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Hsp90 JAK-hammer

By Joanne Kotz, Senior Editor

Researchers at **Memorial Sloan-Kettering Cancer Center** and the **Dana-Farber Cancer Institute** have found a mechanistic link between janus kinase-2 and heat shock protein 90 that opens up the possibility of using inhibitors of the latter in myelofibrosis. There are no approved drugs for the disease, and heat shock protein 90 inhibitors may offer a better therapeutic window than janus kinase-2 inhibitors.

Myelofibrosis is a malignancy that involves proliferation of abnormal bone marrow stem cells. As a reaction to the presence of growing malignant cells, collagenous connective tissue fibers develop in the bone marrow and decrease its function, shifting at least part of the burden of hematopoietic stem cell formation to the spleen and liver. The result is substantial enlargement of these organs. Survival following diagnosis is typically 2–11 years.

Following the 2005 discovery that mutations in janus kinase-2 (JAK-2) were linked to myeloproliferative neoplasms including myelofibrosis, a handful of companies began testing their JAK-2 inhibitors in these indications.[1] One challenge has been that direct JAK-2 inhibitors block the signaling pathway in both malignant and nonmalignant cells.

Incyte Corp. and partner **Novartis AG** have the most advanced JAK-2 inhibitor. INCB18424, which also inhibits JAK-1, is in Phase III testing for myelofibrosis, and the companies hope to announce top-line data in December.[2]

This month, results from a Phase I/II trial of INCB18424 were published in the *New England Journal of Medicine* showing that about half of patients had at least a 50% decrease in spleen size and a 50% decrease in other disease-related symptoms.[3]

JAK-2 allele burden—the percentage of mutated JAK-2 within total JAK-2 DNA in patient hematopoietic cells—was lowered by an average of 13%, to 71% from 84%. Overall, myelosuppression occurred in less than 10% of patients given a 15 mg dose of INCB18424 twice a day. However, the highest dose tested (25 mg twice daily) had to be decreased or interrupted because of hematological toxicities in 60% of patients.

There are at least five other JAK-2 or JAK-1 and 2 inhibitors in Phase I/II or Phase I trials for myelofibrosis, and side effects have ranged from gastrointestinal issues to hematological toxicities.

According to Srdan Verstovsek, principal investigator on the Phase I/II trial of INCB18424, hematological toxicities such as myelosuppression result from on-target effects of inhibiting JAK-2 signaling in nonmalignant hematopoietic cells.

Verstovsek is an associate professor in the Department of Leukemia at **The University of Texas M.D. Anderson Cancer Center**.

Gastrointestinal toxicities, which were not seen in the INCB18424 trial but have been seen with other JAK-2 inhibitors, may be the result of inhibiting off-target kinases, he added.

Given these issues, a team led by Ross Levine, Gabriela Chiosis and James Bradner have been looking for agents that complement or improve upon the JAK-2 inhibitors. In a paper published in *The Journal of Clinical Investigation*, the group suggests that inhibitors of heat shock protein 90 (Hsp90) could be the answer.[4]

Levine is an assistant member in the Human Oncology and Pathogenesis Program and Chiosis is the Frederick Adler Chair of Molecular Pharmacology and Chemistry at Memorial Sloan-Kettering. Bradner is an assistant professor in the Department of Medical Oncology at Dana-Farber.

The group tested an Hsp90 inhibitor, PU-H71, which was previously developed in Chiosis' laboratory. PU-H71 increased degradation of JAK-2 in cells compared with vehicle control, suggesting that JAK-2 is indeed an Hsp90 client protein.

In a mouse model of myeloproliferative neoplasms, PU-H71 increased both degradation of JAK-2 and survival ($p<0.0004$) and decreased both myeloproliferation ($p<0.01$) and spleen weight ($p<0.01$) compared with vehicle control.

PU-H71 appears to act preferentially on activated JAK-2 at the site of malignancy. PU-H71 selectively accumulated in the spleen and bone marrow of the mice and led to greater degradation of JAK-2 in the spleen than that in similarly treated healthy animals. In erythroid cells derived from patients with JAK-2-dependent myeloproliferative neoplasms, PU-H71 increased JAK-2 degradation and decreased cell viability compared with those in similarly treated cells from healthy patients.

"When the results on JAK-2 mutations in myeloproliferative neoplasms first came out in 2005, we immediately thought that JAK-2 could be an Hsp90 client protein" because many other mutant oncoproteins are dependent on Hsp90, said Christian Fritz, vice president of biology at **Infinity Pharmaceuticals Inc.** "But at the time there were not the tools to prove this. This paper now provides the proof."

Infinity has two Hsp90 inhibitors in clinical testing: IPI-504, an i.v. inhibitor, is in a Phase II trial for non–small cell lung cancer (NSCLC) and a Phase Ib trial in combination with Taxotere for solid tumors. IPI-493, an oral Hsp90 inhibitor, is in Phase I testing for both solid tumors and advanced hematological malignancies. Patients with JAK-2-dependent myeloproliferative neoplasms are being included as part of the advanced hematological malignancy trial, which includes measurement of client protein degradation.

One punch or two?

The consensus among researchers contacted by *SciBX* was that Hsp90 inhibitors are ready for the clinic in myeloproliferative neoplasms. An open question, however, is whether they should be tested alone or in combination with JAK-2 inhibitors.

"We were surprised that Hsp90 inhibitors had such impressive activity in degrading JAK-2," said Levine. "The next step will be a targeted trial to really assess efficacy in patients."

Chiosis told *SciBX* that IND-enabling studies have been completed

for PU-H71 and a Phase I trial in partnership with the **National Cancer Institute** Experimental Therapeutics (NexT) Program within the Division of Cancer Treatment and Diagnosis could begin as early as the end of this year. Although the initial study would look at patients with solid tumors and lymphomas, the investigators hope that patients with myeloproliferative neoplasms can be included in an expanded cohort after the best dosing regimen is established.

What is attractive about the *in vivo* results with Hsp90 inhibitors, said Verstovsek, is the potential for Hsp90 as a single agent to more selectively degrade JAK-2 in malignant cells, which may result in less toxicity than direct JAK-2 inhibitors.

In addition to the potential for less toxicity, Chiosis and Levine also suggested that Hsp90 inhibitors may block JAK pathway signaling more effectively than JAK-2 inhibitors. JAK-2 inhibitors reversibly block the enzyme, whereas Hsp90 inhibitors cause JAK-2 to be degraded entirely.

Although the investigators believe that Hsp90 inhibitors should first be tried as single agents, ongoing preclinical research in their labs includes looking at the effects of combined Hsp90 and JAK-2 inhibitors *in vivo*.

Nicola Wallis, director of biology, and John Lyons, vice president of translational R&D at **Astex Therapeutics Ltd.**, think the best approach might be the combination.

"The biggest shortcoming to date with Hsp90 inhibitor work has been its lack of translation into real clinical efficacy. To date, no appreciable single agent activity has been reported with Hsp90 inhibitors in clinical trials," said Wallis and Lyons in a joint e-mail to *SciBX*.

In the *JCI* paper, Levine and colleagues only reported *in vitro* combination results. In cell lines, adding a JAK-2 inhibitor to PU-H71 led to an additive effect in inhibiting cell proliferation but no additional benefit in modulating a JAK pathway gene signature compared with PU-H71 alone.

Wallis and Lyons thus wanted to see tests of the combination in animal models to see if the additive antiproliferative effects seen in cells translate *in vivo*. "Combining these two complementary agents with differing mechanisms of action might bring a halt to this progressive disease since they confer additive antiproliferative effects on the critical signaling pathway," they said.

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—Ross Levine, Memorial Sloan-Kettering Cancer Center

Astex's AT13387, an Hsp90 inhibitor, is in Phase I testing for solid tumors.

Wallis and Lyons also highlighted recent data by a group at Memorial Sloan-Kettering showing that Hsp90 inhibitors may resensitize a kinase-refractory population of cells to the original kinase inhibitor.[5]

Although Fritz believes that it will make sense to test Hsp90 as a single agent first, he agreed that Hsp90 inhibitors may help overcome kinase resistance as part of a combination treatment. Indeed, mutations that make a kinase resistant to an inhibitor may decrease the stability of the kinase and increase its dependence on Hsp90.

At least 11 companies have Hsp90 inhibitors in clinical testing for various cancers.

Memorial Sloan-Kettering Cancer Center is pursuing patent protection covering PU-H71 in cancer. The licensing status of PU-H71 is undisclosed.

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   Contact: Ross L. Levine, Memorial Sloan-Kettering Cancer Center, New York, N.Y.
   e-mail: leviner@mskcc.org
   Contact: Gabriela Chiosis, same affiliation as above
   e-mail: chiosisg@mskcc.org
   Contact: James E. Bradner, Dana-Farber Cancer Institute, Boston, Mass.
   e-mail: james_Bradner@dfci.harvard.edu
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COMPANIES AND INSTITUTIONS MENTIONED

Astex Therapeutics Ltd., Cambridge, U.K.
Dana-Farber Cancer Institute, Boston, Mass.
Incyte Corp. (NASDAQ:INCY), Wilmington, Del.
Infinity Pharmaceuticals Inc. (NASDAQ:INFI), Cambridge, Mass.
Memorial Sloan-Kettering Cancer Center, New York, N.Y.
National Cancer Institute, Bethesda, Md.
Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland
The University of Texas M.D. Anderson Cancer Center, Houston, Texas