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Pinpointing the Mutations that Cause Resistance to Gleevec

Researchers have identified specific mutations in a rogue gene that render the drug Gleevec ineffective in some patients who have chronic myeloid leukemia.

The studies could provide new information that may improve the effectiveness of second-generation drugs for chronic myeloid leukemia (CML). The researchers said their findings suggest that these mutations are central to resistance to Gleevec observed in some patients with CML.

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CML develops when an enzyme called Abelson tyrosine kinase (Abl) becomes overactivated by a chromosomal mixup 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 abnormal activity of the enzyme causes the overproliferation of white blood cells that is the hallmark of CML.

In an article published in the August 2002 issue of the journal *Cancer Cell*, researchers led by Charles Sawyers at the University of California, Los Angeles, and Howard Hughes Medical Institute (HHMI) investigator John Kuriyan at the University of California, Berkeley, reported that they identified 15 mutations in the *BCR-ABL* gene that cause resistance to Gleevec. Sawyers was selected as an HHMI investigator in May 2002 in a national competition to identify outstanding investigators who are conducting patient-oriented research.

The latest findings build on earlier work by Sawyers group that showed that mutations in *BCR-ABL* underlie resistance to Gleevec, a drug that has shown remarkable potency against CML. "We had studied a handful of CML patients who had a beautiful initial response to Gleevec, but who then relapsed quite dramatically," Sawyers said. "Our biochemical studies showed that the Bcr-Abl target protein, which was inhibited when the patients responded to the drug, was turned back on. Then, we found in a subset of those patients, one mutation in the kinase domain. John Kuriyan's structural studies revealed that this mutation was right at the point where Gleevec would bind to Bcr-Abl to inhibit it. Given this initial study, we believed it was critical to mount a much more comprehensive search for mutations in *BCR-ABL* .

In their current study, Sawyers and his colleagues searched for mutations in 32 patients in differing stages of CML. Each of the patients had developed resistance to Gleevec. Kuriyan and his colleagues at the University of California at Berkeley analyzed the mutations to reveal how they might affect the overall structure of the Bcr-Abl protein.

"The essential discovery was that the mutations fall into two categories, said Kuriyan. An obvious category would be mutations located directly at the binding site of the drug and which prevent binding. But especially interesting was that most of the mutations are scattered throughout the protein and at first glance don't have anything to do with occluding the drug's binding site." According to Kuriyan, these mutations might have altered the flexibility of the Bcr-Abl enzyme in a way that thwarted Gleevec. "In order to bind to Gleevec, the kinase domain of the enzyme has to distort itself into an inactive conformation, what is termed a 'closed' state," said Kuriyan. "And Gleevec acts to jam the machinery that cycles between the different conformational states. However, the mutations might lock Bcr-Abl into the on conformation, not giving Gleevec a chance to bind the enzyme and keep it in the off state."

Sawyers and his colleagues believe that the cells develop resistance to Gleevec through a process called "clonal selection." Their assumption is that although most of the cancer cells that harbor mutations are eliminated during treatment with Gleevec, a small number of those cells may survive and cause relapse.

Other theories for drug resistance have been proposed — including that the cancer cells learn to eliminate the drug, or that the drug is inactivated by other proteins. Sawyers emphasized that the new finding strongly suggests that mutations in Bcr-Abl are the primary cause of drug resistance. "I can't say that we have ruled out other resistance mechanisms, but to my mind, finding these mutations is the smoking gun, and we can't ignore them," he said.

Sawyers said that studies of the mutations could yield new information that may improve the next generation of drugs, and thereby minimize drug resistance. "The silver lining in this cloud is that now we know exactly what the drug target has to be for the second-generation drugs for CML, instead of

it being some mysterious bypass mechanism that we'd have to figure out," he said.

According to Sawyers, screening for mutations in the near-term will also likely become a method for determining the best therapies for patients. "However, over the longer term, we will need better Gleevecs — kinase inhibitors that work through other mechanisms that overcome resistance."

Discovering that multiple mutations can alter the conformation of Bcr-Abl offers important lessons for overcoming drug resistance, said Kuriyan. "In his novel *Anna Karenina*, Leo Tolstoy wrote that 'Happy families are all alike; every unhappy family is unhappy in its own way.' Similarly, we are finding that a kinase is active in only a single conformation, but it can be inactive in many conformations, he said. Gleevec specifically targets only one inactive conformation of Bcr-Abl, but these mutations presumably create different active conformations that the drug cannot bind to."

Kuriyan believes that overcoming drug resistance in CML, HIV and other diseases will require a deeper understanding of conformational changes in proteins. "We understand a considerable amount about how proteins are formed," he said. "But we understand very little about the dynamics of how proteins distort themselves from one structure to another, and what controls those distortions. These findings emphasize how crucial it is that we as structural biologists understand the factors that control the ability of protein kinases to distort themselves."