Proteasome DUBstep

By Chris Cain, Staff Writer

Karolinska Institute researchers have identified a small molecule proteasome inhibitor for cancer that has a distinct mechanism from other compounds in the class.[1] **Vivolux AB** has licensed the candidate, which blocks two proteasome-associated deubiquitinating enzymes, and is performing lead optimization.

Ubiquitin ligases and deubiquitinating enzymes (DUBs) act in concert with the proteasome to maintain cellular protein homeostasis. Ligases covalently link ubiquitin to protein substrates and direct them to the proteasome for degradation. DUBs reverse this process by removing ubiquitins, and a subset of proteasome-associated DUBs aid in protein digestion by the proteasome.

The only approved therapy that targets the ubiquitin-proteasome pathway is Velcade bortezomib, a small molecule inhibitor of the proteolytic activity of the proteasome.

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

The Karolinska team's small molecule, b-AP15, takes a different tack and inhibits the proteasome by specifically blocking proteasome-associated DUB activity.

> **"This was very surprising—we thought, okay, we have a proteasome inhibitor, but it doesn't inhibit the proteolytic activity, so what does it do?"**
> ***—Stig Linder, Karolinska Institute***

A team led by Karolinska's Stig Linder identified b-AP15 in a 2005 screen for small molecules that induce p53-independent apoptosis in cancer cells; however, its molecular targets were unknown.[2] Linder and his team thus set out to determine how the compound kills cancer cells.

Linder is a professor of experimental oncology and vice chair of the Department of Oncology-Pathology.

His group performed whole-genome gene expression analysis of cells treated with b-AP15 and compared the resulting profile to a database of known drug-induced expression profiles. The compound's signature was very similar to that of known proteasome inhibitors. Indeed, subsequent experiments showed that b-AP15 caused the accumulation of proteasome substrates in cells, a hallmark of proteasome inhibition.

In vitro, however, b-AP15 had no effect on the proteolytic activity of the proteasome, whereas control assays confirmed Velcade was a potent inhibitor.

"This was very surprising," Linder told *SciBX*. "We thought, okay, we have a proteasome inhibitor, but it doesn't inhibit the proteolytic activity, so what does it do?"

Previous genetic studies suggested inhibiting proteasome-associated DUB activity could prevent protein degradation.[3] Thus, Linder's team hypothesized that because b-AP15 shares chemical similarity with existing DUB inhibitors, the compound could be inhibiting the proteasome by blocking DUB activity.

In vitro studies with purified proteasomes showed b-AP15 reversibly inhibited proteasome-associated DUB activity at low micromolar concentrations. To identify the specific DUB targets of the compound, Linder used a chemical probe that labels active DUBs and showed that b-AP15 blocked labeling of two DUBs known to interact with the proteasome: ubiquitin specific peptidase 14 tRNA-guanine transglycosylase (USP14; TGT) and ubiquitin carboxyl-terminal hydrolase L5 (UCHL5).

Finally, the team tested b-AP15 in multiple mouse models of cancer. In a xenograft mouse model of squamous cell cancer and syngeneic mouse models of lung and breast cancer, injection of b-AP15 lowered tumor growth compared with injection of vehicle control. In a mouse model of acute myelogenous leukemia (AML), injection of b-AP15 led to remission in 8 of 10 animals, whereas injection of vehicle did not cause remission in any animals.

Results were published in *Nature Medicine*.

Specifically speaking

Researchers contacted by *SciBX* suspect b-AP15 is a nonspecific inhibitor of cellular DUBs and want to see more biochemical evidence that b-AP15 specifically inhibits proteasome activity and spares DUBs that play critical roles in regulating a host of cellular functions.

Randall King, associate professor in the Department of Cell Biology at **Harvard Medical School**, found it surprising that USP14 and UCHL5 are b-AP15's only targets. He said that a major limitation of this paper is that "there is no insight into the mechanism of specificity of this compound. This is problematic because USP14 and UCHL5 are evolutionarily distant from one another and it is not clear how the inhibitor is bispecific without inhibiting other DUB enzymes. It just doesn't make a lot of sense until they can figure out the rationale."

King also noted that a compound with a chemical structure that differs at one position on a ring from b-AP15 was previously described as a nonspecific and irreversible DUB inhibitor with anticancer activity.[4]

King was co-senior author of a 2010 paper that suggested inhibiting USP14 could actually increase the proteolytic activity of the proteasome.[5] He said inhibiting both UCHL5 and USP14 would likely have distinct effects from inhibiting either protein alone. King's USP14-specific compounds are licensed to **Proteostasis Therapeutics Inc.**

Linder acknowledged it was unexpected that USP14 and UCHL5 were identified as the targets of b-AP15 but noted that the compound did not significantly inhibit total DUB activity in cells. Nor did the compound inhibit a panel of six additional DUBs that had no association with the proteasome.

He thinks the compound could be specifically interacting with USP14 and UCHL5 because unlike other DUBs, they are only active once they are associated with the proteasome. Therefore he speculated they could undergo a conformational change that enables them to be specifically recognized by the molecule.

He added that it is unknown how the compound interacts with its targets and that there are no structural data for USP14 and UCHL5 in association with the proteasome.

Raymond Deshaies, professor of biology at **California Institute of Technology**, was concerned about the structure of b-AP15 itself. He said the molecule has three sites with which it could covalently and irreversibly react with cysteine residues and thus appears as if it should be a nonspecific irreversible inhibitor of DUBs. Deshaies wanted to see additional cell-based experiments to confirm the compound's specificity.

> **"Obviously a more specific drug is predicted to be better tolerated, but in the absence of any DUB inhibitors in the clinic, limited data are available as to how specific a drug would really have to be."**
> ***—Tauseef Butt, Progenra Inc.***

Deshaies cofounded Proteolix Inc., a company developing proteasome inhibitors that was acquired by **Onyx Pharmaceuticals Inc.** in 2009. This year, Deshaies cofounded **Cleave Biosciences Inc.**, a company developing small molecules targeting protein homeostasis to treat cancer.

Deshaies and King also were concerned that no SAR series was published around the compound and said such data would help shed light on how the compound interacts with its targets.

Progenra Inc. president and CEO Tauseef Butt said regardless of the questions about b-AP15's precise molecular targets, the compound or optimized derivatives may have potential in cancer. "Obviously a more specific drug is predicted to be better tolerated, but in the absence of any DUB inhibitors in the clinic, limited data are available as to how specific a drug would really have to be," he said.

Progenra is developing small molecule inhibitors of specific DUBs. Its lead compound is a small molecule inhibitor of ubiquitin specific peptidase 7 (USP7; HAUSP) that is in preclinical development for cancer.

Viva Vivolux

Vivolux is pursuing SAR studies, lead optimization and preclinical development of b-AP15. The company was founded in 2006 to screen for cancer drugs using a 3D cell screening system licensed from **Uppsala University**. Linder joined the company's board in 2009, and this year the company exclusively licensed b-AP15 after an infusion of undisclosed funds from VC firm **Nxt2b AB**.

Nxt2b was founded by Bengt Ågerup this year after his former company, Q-Med AB, was sold to **Galderma S.A.** in December 2010 for $1.1 billion. At the time of the sale, Ågerup's holding company, Lyftet Holding, owned 47.5% of Q-Med.

Hans Rosén, CEO of Vivolux, told *SciBX* Ågerup founded Nxt2b to use the proceeds from that sale to fund further research, including in cancer, and that Ågerup had previously worked with Vivolux's Uppsala founders. Nxt2b has invested in more than 20 companies this year.

In addition to optimizing b-AP15, Linder is planning to test b-AP15 in mouse models of Velcade-resistant MM. In cell culture studies using Velcade-resistant colon cancer cells, b-AP15 induced apoptosis at submicromolar concentrations.

Rosen said Vivolux hopes to advance a compound into the clinic by 2013.

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COMPANIES AND INSTITUTIONS MENTIONED

California Institute of Technology, Pasadena, Calif.
Cleave Biosciences Inc., Burlingame, Calif.
Galderma S.A., Lausanne, Switzerland
Harvard Medical School, Boston, Mass.
Karolinska Institute, Stockholm, Sweden
Millennium Pharmaceuticals Inc. (NASDAQ:MLNM), Cambridge, Mass.
Nxt2b AB, Uppsala, Sweden
Onyx Pharmaceuticals Inc. (NASDAQ:ONXX), South San Francisco, Calif.
Progenra Inc., Malvern, Pa.
Proteostasis Therapeutics Inc., Cambridge, Mass.
Takeda Pharmaceutical Co. Ltd. (Tokyo:4502), Osaka, Japan
Uppsala University, Uppsala, Sweden
Vivolux AB, Uppsala, Sweden