Purely proteasome

By Kai-Jye Lou, Staff Writer

The tumor suppressor cyclin-dependent kinase inhibitor 1B has the characteristics of a good cancer target—it often malfunctions in tumors, and high levels of it correlate with improved patient prognosis. With that in mind, researchers at **Hannover Medical School** have been hunting for a molecule that increases the tumor suppressor's levels. In a paper in *Cancer Cell*, the group reported on argyrin A, a proteasome inhibitor that modulates the target and could have comparable efficacy and potentially better safety than the marketed proteasome inhibitor Velcade bortezomib.

One caveat is that increasing levels of cyclin-dependent kinase inhibitor 1B (p27^{Kip1}) has the potential to actually increase cell proliferation. As a result, some researchers would like to see additional *in vivo* pharmacokinetic and safety data on argyrin A before it enters the clinic.

Proteasome inhibition was a logical choice for increasing p27^{Kip1} expression. Low levels of p27^{Kip1} in cancer tissues is believed to be due to increased enzymatic degradation of the protein rather than mutations in the gene. The lower levels result from aberrant turnover caused by proteasome-dependent pathways.¹⁻³

Enter argyrin A, a cyclic peptide derived from the soil bacterium *Archangium gephyra*. Short interfering RNA studies showed that the effects of argyrin A were dependent on the stabilization and thus low turnover of p27^{Kip1}.⁴ In multiple cancer cell lines, nanomolar concentrations of argyrin A increased p27^{Kip1} expression and resulted in higher rates of apoptosis and cell-cycle arrest than those seen in untreated controls.

In colon carcinoma murine xenograft models, argyrin A minimized tumor proliferation compared with that seen in saline-treated controls,

and it had efficacy comparable to Velcade. Argyrin A also had more disruptive effects on existing tumor blood vessels and showed fewer side effects than Velcade.

Velcade is marketed by **Takeda Pharmaceutical Co. Ltd.**'s **Millennium Pharmaceuticals Inc.** subsidiary and **Johnson & Johnson** to treat multiple myeloma (MM) and mantle cell lymphoma.

The work performed by the Hannover researchers suggests that argyrin A affects tumors through p27^{Kip1} on multiple fronts: inducing apoptosis or G1 cell-cycle arrest, minimizing angiogenesis and disrupting existing tumor blood vessels.

"With bortezomib, we have seen some of the mice die at the highest published doses. Conversely, we have not seen a single side effect with argyrin," said Nisar Malek, principal investigator on the *Cancer Cell* paper and a professor of internal medicine, gastroenterology and molecular biology at Hannover. "Compared to bortezomib, the primary observed advantage in mice is less toxicity, less discomfort, fewer disease symptoms and no weight loss."

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**—Nisar Malek,
Hannover Medical School**

A potential explanation for the improved safety profile, said Malek, is that "between the two drugs, argyrin is the purer proteasome inhibitor." Indeed, a genome-wide gene expression profile showed that Velcade treatment led to changes in the expression of more than 10,900 genes, whereas argyrin A modulated the expression of about 500 genes.⁴

"At this point, it is not clear if broader activity is better or worse," said Malek. He noted Velcade's off-target effects likely contribute to its activity.

"This is probably the reason that bortezomib can still exhibit antitumor effects independent of p27," he said.

Julian Adams, who discovered Velcade and is now CSO and president of R&D at **Infinity Pharmaceuticals Inc.**, said the *Cancer Cell* paper does not give a clear picture of Velcade's safety and efficacy profile. "The negative effects observed in mice are possibly due to an overdosing of bortezomib," he told *SciBX*. As a result, Adams wanted to see additional data showing the compounds tested at varying dosages *in vivo*.

Adams also said the dichotomous results may be due to potency and dosage differences between the two compounds. He said the expression

Table 1. Targeting the proteasome. Selected proteasome inhibitors on the market and in development for cancer indications.

Company	Product	Indication	Status
Millennium Pharmaceuticals Inc., a subsidiary of Takeda Pharmaceutical Co. Ltd. (Tokyo:4502)/ Johnson & Johnson (NYSE:JNJ)	Velcade bortezomib	Multiple myeloma (MM); mantle cell lymphoma	Market
Proteolix Inc.	Carfilzomib (PR-171)	MM; solid tumors	Phase II
Cephalon Inc. (NASDAQ:CEPH)	CEP-18770	Cancer	Phase I
Nereus Pharmaceuticals Inc.	NPI-0052	Cancer	Phase I
4SC AG (Xetra:VSC)	SC68896	Cancer	Preclinical
Millennium, a subsidiary of Takeda	MLN2238	Cancer	Preclinical

profile needs to be bolstered with dose-response curves before conclusions on selectivity can be drawn.

David McConkey, a professor of cancer biology and urology at the **MD Anderson Cancer Center**, agreed. He said he would like to see the gene expression studies expanded to include additional cell lines and proteasome inhibitors, such as NPI-0052 from **Nereus Pharmaceuticals Inc.** and carfilzomib from **Proteolix Inc.** (See Table 1, “Targeting the proteasome.”)

If the safety results do hold up, Malek suggested argyirin A's primary use is likely to be in combination therapies.

“By being less toxic, you may be able to use argyirin in combination with more aggressive regimens of chemo drugs,” he told *SciBX*. “Its main potential is in combination therapies, for example, as a sensitizer for tumors that are in a resistant state.”

Given that Velcade is so well entrenched in MM, it would be good to look at argyirin in cases where Velcade has failed, which includes certain solid tumors, said Adams. “I would be very interested to see a Phase I clinical study testing argyirin in solid tumors,” he said.

In 2004 and 2007, Millennium discontinued development of Velcade in colorectal cancer and non-small cell lung cancer (NSCLC), respectively.

“Clearly you can imagine applying this drug in cases where tumors are resistant to bortezomib and other proteasome inhibitors,” said McConkey.

The caveats

Although stabilizing p27^{Kip1} expression generally shows a positive benefit in cancer, the target does pose obstacles for both single and combination therapies.

For example, Malek cautioned that p27^{Kip1} can behave as an oncogene, and that “in certain cancers, having intermediate levels of p27 is worse than not having it at all.”

Indeed, past studies have shown that losing one copy of the *p27^{Kip1}* gene can increase disease risk or accelerate disease progression, whereas losing both copies produced the opposite effect.^{1,2,5,6}

James Roberts, director of the Basic Sciences Division at the **Fred Hutchinson Cancer Research Center**, also noted that cytoplasmic expression of p27^{Kip1} can be oncogenic. Thus, he said, “stabilization of p27, by argyirin for example, may make matters worse by increasing the

amount of p27 available for cytoplasmic export.”

As a result, Roberts recommended combining argyirin A with a generic inhibitor of cytoplasmic export or a compound that specifically targets the p27^{Kip1} export pathway.

Malek did say his group has yet to see a case where argyirin A treatment increased cellular proliferation.

In terms of combination therapy, Malek noted that “many chemo drugs need cycling cells to work, so inducing G1 arrest is not always desirable.”

McConkey also warned that “increasing p27 can actually lead you to trouble when you combine the compound with drugs that require cell cycling, like Taxol.” Taxol paclitaxel is a promoter of microtubule stabilization marketed by **Bristol-Myers Squibb Co.** for breast and ovarian cancer.

Malek's group already has data showing efficacy of argyirin A in additional tumor types. The next steps, he said, are to perform formalized toxicology and pharmacology studies in additional animal tumor models and to submit a proposal for a Phase I trial in about 12 months.

Because the compound is derived from a living organism, Malek expects minimal issues with argyirin A's solubility and bioavailability.

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COMPANIES AND INSTITUTIONS MENTIONED

Bristol-Myers Squibb Co. (NYSE:BMJ), New York, N.Y.
Fred Hutchinson Cancer Research Center, Seattle, Wash.
Hannover Medical School, Hannover, Germany
Infinity Pharmaceuticals Inc. (NASDAQ:INF), Cambridge, Mass.
Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.
MD Anderson Cancer Center, Houston, Texas
Millennium Pharmaceuticals Inc. (NASDAQ:MLNM), Cambridge, Mass.
Nereus Pharmaceuticals Inc., San Diego, Calif.
Proteolix Inc., South San Francisco, Calif.
Takeda Pharmaceutical Co. Ltd. (Tokyo:4502), Osaka, Japan