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Studies Reveal How New Drug May Overcome Deadly Mutation that Causes Leukemia

HHMI researchers have discovered how a new generation of drugs thwarts a deadly mutation that causes chronic myelogenous leukemia (CML).

Although the drug Gleevec, which is manufactured by Novartis, is considered the standard of treatment for CML, certain patients develop resistance to the drug. Resistance is usually caused by mutations that alter the shape of an enzyme called Abl that is targeted by Gleevec. Once the shape of the enzyme is changed, the drug does not bind as effectively to its target.

Using x-ray crystallography, the scientists produced detailed molecular images that reveal how VX-680, a drug developed by Vertex Pharmaceuticals and Merck, interacts with a mutant form of Abl. The new studies, published in the January 15, 2006, issue of the journal *Cancer Research*, hint at how VX-680 jams the mutant Abl enzyme and stops proliferation of cancerous cells. HHMI researchers John Kuriyan at the University of California, Berkeley, and Charles Sawyers at the Jonsson Comprehensive Cancer Center at UCLA, led the research teams that collaborated on the studies.

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- Charles L. Sawyers

One of the more problematic mutations in Abl is called T315I, and it is responsible for about 15 percent of cases in which CML patients develop resistance to Gleevec. The T315I mutation also confers resistance to dasatinib, a newer drug developed by Bristol-Myers Squibb that has been effective in phase I and phase II trials as a second line of defense in patients who develop resistance to Gleevec. The T315I mutation is particularly troublesome because it sits at the heart of the enzyme's catalytic center, said

Kuriyan, and blocks the binding of Gleevec and dasatinib.

“More than fifty mutations have been described that cause resistance to Gleevec,” said Sawyers. “We found that dasatinib overcomes all of these Gleevec-resistant mutants, with the exception of T315I. So, the remaining unturned stone has been to find a drug to treat T315I.

“By good luck, in collaboration with Ambit Biosciences, we found that VX-680, now in clinical trials as an inhibitor of another kinase called Aurora, also inhibits the T315I mutant. However, it is important to understand how VX-680 binds to this mutant to inhibit it, whereas the other drugs do not,” said Sawyers. That new knowledge could aid in developing a strategy for searching for other drugs that could inhibit resistant mutants.

Abl is an enzymatic switch called a kinase that becomes overactivated by a chromosomal mix-up that occurs during blood cell development. The genes *ABL* and *BCR*, which are located on different chromosomes, become fused and express a hybrid Bcr-Abl enzyme that is always active. The hyperactive Bcr-Abl, in turn, drives the overproliferation of white blood cells that is the hallmark of CML. In earlier studies, Sawyers and his colleagues showed that dasatinib could overcome a significant proportion of the resistant mutants.

In the experiments reported in the *Cancer Research* article, Kuriyan and his colleagues used x-ray crystallography to produce molecular images that give researchers a better idea of how VX-680 binds to the T315I mutant version of Bcr-Abl. In x-ray crystallography, x-rays are directed through crystals of a protein or a drug-and-protein complex, and the resulting diffraction pattern is analyzed by researchers and used to deduce the structure of the complex under study.

While the researchers were not able to create useful crystals of the complex of VX-680 with a T315I mutant Bcr-Abl, the current studies show the structure of VX-680 in complex with another Abl mutant, H396P, which is resistant to Gleevec.

The Abl kinase, like other kinases, can assume active or inactive conformations, and the researchers' structural analysis revealed that the H396P mutation caused the enzyme to favor an active conformation. The researchers found that VX-680 was effective at blocking this mutant because it could recognize and bind to an active conformation, said Kuriyan. In contrast, Gleevec and dasatinib act by recognizing an inactive conformation and binding to it, rendering the Abl kinase inactive.

“Thus, this structure does help explain why VX-680 has activity against the T315I mutation, and that arises from the shape of VX-680,” said Kuriyan. “While Gleevec penetrates deeply into the kinase domain of Abl, VX-680 stays away from the position of the T315I mutation, leaving a significant gap.” This gap allows VX-680 to accommodate the mutation and still bind to

the mutant Abl.

In additional experiments, Sawyers and his colleagues tested VX-680's effectiveness against CML cells isolated from a patient with the T315I mutation. The studies showed that the drug inhibited T315I mutant BCR-ABL in the cancer cells.

“This offers a very useful predictive tool that tells us that the drug does work against the mutation,” said Sawyers. “It gives us confidence that we can now take the drug into clinical trials.” Tests are already underway to determine the effectiveness of the drug in leukemia patients, including those with the T315I mutation.