia cell proliferation, there was no significant prolongation of survival in mice because animals treated with the compound died as a result of drug-induced anemia. Somervaille suggested this could be managed in humans by adjusting dose or performing blood transfusions, a common procedure in AML patients.

Tamara Maes, cofounder and CSO of Oryzon, told *SciBX* it was important to distinguish the side effects of LSD1 inhibition from outright toxicity.

"LSD1 inhibition will reduce the proliferation of hematopoietic stem cells in the bone marrow in a dose-dependent manner, and as a consequence, complete and sustained inhibition will lead to thrombocytopenia, neutropenia and erythropenia," she said. "The effect of LSD1 should not, however, be confused with the effect of older, highly cytotoxic drugs that killed the bone marrow cells. We know that the effect on normal blood cells is perfectly reversible."

Epigenetic cocktail party

Whereas Somervaille was investigating the use of highly potent LSD1 inhibitors, the other U.K. team took a different tack and showed that even mild LSD1 inhibition could be enough to treat leukemia.

Arthur Zelent, reader in biochemistry in the Division of Molecular Pathology at **The Institute of Cancer Research**, led a group that came upon LSD1 while seeking an epigenetic explanation for the long-standing question in leukemia treatment of why APL responds to all-*trans* retinoic acid (ATRA) and other AML subtypes do not.

ATRA is a generic drug that reduces the proliferation of promyelocytic leukemia cells by inducing their differentiation into a less-proliferative cell type.

Because LSD1 overexpression in AML is correlated with alterations in the histone methylation status of ATRA-responsive genes, Zelent hypothesized that inhibiting LSD1 could improve sensitivity to ATRA in unresponsive leukemia samples.

In AML cell lines or patient samples, ATRA plus LSD1 shRNA or tranylcypromine increased the differentiation of leukemia-initiating cells compared with either agent alone.

In a xenograft mouse model of AML, tranylcypromine plus ATRA decreased leukemic engraftment and tumor burden compared with either agent alone.

Results were published in *Nature Medicine*.

Zelent told *SciBX* he used tranylcypromine because it could be immediately translated to the clinic.

“Tranylcypromine has been safely used to treat depression for many years,” he said. “We have initiated a Phase II pilot study in collaboration with Carsten Müller-Tidow, head of the lab for molecular hematology and oncology at **University Hospital Muenster**. Additional clinical trials are planned in the U.K.” The German study is testing ascending doses of tranylcypromine in combination with ATRA in patients with treatment-refractory AML.

Researchers contacted by *SciBX* were skeptical that tranylcypromine would be effective.

“It’s a difficult drug—it is used as one of the last-line treatments for depression, it can have serious side effects and it is contraindicated for use with many other drugs,” noted Maes. She added that the dose of tranylcypromine used in the study would translate to about the maximum dose of the drug indicated for use in humans.

Side effects of tranylcypromine include hypotension or hypertension, drowsiness and nausea.

Zelent acknowledged that “in the future we hope to replace tranylcypromine with more specific LSD1 inhibitors.” But he added that because of the effects seen with clinically relevant concentrations in mouse models, it was important to test the approach in the clinic.

Kapil Bhalla, deputy director of **The University of Kansas Cancer Center**, chief of personalized cancer medicine and coleader of the center’s drug discovery, delivery and experimental therapeutics program, thinks the most important aspect of the paper was not the potential of tranylcypromine but the message that LSD1 inhibitors should be tested in combination with other leukemia drugs.

Bhalla is collaborating with **Salarius Pharmaceuticals LLC**, a company founded in 2011 to license and develop small molecule LSD1 inhibitors discovered at the **Huntsman Cancer Institute at the University of Utah**. This week he is presenting data at the American Association for Cancer Research annual meeting showing a synergistic effect of the specific LSD1 inhibitor SP-2059 with histone deacetylase (HDAC) or EZH2 inhibitors in AML cell lines. He serves as scientific advisor to Salarius.

LSD1 lineup

At least four biotech companies have LSD1 inhibitor programs. In addition to Oryzon and Salarius, **Epi Pharmaceuticals Inc.** is developing specific LSD1 inhibitors. The company was spun out of **Progen Pharmaceuticals Ltd.** in 2011 to develop epigenetic and cell proliferation assets obtained when Progen bought CellGate Inc. for \$1.5 million in stock and up to \$19.5 million in milestones.

Robert Casero, professor of oncology at The Johns Hopkins University School of Medicine and former consultant to CellGate, told *SciBX* that Epi is operating in a virtual mode and said that polyamine-based specific inhibitors of LSD1 have been tested in mouse models of breast, prostate and lung cancer. The company has not yet selected

a lead candidate or indication.⁸ Casero is co-inventor of the polyamine analogs licensed by the company and was a senior author on the 2004 paper that first described LSD1’s function.

Frontier Pharma Inc., a Japanese biotech located in the Nagahama Bio Incubation Center, confirmed to *SciBX* that the company is also actively developing preclinical LSD1 inhibitors. In 2009, a research team from the company, **Nagahama Institute of Bio-Science and Technology** and **Nagoya City University** published a description of selective LSD1 inhibitors with low micromolar potency and activity against a panel of cancer cell lines.⁹

Maes said Oryzon has an advanced lead candidate and hopes to begin Phase I testing to treat hematological malignancies next year.

Salarius CEO Jon Northrup said the company has two clinical candidates and is planning to enter the clinic in 12–18 months. The company has selected myelodysplasia and prostate cancer as its lead indications and is also testing the compounds in preclinical models of triple-negative breast cancer.

“It seems that there is a rather broad consensus on the beneficial effect of LSD1 inhibition in cancer in general. In addition to AML, elevated expression levels of LSD1 and/or a growth-promoting function of LSD1 has been demonstrated for prostate, breast, bladder, lung and colorectal cancers and neuroblastomas,” said Peter Staller, director of oncology research at **EpiTherapeutics ApS**.

In addition, late last year a **Nevada Cancer Institute**–led team used their own potent LSD1 inhibitors to show that blocking the enzyme could enable specific targeting of cancer stem cells.¹⁰

EpiTherapeutics does not have an LSD1 inhibitor program, but Staller said the target is on their radar. The company is pursuing histone demethylases as drug targets but is focusing on a different enzymatic class known as jumonji domain-containing proteins.

Staller thinks there is reason to suspect inhibiting LSD1 may have an on-target anemia effect but said more detailed side effect data need to be seen with distinct classes of LSD1 inhibitors.

Bhalla said he has not seen any side effects—including anemia and thrombocytopenia—for Salarius’ compound in mouse models and questioned whether the adverse events reported in the *Cancer Cell* paper were compound specific.

“It seems that there is a rather broad consensus on the beneficial effect of LSD1 inhibition in cancer in general. In addition to AML, elevated expression levels of LSD1 and/or a growth-promoting function of LSD1 has been demonstrated for prostate, breast, bladder, lung and colorectal cancers and neuroblastomas.”

**—Peter Staller,
EpiTherapeutics ApS**

At least two larger companies have published data indicating they may have an interest in LSD1. In 2011, **Amgen Inc.** published at least two papers describing screening techniques to identify specific LSD1 inhibitors.^{11,12} The company declined to comment. **Novartis AG** has published at least two papers since 2009 characterizing the development of mice with defects in LSD1 function.^{13,14} Novartis did not disclose additional details.

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COMPANIES AND INSTITUTIONS MENTIONED

Amgen Inc. (NASDAQ:AMGN), Thousand Oaks, Calif.
Cancer Research UK, London, U.K.
Epi Pharmaceuticals Inc., no location yet
EpiTherapeutics ApS, Copenhagen, Denmark
Frontier Pharma Inc., Nagahama City, Japan
Harvard Medical School, Boston, Mass.
Huntsman Cancer Institute at the University of Utah, Salt Lake City, Utah
The Institute of Cancer Research, Sutton, U.K.
The Johns Hopkins University School of Medicine, Baltimore, Md.
Nagahama Institute of Bio-Science and Technology, Shiga, Japan
Nagoya City University, Nagoya, Japan
Nevada Cancer Institute, Las Vegas, Nev.
Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland
Oryzon Genomics, Cornellà de Llobregat, Spain
Paterson Institute for Cancer Research, Manchester, U.K.
Progen Pharmaceuticals Ltd. (ASX:PGL; Pink:PGLA), Brisbane, Queensland, Australia
Salarius Pharmaceuticals LLC, Salt Lake City, Utah
University Hospital Muenster, Muenster, Germany
The University of Kansas Cancer Center, Kansas City, Kan.