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Proteasome progress

By Lev Osherovich, Senior Writer

Although animal studies have shown that proteasome inhibitors can prevent self-antigen and cytokine production and thus may have potential utility in autoimmune diseases, the toxicity of the drugs makes them unsuitable for such chronic conditions.[1] Researchers at **Proteolix Inc.** may have found a way around the problem with the discovery of PR-957, a small molecule that inhibits a subunit of the proteasome—LMP7—that is involved in immune cell activation.

Proteolix and academic colleagues have proof-of-concept data showing that the compound treated rheumatoid arthritis (RA) in mice.[2]

The proteasome is a multi-subunit complex of proteases that degrades proteins.[3] Because of the proteasome's role in regulating levels of proliferation-promoting proteins, it has been pursued as a cancer target. The one marketed proteasome inhibitor—Velcade bortezomib from **Takeda Pharmaceutical Co. Ltd.**'s **Millennium Pharmaceuticals Inc.** subsidiary—is indicated for multiple myeloma (MM) and mantle cell lymphoma. In fact, all proteasome inhibitors in the clinic are being developed in oncology.

PR-957 also began life as a cancer therapeutic. Proteolix was looking for compounds that inhibited specific subunits of the immunoproteasome, a variant of the proteasome that is present in certain blood cancer cells and in immune system cells. One screen turned up PR-957, an inhibitor of proteasome prosome macropain subunit-β type 8 (PSMB8; LMP7), a catalytic subunit of the immunoproteasome.

The problem, said Christopher Kirk, director of biology and pharmacology at Proteolix, was that "we tested PR-957 in a tumor model and found that LMP7 inhibition did not cause cell death."

However, the compound did block the production of cytokines—an observation that suggested PR-957 would be better suited in the autoimmunity field.

Proteolix then collaborated with Marcus Groettrup, professor of immunology at the **University of Constance**, to test the compound in *in vitro* and mouse models of antigen presentation. Groettrup's team found that because PR-957 prevented cytokine production, the compound blocked immune activation in a mouse model of viral infection.

Moreover, in a mouse model of RA, PR-957 prevented inflammatory cytokine production and joint inflammation compared with mock treatment. A single dose of the compound completely reversed the disease, with the effects being noticeable a day after administration. The effect was faster and stronger than that of Enbrel etanercept, a soluble tumor necrosis factor receptor that is marketed by **Amgen Inc.** and **Wyeth** for multiple autoimmune diseases.

Kirk noted that, unlike typical anti-inflammatory therapies, PR-957 was highly potent in small doses and had a long-lasting effect. A single dose of PR-957 protected mice from experimentally induced RA for up to two weeks.

Kirk suspects the long duration of action is because PR-957's irreversible inhibition of LMP7 blocks T helper type 17 (Th17) cell activation during a critical early phase of autoimmunity. According to Kirk, proteasome activity eventually recovers thanks to new protein synthesis, but only after the time window for autoimmunity has closed.

"If you're targeting the Th17 cells, once you've exerted an anti-inflammatory effect, you fundamentally reset the immune response," said Kirk.

"People have been looking for a drug to inhibit these processes," said Groettrup.

Nonselective proteasome inhibitors, including Velcade as well as Proteolix's lead compound, carfilzomib, are not well suited to the task because of their side-effect profiles, noted Kirk.

"For more than 10 years, it's been known that the proteasome can regulate the production of cytokines throughout the immune system," he said. "Velcade affects this, but comes at the cost of cell viability and toxicity *in vivo*."

Because PR-957 blocked the immunoproteasome at low concentrations without affecting regular proteasome activity, Kirk thinks the new compound can bypass the toxicity problem. Indeed, the maximum tolerated dose of PR-957 in mice was about 10-fold higher than that of carfilzomib and Velcade.

Results were published in *Nature Medicine*; Kirk and Groettrup were authors on the paper.

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Groettrup now plans to examine the specific proteins that are cleaved by LMP7 to kick off autoimmunity. He suspects that the immunoproteasome is involved in activating the proinflammatory NF-κB pathway.

For Proteolix, the challenge now is to make an orally available version of PR-957 and to persuade rheumatologists that a proteasome inhibitor will be safe and effective. The company expects to submit an IND to treat RA in mid-2010.

"Given the profile of the cytokines that are inhibited by PR-957, it's likely that there are applications for this molecule in a number of other autoimmune indications," said Kirk. "Th17 cells are thought play roles in psoriasis and MS."

Proteolix has filed for a patent on PR-957 and has no plans to partner the compound at this stage.

"Our thought is to get a little bit of Phase I data to provide evidence of clinical activity," said Craig Parker, CFO and SVP of finance and corporate development. "Good clinical data lead one to do as much internal development as possible" before seeking partners.

Proteolix isn't hurting for cash, as the company raised $78 million in a C series round last September. Carfilzomib is in Phase II trials for MM and solid tumors.

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COMPANIES AND INSTITUTIONS MENTIONED

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