Giving T_{reg} cells a boost

By Lauren Martz, Staff Writer

Toxicity due to widespread epigenetic effects has prevented the use of pan-HDAC inhibitors in indications beyond advanced cancer, in which at least one such drug has reached the market. A team at the **Perelman School of Medicine at the University of Pennsylvania** looking for a way around this has found that specific inhibition of HDAC6 increases the immune suppressive functions of T_{reg} cells.¹

Histone deacetylase 6 (HDAC6) is one of the few isoforms for which highly selective inhibitors have been developed, and the findings could therefore point to new indications in autoimmune diseases and transplant rejection for the trio of biotechs that have disclosed programs against the target.

HDACs are a class of enzymes that predominantly act on histones to regulate gene expression. Pan-HDAC inhibitors, such as Zolinza vorinostat, promote tumor cell-cycle arrest and apoptosis.² Zolinza is marketed by **Merck & Co. Inc.** to treat advanced cutaneous T cell lymphoma (CTCL).

HDAC6 is unusual among HDACs, primarily modulating the activity of nonhistone proteins including heat shock protein 90 (Hsp90).

Previous preclinical studies by the UPenn group showed that pan-HDAC inhibitors could have therapeutic effects outside of the oncology space, including in autoimmunity and transplant rejection, but toxicity prevented chronic use of pan-HDAC inhibitors in the clinic.^{3,4}

Now, in a paper published in *Molecular and Cellular Biology*, a team led by Wayne Hancock has found that the HDAC6 isoform is likely responsible for the anti-inflammatory properties of pan-HDAC inhibitors. Indeed, inhibition of that specific isoform ameliorated autoimmune diseases and transplant rejection in mice.

Hancock is professor of pathology and laboratory medicine at the UPenn School of Medicine and chief of the Division of Transplantation Immunology in the Department of Pathology and Laboratory Medicine at **The Children's Hospital of Philadelphia**.

The paper also included researchers from the **University of Illinois at Chicago, Harvard Medical School, Broad Institute of MIT and Harvard and Friedrich Miescher Institute for Biomedical Research**.

In culture, the team first showed that forkhead box P3 (FOXP3)-expressing T_{reg} cells deficient in HDAC6 suppressed the proliferation of T cells more than wild-type T_{reg} cells. Proliferation of T cells can lead to the exaggerated immune response that occurs during autoimmune diseases and organ transplant.

Also in culture, the research-grade HDAC6 inhibitors tubacin or

tubastatin A increased the suppressive function of wild-type T_{reg} cells against T cells compared with an inactive control compound or vehicle, respectively.

Inhibiting Hsp90 increased T_{reg} cell activity similarly to inhibiting the upstream target HDAC6.

In a mouse model of ulcerative colitis (UC), tubacin protected the animals from weight loss and diarrhea compared with control. Tubacin also reversed weight loss and decreased colon inflammation in established T cell-induced colitis compared with an inactive control compound.

In another mouse model of colitis, tubastatin A or an Hsp90 inhibitor allowed body weight recovery and normal stool formation compared with vehicle.

Finally, in a model of cardiac allograft transplant rejection, administration of HDAC6^{-/-} T_{reg} cells or wild-type T_{reg} cells plus tubacin, tubastatin A or Hsp90 inhibitors allowed long-term allograft survival compared with administration of wild-type or vehicle controls.

"We already knew that pan-HDAC inhibitors have anti-inflammatory properties. This paper suggests that HDAC6 might be the specific enzyme responsible for the activity," said Jeffrey Besterman, CSO and EVP of R&D at **MethylGene Inc.**

MethylGene's HDAC inhibitor program includes mocetinostat (MGCD0103), an inhibitor of HDAC1 and HDAC2, which is in Phase II testing to treat cancer. **Otsuka Pharmaceutical Co. Ltd.**'s **Taiho Pharmaceutical Co. Ltd.** subsidiary has rights to the compound in certain Asian countries.

Hancock told *SciBX* the team originally hoped that Hsp90 inhibitors, which are already in the clinic, would obviate the need for selective blockers of HDAC6. However, "our ongoing studies indicate that this is not the case and that there are significant anti-inflammatory effects of HDAC6 inhibitors that occur independently of the Hsp90/HSF1 [heat shock transcription factor 1] pathway," he said.

The researchers now want to determine the precise types of cells that HDAC6 inhibitors target. "We have focused to date on aspects of HDAC6 regulation of FOXP3-expressing T_{reg} function but recognize that there are many other types of cells that express HDAC6, including dendritic cells and macrophages," noted Hancock.

Testing toxicity

Hancock said in terms of development, further optimization of the potency of tubacin or tubastatin A is required, "along with a detailed assessment of pharmacokinetics and toxicity, though initial data using pharmacological inhibitors as well as the normal phenotype of HDAC6-deficient animals are highly encouraging."

"No specific mechanism-related toxicities associated with HDAC6 inhibition have been uncovered to date. As such, potent and selective inhibitors of this isoform have the potential to represent a new class of anti-inflammatory and immunosuppressant agents lacking the side effects of other competing therapeutics with alternative modes of action," said Stephen Shuttleworth, CSO of **Karus Therapeutics Ltd.**

Karus' KAR3166, an HDAC6 inhibitor, is in preclinical testing to treat inflammation.

“We are learning that the bad players in the HDAC family are the class I HDACs, particularly HDAC3, which when inhibited can lead to extensive adverse side effects,” suggesting that a specific way to inhibit the HDAC culprit without inhibiting other HDAC isoforms that cause toxicity would be ideal, added Walter Ogier, president and CEO at **Acetylon Pharmaceuticals Inc.**

Acetylon's small molecule HDAC6 inhibitor, ACY-1215, has completed preclinical testing. Ogier said the company is currently initiating a clinical trial in multiple myeloma (MM). The company also is evaluating HDAC6 inhibitor candidates in late stage preclinical studies for autoimmune disease indications including rheumatoid arthritis (RA) and other inflammatory disorders.

Another biotech working on selective HDAC6 inhibitors is Cellzome AG, which is currently in the discovery phase.

Back to oncology

The most exciting applications for HDAC6 inhibition may be treating transplant rejection and graft-versus-host disease (GvHD).

According to Simon Jones, VP of biology and preclinical development at Acetylon, “The quite astonishing work in the *Molecular and Cellular Biology* paper is the transplant rejection model. Reducing chronic—as well as acute—immune rejection is so key.”

He wanted to see preclinical studies that determine whether HDAC6 inhibition could be effective against GvHD, specifically for bone marrow transplant in oncology indications.

For patients receiving bone marrow transplantation for cancer and noncancer indications, decreasing chronic transplant rejection is an important part of the GvHD treatment, Jones noted. “You can manage to some extent acute GvHD with steroids, but chronic rejection has not yet been resolved. The results in the cardiac transplant rejection model are

quite profound and suggest that this mechanism could apply to GvHD in bone marrow transplantation,” thus allowing broader use in patients to be treated with bone marrow transplantation, he said.

Hancock said UPenn did not file for patents covering the role of HDAC6 inhibitors in autoimmune diseases and transplant rejection. Tubacin is patented by the University of Illinois at Chicago, and tubastatin A is patented by Harvard Medical School. Acetylon has exclusively licensed IP covering commercial use and development of tubacin.

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