Tapping TOPK

By Benjamin Boettner, Senior Writer

Although targeting proteins involved in cell division is falling out of fashion in cancer research because of the difficulty of distinguishing between diseased and normal cells, several companies are still exploring enzymes that could be tractable targets. A new inhibitor of TOPK—an enzyme involved in mitosis—could provide an option for cancers unserved by targeted therapies, based on findings in mice that liposomal delivery reduces the compound's toxicity, keeps it away from hematopoietic cells and increases its therapeutic index.[1]

The inhibitor was developed by Japanese biotech **OncoTherapy Science Inc.** in collaboration with researchers at **The University of Chicago**. The team is optimizing the formulation for use in solid tumors but is starting with a Phase I trial in acute myelogenous leukemia (AML), in which the potential for hematopoietic toxicity would not pose a significant risk.

TOPK (PDZ binding kinase; PBK)—also known as T lymphocyte–activated killer cell-originated protein kinase—is involved in cytokinesis, the process by which two daughter cells split from one another at the end of mitosis.

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> ***—Chao Zhan, Daiichi Sankyo Co. Ltd.***

Inhibitors of cytokinesis were originally touted as safer alternatives to other drugs affecting cell division such as microtubule-targeted therapies because microtubules are involved in a wide array of cellular functions in both cancer and normal cells.

However, some compounds targeting polo-like kinase 1 (PLK1; STPK13) and aurora kinases—key enzymes involved in cytokinesis—have shown toxicity in clinical studies, most likely because they were not selective for cancer cells over normal cells.

Nevertheless, several PLK1 and aurora programs are still alive, and a number of candidates are in clinical development.

There are two compounds in the clinic specific for PLK1: **Boehringer Ingelheim GmbH**'s BI-6727, a small molecule in Phase I to III studies for various cancers, and **Tekmira Pharmaceuticals Corp.**'s TKM-080301, an siRNA in Phase I and Phase I/II trials for various solid tumors.

In addition, there are at least five compounds in the clinic that act specifically on aurora kinases: **AbbVie Inc.**'s ABT-348, **Amgen Inc.**'s AMG 900, **GlaxoSmithKline plc**'s GSK1070916, **Nerviano Medical Sciences s.r.l.**'s danusertib and **Takeda Pharmaceutical Co. Ltd.**'s alisertib. All are small molecules in Phase I to III testing for various cancers.

Unlike PLK1 and aurora kinases, TOPK has been relatively unexplored in drug development because its role in cancer cell cytokinesis is poorly understood.

The enzyme shows highly elevated expression in several cancers including lymphomas, breast cancer and lung cancer. Its activity peaks in the M phase of the cell-division cycle, during which it phosphorylates several proteins involved in cancer cell cytokinesis, such as histone H3, protein regulator of cytokinesis 1 (PRC1), G protein signaling modulator 2 (GPSM2) and valosin containing protein (VCP; p97).

Many cancer cells upregulate TOPK and require its activity to drive their rapidly progressing cell cycles. Because the kinase is either not expressed or expressed at relatively low levels in most healthy tissues, targeting it in cancer cells could eliminate some of the risks associated with other cytokinesis enzymes.

In 2011, the OncoTherapy and University of Chicago team, led by Yusuke Nakamura, identified a potent TOPK inhibitor—dubbed OTS514—through SAR and screening studies but found it induced severe hematopoietic toxicity.

Now, Nakamura and colleagues have optimized the compound using medicinal chemistry and circumvented its toxicity by using a liposomal formulation that shields the drug and reduces its effects on normal cells.

Nakamura is a professor of medicine at the University of Chicago.

Top delivery

To increase the potency and bioavailability of OTS514, the team employed SAR to design and synthesize a dimethylated analog, OTS964. The optimized compound inhibited TOPK in a panel of TOPK-expressing cancer cell lines with low nanomolar IC_{50} values and high selectivity for TOPK over 60 other human protein kinases.

In lung cancer cell lines, OTS964 suppressed phosphorylation of TOPK and its downstream mediator histone H3, disrupted cytokinesis and caused apoptosis. Those effects mimicked results from another team that used siRNA to knock down TOPK.[2]

Next, Nakamura and colleagues administered OTS964 to tumor-bearing mice and observed that although it had potent antitumor activity, it caused notable hematopoietic toxicity. Specifically, the compound diverted differentiation of hematopoietic stem cells away from white and red cell lineages and toward megakaryocytes and platelets, which led to anemia and leukocytopenia in the animals.

The team thought that liposomal delivery could alter the therapeutic index because that strategy has improved the safety profile of several other chemotherapeutics, such as DaunoXome daunorubicin from **Gilead Sciences Inc.** and **Galen Ltd.** and Myocet liposomal doxorubicin from **Teva Pharmaceutical Industries Ltd.** and **Sopherion Therapeutics LLC**.

Liposomes increase the accumulation of compounds within tumors over normal tissues by a phenomenon known as the enhanced permeation and retention (EPR) effect, which stems from the increased

leakiness and highly abnormal vascularization typically found in tumor tissues.

In tumor-bearing mice, liposomal OTS964 caused no hematopoietic abnormalities when delivered intravenously, but with oral delivery it provoked a temporary decrease of white blood cell numbers that returned to normal within two weeks.

In addition, the liposomal formulation of OTS964 induced complete regression of tumors formed from an aggressive human lung cancer that was faster than the regression produced by the free inhibitor. The liposomal formulation did not cause any body weight loss when given either intravenously or orally.

"The significance of the findings lies in the identification of a delivery system that enhanced the absorption of the drug into the tumor while shielding it from blood cells in circulation," said Chao Zhan, VP of research at **Daiichi Sankyo Co. Ltd.**'s Plexxikon Inc. subsidiary. "Although this approach has been used in the clinic, including the clinical development of anticancer agents, it is the first time this was applied to extend the therapeutic index of an experimental antimitotic drug."

Blood lines

Although the compound will need to go through standard testing for safety and tolerability, the emphasis will likely be on its potential hematopoietic liability.

Dominique Bonnet told *SciBX*, "Pushing normal hematopoietic stem cells into megakaryocyte differentiation might exhaust the stem cell pool, especially in patients with less functional hematopoietic stem cells, like aged individuals with myelodysplastic syndrome."

She said that orthotopic xenografts from patient primary samples transplanted directly into the lungs of mice would allow the compound to be tested on cells in their endogenous location. That would provide valuable information beyond the xenografts used thus far, which were all subcutaneous. Bonnet is a laboratory head at the **London Research Institute** and studies regulation of normal hematopoietic and leukemic stem cells.

According to Zhan, the team needs to focus on selecting the right type of cancer. "Although TOPK is upregulated in a spectrum of tumor types, it is unrealistic to expect that OTS964 will be effective against all cancers or that the liposome formulation can help deliver the drug to tumors at all anatomical locations," he said.

He added, "Screening a large panel of preclinical tumor models and a better understanding of the exact role of TOPK in controlling cytokinesis may help them zoom in on the tumor types most vulnerable to TOPK inhibition."

According to Nakamura, the inhibitor can be applied to 50%–80% of common cancer types, but his team is going to focus on patients with AML in its first clinical studies. This is because the team thinks that in cases in which leukemia is accompanied by leukocytopenia and reduced platelet numbers, the thrombocytotic effect of the TOPK inhibitor could be an advantage, he said.

He also noted that OTS964 was effective in a subset of AML samples in preclinical testing.

Nakamura said that the team is continuing its mechanistic studies on TOPK and wants to identify the enzyme's substrates and downstream pathways.

The researchers are planning to start a trial in AML by next summer using the free TOPK inhibitor. They also are preparing a clinically usable liposomal formulation for studies in solid cancers and plan for it to be ready by YE15. OncoTherapy has started GMP manufacturing of the inhibitor for its own preclinical studies.

In addition, Nakamura thinks that because inhibition of TOPK induces the differentiation to megakaryocytes and platelets, the compound may help overcome thrombocytopenias in myelodysplastic syndrome (MDS) or idiopathic thrombocytopenia purpura (ITP).

However, Bonnet cautioned, "it is too early to speculate about a potential use of this drug in thrombocytopenias. Evaluating the mechanisms of action and exactly which cells in the hematopoietic lineages are affected will be necessary before any potential clinical use" in noncancer indications.

OncoTherapy has filed a patent, and Nakamura told *SciBX* that OTS964 is available under a material transfer agreement.

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COMPANIES AND INSTITUTIONS MENTIONED

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