Selecting CDK7

By Chris Cain, Senior Writer

Selective inhibitors of cyclin dependent kinases have been hard to develop because the active sites of many family members are so similar. Now, a **Harvard Medical School** team has found a cyclin dependent kinase 7 inhibitor that covalently binds a cysteine residue outside the enzyme's kinase domain and suppresses leukemia in mice.¹

The strategy could pave the way for other specific inhibitors, but the key question is whether selective targeting results in a better therapeutic index.

Syros Pharmaceuticals Inc. has licensed the new compound, dubbed THZ1, and is incorporating it into the company's preclinical cyclin dependent kinase 7 (CDK7) inhibitor program.

CDKs are key regulators of cell proliferation. However, because CDK inhibitors have been designed to interact with the ATP-binding domain—which is conserved across many kinases—it has been difficult to target the right pathway without triggering off-target effects. There are a handful of multi-target CDK inhibitors in development, most notably inhibitors of CDK4 and CDK6, as well as additional compounds that inhibit CDK2, CDK7 and CDK9.

Thus, according to Bert Klebl, the high selectivity of THZ1 is a major achievement.

"The major challenge for CDK inhibitors in general has been the lack of a therapeutic window," he said. "In most of the pharmacological experiments, efficacy and toxicity were hard to separate from one another. The lack of CDK-family selectivity has been the biggest hurdle for most of the first-generation CDK inhibitors." Klebl is CSO and managing director of **Lead Discovery Center GmbH**, which also has a selective CDK7 inhibitor program.

Existing CDK inhibitors in development include **Cyclacel Pharmaceuticals Inc.**'s seliciclib, **Sunesis Pharmaceuticals Inc.**'s SNS-032 and **Tolero Pharmaceuticals Inc.**'s alvocidib (flavopiridol).

Seliciclib inhibits CDK2, CDK7 and CDK9 and has completed Phase II testing in non-small cell lung cancer (NSCLC), in which it missed the primary endpoint of improving progression-free survival. It is also in development in additional indications. SNS-032 also is a CDK2, CDK7 and CDK9 inhibitor and completed Phase I testing in advanced B-lymphoid malignancies. Alvociclib is a pan-CDK inhibitor in Phase II testing for acute myelogenous leukemia (AML). The compound was licensed from **Sanofi** last year and was previously tested in multiple Phase II trials.

Nathanael Gray, lead investigator of the Harvard study, told *SciBX* that his team was not specifically looking for an inhibitor of CDK7 and instead was looking for cancer research tools.

The group used phenotypic screening to identify covalent inhibitors for any kinase that could suppress proliferation, regardless of the target. To do that, the team screened cancer cell lines for inhibitors of proliferation using a small molecule library that was enriched in covalent kinase inhibitors from Gray's earlier work in that space.

Gray is a professor of cancer biology at the **Dana-Farber Cancer Institute** and a professor of biological chemistry and molecular pharmacology at Harvard Medical School.

In the screen, THZ1 inhibited T cell acute lymphoblastic leukemia (T-ALL) cell lines at low nanomolar concentrations. Its structure showed that it had a chemical group that could interact with a reactive cysteine, whereas a modified compound lacking that ability was ineffective. Using kinome profiling, the team was surprised to find CDK7 as the primary target of THZ1.

"We had done a careful bioinformatics inventory on kinases with a reactive cysteine residue in the ATP site, and CDK7 did not have one, so it was unexpected that CDK7 should be a target," said Gray. "It turns out the compound was alkylating a cysteine residue outside of the canonical ATP-binding domain, and the available crystal structure stopped one residue short."

THZ1 inhibited cell growth in 527 diverse cancer cell lines—over 50% of the total number tested—with IC₅₀ values below 200 nM. The results suggested that the compound has broad anticancer activity. It was particularly potent in T-ALL cells, in which it worked at low nanomolar concentrations.

In a xenograft mouse model of T-ALL, THZ1 decreased tumor growth compared with modified control compound and did not cause weight loss or other toxic effects.

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—Bert Klebl,
Lead Discovery Center GmbH

ALL about super-enhancers

To discover how THZ1 induced cell death, the researchers focused on RNA polymerase,

a known target of CDK7. In cancer cells, THZ1 blocked phosphorylation of RNA polymerase and decreased the overall mRNA levels compared with the inactive, modified compound.

However, that raised the question of how THZ1 could be more potent in some cells if it acted by blocking transcription globally. To answer this, Gray teamed up with Richard Young, a member of the **Whitehead Institute for Biomedical Research** and a professor of biology at the **Massachusetts Institute of Technology**, who specializes in global transcriptional analysis. Last year Gray and Young cofounded Syros together with James Bradner, a BET bromodomain expert.²

Bradner is an investigator in the Department of Medical Oncology at Dana-Farber, an associate professor in the Department of Medicine at Harvard Medical School and associate director of the Center for the Science of Therapeutics at the **Broad Institute of MIT and Harvard**.

In T-ALL cells, Gray and Young showed that a subset of genes was sensitive to low concentrations of THZ1, and the oncogene runt-related transcription factor 1 (RUNX1) stood out as particularly sensitive.

Chromatin immunoprecipitation coupled with high throughput sequencing (ChIP-seq) of multiple transcription factors including

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**—Nancy Simonian,
Syros Pharmaceuticals Inc.**

The authors proposed that because super-enhancers are particularly sensitive to perturbation, THZ1’s potency in T-ALL cells might be due to its action on the RUNX1 super-enhancer causing large-scale disruption of gene expression and cell death.

Results were published in *Nature*.

Syros CEO Nancy Simonian told *SciBX*, “What these data demonstrate is the critical role this transcriptional kinase plays in enabling the dominant expression of oncogenes by super-enhancers.”

Steven Warner, VP of drug discovery and development at Tolero, said that alvocidib also affects the transcriptional elongation of genes such as *RUNX1* that are regulated by super-enhancers. Indeed, Gray’s team tested both alvocidib and THZ1 and saw distinct biochemical effects, although both compounds had efficacy in T-ALL cells.

Gray told *SciBX* that this work provides further support for using super-enhancer mapping to predict drug response. “Super-enhancer analysis is now going to be applied broadly to drugs inhibiting transcription. I think the challenge in the field has been to understand where you are going to see the maximum efficacy vs. toxicity with the inhibitors, and there is no simple way to predict that based on sequence alone,” he said.

Getting specific

According to Klebl, the most striking finding from the paper was the specificity of THZ1.

“I consider high selectivity to be the major achievement. Since not all of the CDK family members do have a cysteine just outside the kinase domain, selectivity might be improved by addressing this cysteine. Therefore, it is an important result that the affinity of CDK12 for THZ1 is approximately two orders of magnitude lower than for CDK7, emphasizing the selectivity of THZ1 for CDK7 over other CDKs,” he said. He added that this could differentiate the compound from other CDK inhibitors in clinical development.

Klebl said that the key next step for THZ1 will be to detail its pharmacological properties. “I would like to see many more pharmacological experiments; DMPK [drug metabolism and pharmacokinetics] studies, dose dependencies, dose escalation studies, toxicity and tolerability studies,” he said. “We need to see a lot more pharmacological data to understand if THZ1 is just a tool compound or if it is a lead or drug candidate.”

RUNX1 identified a super-enhancer upstream of *RUNX1* that could explain its sensitivity to THZ1. Super-enhancers are regulatory elements that bind to more proteins and control the expression of larger numbers of genes than regular enhancers.^{3,4}

Syros is not disclosing many details about next steps for its CDK7 program. Simonian told *SciBX* that the company is now looking at a wide range of cancers. “We are using our platform to determine which tumors are most dependent on transcription for their survival. There are several cancers and subtypes that are most sensitive to CDK7 inhibition, and we are deciding which indications to pursue, but for competitive reasons we aren’t disclosing what those are at this time.”

While the biotech is deciding which cancers to prioritize, the Lead Discovery Center is pushing forward with developing its own CDK7-selective inhibitors.

Klebl told *SciBX* that unpublished results from their program corroborate the *Nature* findings. “The authors strongly focus on a potential therapeutic use for T-ALL and potentially other leukemias/lymphomas. Indeed—and this matches our own data with our highly selective, proprietary CDK7 inhibitors—the effects of CDK7 inhibition are particularly strong in leukemias and lymphomas.”

He said that his organization will now compare its proprietary CDK7 inhibitors with THZ1 “to get more insight into pharmacology and the potential impact of an extended residence time of a covalent CDK7 inhibitor on target.”

He added that data from both his and Gray’s labs suggest that inhibiting CDK7 can work in solid tumors too.

The Dana-Farber Cancer Institute has filed patents covering THZ1, and the Whitehead Institute has filed patents covering bioinformatics approaches used to identify super-enhancers. All of the patents have been exclusively licensed to Syros. The Lead Discovery Center’s CDK7 compounds are available for licensing or partnering.

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Contact: Nathanael S. Gray, Dana-Farber Cancer Institute, Boston, Mass.
e-mail: nathanael_gray@dfci.harvard.edu
Contact: Richard A. Young, Whitehead Institute for Biomedical Research, Cambridge, Mass.
e-mail: young@wi.mit.edu
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COMPANIES AND INSTITUTIONS MENTIONED

Broad Institute of MIT and Harvard, Cambridge, Mass.
Cyclacel Pharmaceuticals Inc. (NASDAQ:CYCC), Berkeley Heights, N.J.
Dana-Farber Cancer Institute, Boston, Mass.
Harvard Medical School, Boston, Mass.
Lead Discovery Center GmbH, Dortmund, Germany
Massachusetts Institute of Technology, Cambridge, Mass.
Sanofi (Euronext:SAN; NYSE:SNY), Paris, France
Sunesis Pharmaceuticals Inc. (NASDAQ:SNSS), South San Francisco, Calif.
Syros Pharmaceuticals Inc., Watertown, Mass.
Tolero Pharmaceuticals Inc., Lehi, Utah
Whitehead Institute for Biomedical Research, Cambridge, Mass.