ITK: TKO for HIV?

By Michael J. Haas, Senior Writer

HIV's resistance to antiretrovirals has led researchers to target host proteins upon which the virus depends for survival. Compounds targeting these proteins are harder for HIV to resist but are potentially more toxic to the host than drugs that target viral proteins. Scientists now report that inhibition of IL-2–inducible T cell kinase in T cells blocks three stages of HIV replication without compromising T cell function.[1] However, companies contacted by *SciBX* want to know more about how IL-2–inducible T cell kinase inhibitors work—and how well—before deciding on the viability of the target.

The paper, published in the *Proceedings of the National Academy of Sciences* by a team of researchers at the **NIH**, **Pennsylvania State University** and **Boston University School of Medicine**, shows that IL-2–inducible T cell kinase (ITK) plays a role in the release of newly replicated viruses—called virus-like particles (VLPs) or virions—from infected cells. The team was led by Pamela Schwartzberg, senior investigator at NIH's **National Human Genome Research Institute**, and Andrew Henderson, associate professor of medicine at BU School of Medicine.

There are no marketed HIV treatments that block this stage of the replication cycle. The only related approach is currently being pursued by **Panacos Pharmaceuticals Inc.** and **Myriad Genetics Inc.**, which have two maturation inhibitors in clinical testing that bind the HIV gag polyprotein and thus prevent virions from maturing into infectious viruses. Panacos' bevirimat (PA-457) is in a Phase II trial, and Myriad's Vivecon (MPC-9055) is in a Phase I trial.

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> *–Andrew Henderson*
> *Boston University School of Medicine*

"Understanding how HIV exits the cell is an area of very active research," Schwartzberg told *SciBX*. "Clearly, it would be an important advance and a complement to current treatments to target early events of the HIV life cycle."

Henderson added that several institutions are researching the processes involved in virus release, including the NIH, the **Aaron Diamond AIDS Research Center**, **Northwestern University** and the **University of Utah**.

Knocking ITK down

ITK plays a role in regulating both the rearrangements of a cell's cytoskeleton and the interactions between the cytoskeleton and the cell membrane.[2] The processes of HIV infection and virion release both involve disruption of the cell membrane, and as a result the Schwartzberg-Henderson team decided to investigate whether ITK was a player in these processes.

ITK is a member of the Tec family of tyrosine kinases and is expressed in T cells, NK cells and mast cells. It is involved in T cell receptor–mediated activation of T cells and may play a role in T cell proliferation and differentiation.[3]

First, the team showed that in primary human $CD4^+$ T cells, ITK knockdown with short interfering RNA or an ITK inhibitor blocked HIV entry without altering the cells' viability. Additional experiments demonstrated that the gp120 HIV envelope protein required ITK to rearrange the cytoskeleton of a T cell and invade it.

Next, the team found that ITK deficiency in mouse T cells impaired the activation of three transcription factors essential to HIV transcription and replication.

Last, the team showed that enhanced expression of ITK in T cells increased the production and release of VLPs compared to that seen with normal ITK expression. ITK's role in this process was independent of its kinase function. In contrast, ITK's role in viral entry and transcription requires kinase function.

Collectively, the researchers said the findings suggest that targeting ITK could be an effective addition to combination therapies for HIV.

"ITK may have multiple activities and may act in different ways at different stages of the virus's life cycle," Henderson told *SciBX*. "And ITK may be more attractive than other cellular targets because the animals appear to tolerate ITK knockdown."

Power and function

Earlier results by researchers at the **University of Zurich** showed that ITK-deficient mice did not have compromised immune system function.[4] This led the *PNAS* team to suggest that ITK inhibition might not have significant side effects and thus could constitute a promising therapeutic strategy. But company representatives contacted by *SciBX* said the reported effects of the ITK inhibitor on HIV replication itself did not yet make the case for ITK inhibition as a therapeutic approach to HIV. They would like to see more quantitative data linking the potency of the ITK inhibitor to its effect on HIV replication and to learn more about ITK's other functions.

Amy Espeseth, director of RNAi therapeutics at **Merck & Co. Inc.**'s Merck Research Laboratories unit, noted the authors of the *PNAS* paper did not numerically correlate the amount of ITK inhibition to the observed reductions in HIV replication.

For instance, "if a complete loss of ITK function gives you only a 50% decrease in HIV replication, then it is hard to see how an anti-ITK drug would be a good addition to HIV combination therapies," she said.

Although the team showed that inhibiting ITK in healthy cells reduced HIV infection and that inhibiting ITK in infected cells lowered the release of VLPs, Espeseth also wants to see whether the two reductions are cumulative.

"I would have liked to see the virus used to infect cells where ITK activity was already reduced, either by the chemical inhibitor or the siRNA, and then see what happens" over the course of several days, she said. "That would provide insight into the overall effect of ITK knockdown on a spreading viral infection."

Espeseth added that although the results show ITK is a druggable target, it was not necessarily a good one. "Overall we need to be careful about which host factors we choose for drug discovery research" because of the functions of those proteins in normal cells, she said.

Graham Allaway, COO of Panacos Pharmaceuticals, said studies such as the one in *PNAS* "elucidate the HIV replication process and may open doors to new therapeutic approaches." But like Espeseth, he wants to see more data on the potency, specificity and toxicity of the team's ITK inhibitor, which the team synthesized in-house based on research published by **Bristol-Myers Squibb Co.** (BMS).[5]

"Obviously this was a proof-of-concept study," Allaway told *SciBX*. "But few data were presented on the potency of the BMS compound."

He noted that the relatively large 10 μM concentration of the inhibitor required for anti-HIV activity indicated that the compound was not very potent and raised the question of its therapeutic index—how close an active dose was to a toxic one.

"We would want to know what concentration begins to give toxicity," Allaway said. "Is it 20 μM, 1 mM?"

He also said the multiple roles of ITK—in both normal and HIV-infected T cells—raise additional concerns about the specificity of ITK inhibitors. "If ITK is important to a range of immune cell functions, it might not be a viable target," Allaway said.

Henderson didn't think that ITK's range of functions in T cells and HIV replication make it a difficult drug target. Instead, he suggested that understanding ITK's different mechanisms of action against HIV could lead to the development of more specific ITK inhibitors.

"For example, VLP formation does not appear to require ITK's kinase activity," Henderson said. "Once we have more information regarding that mechanism, we might be able to target that event" without affecting ITK's other functions.

Allaway noted that although "the whole concept of using an immune system inhibitor as an HIV drug gives some people a shudder," similar concerns were raised about compounds targeting CC chemokine receptor 5 (CCR5) on host T cells.

There is one approved drug that targets CCR5—Selzentry maraviroc from **Pfizer Inc.** Other CCR5 inhibitors in clinical development include **Schering-Plough Corp.**'s vicriviroc, which is in Phase III testing.

In 2005, **GlaxoSmithKline plc** discontinued development of the CCR5 antagonist aplaviroc (GW873140) because of concerns over the compound's hepatotoxicity. In March, **Incyte Corp.** halted development of its CCR5 antagonist, INCB9471, because of the high cost, time and labor requirements of the program.

Allaway said maraviroc has shown "no evidence of significant immune system side effects, but longer-term effects are still unknown."

He also said that targeting a host protein will not entirely avoid the problem of viral resistance. "HIV can and probably will develop resistance to any drug, even one that targets a cellular protein or gene."

Nevertheless, Allaway thinks "the idea of targeting a host protein or gene is a reasonable one." He also said that ITK might have one advantage over other host protein targets.

"Companies have developed ITK inhibitors for other indications such as asthma and autoimmune disorders," he said. "It would be very important to know how far these ITK inhibitors have gone toward development," because that could provide evidence of tolerability and could indicate how likely ITK inhibitors are to become HIV drugs.

Cellzome Inc. previously studied the role of ITK inhibitors in immune and inflammatory indications. Without elaborating, spokesperson Melanie Toyne-Sewell said the company is no longer working in the area of ITK inhibition.

Bristol-Myers, which at one time was studying the role of ITK inhibitors—including the compound used by the *PNAS* team—in immunosuppressive and inflammatory diseases, did not respond to requests for interviews.

AstraZeneca plc has ITK inhibitors in early discovery research according to Chris Yochim, the company's associate director of external relations, who also did not elaborate.

Henderson said the team is now working to more fully elucidate ITK's mechanisms of action in HIV replication.

Both Henderson and Schwartzberg said that it was too soon to conduct *in vivo* studies—such as those that might determine whether ITK deficiency affects the host's ability to clear the opportunistic infections that characterize HIV and AIDS.

"We are concentrating on the biology of how ITK may affect stages of HIV replication," Schwartzberg said. She added that the team hopes drug companies working on ITK inhibitors will take up these findings and further evaluate the role of ITK in HIV's life cycle.

NIH and Pennsylvania State University have filed a patent on the use of inhibiting ITK expression or function to block HIV infection. NIH spokesperson Claire Driscoll said the findings are available for licensing.

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COMPANIES AND INSTITUTIONS MENTIONED

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AstraZeneca plc (LSE:AZN; NYSE:AZN), London, U.K.
Boston University School of Medicine, Boston, Mass.
Bristol-Myers Squibb Co. (NYSE:BMY), New York, N.Y.
Cellzome Inc., Boston, Mass.
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
Incyte Corp. (NASDAQ:INCY), Wilmington, Del.
Merck & Co. Inc. (NYSE:MRK), Whitehouse Station, N.J.
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