Dis*ABL*ing imatinib

By Kai-Jye Lou, Senior Writer

Mutations in *BCR-ABL* tyrosine kinase have long been associated with resistance to Gleevec imatinib, but they only account for about half of all cases. **University of Massachusetts Medical School** researchers have shown that upregulation of protein kinase Cη could be an alternative cause and might provide a new target for bypassing resistance.[1] But blocking the downstream signaling components could be easier than creating specific inhibitors of the relatively unexplored protein kinase C isoform.

In the study, the team showed that upregulation of protein kinase Cη (PRKCH; PKCη) enhances activity of the RAF/MEK/ERK pathway in models of imatinib-resistant chronic myelogenous leukemia (CML) that did not involve *BCR-ABL* tyrosine kinase mutations.

"Normally, when you treat patients with imatinib, you will see inhibition of MEK/ERK signaling, but what we saw in the resistant CML cells is independent activation of PKCη, leading to sustained MEK/ERK signaling even in the presence of imatinib," said corresponding author Michael Green.

Green is a professor of molecular medicine and director of the Program in Gene Function and Expression at the UMass Medical School and a **Howard Hughes Medical Institute** investigator.

Novartis AG markets Gleevec to treat CML, acute lymphoblastic leukemia (ALL) and gastrointestinal stromal tumors (GISTs). Novartis and several other companies also have clinical-stage compounds targeting RAF, MEK or ERK enzymes that could be combined with Gleevec to overcome resistance mechanisms that emerge in CML (*see* **Figure 1, "Circumventing BCR-ABL–independent resistance in CML"**).

Resistance to BCR-ABL tyrosine kinase inhibitors such as Gleevec is broadly categorized as BCR-ABL dependent or BCR-ABL independent. The former cases typically involve mutations or amplification of *BCR-ABL* and are generally treated with second-generation BCR-ABL inhibitors or chemotherapy. But the latter cases are less well understood—which prompted Green's team to investigate how such forms of resistance could develop in CML.

The researchers first carried out shRNA screens on human CML cells to identify genes that could confer sensitivity to Gleevec. They then generated a panel of Gleevec-resistant CML cell lines by knocking down individual sensitivity genes and found that many of the resulting cell lines had sustained activation of the RAF/MEK/ERK pathway following treatment with the drug. That oncogenic pathway is one of several known to operate downstream of BCR-ABL.[2]

Using an *in vitro* kinase assay on purified peptides, Green's group found that PKCη could directly activate CRAF (RAF1). That suggested a direct link between PKCη upregulation and RAF/MEK/ERK signaling.

Next, the researchers showed that the pathway was activated in patient samples as well. Cells from patients with Gleevec-resistant CML with wild-type *BCR-ABL* had higher *PKCη* mRNA levels than samples containing mutated *BCR-ABL*. In addition, resistant CML cells that contained wild-type *BCR-ABL* became more sensitive to Gleevec when *PKCη* was blocked with shRNA.

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—Michael Deininger, Huntsman Cancer Institute at The University of Utah

Finally, the team used cell-based and mouse models of CML with BCR-ABL-independent Gleevec resistance to test whether blocking the PKCη pathway could synergize with Gleevec to improve outcomes. Gleevec combined with either PKCη-targeted shRNA or the MEK inhibitor Mekinist trametinib decreased viability of CML cells and CML stem cells and led to increased survival of CML mouse models compared with control shRNA or either drug alone. **GlaxoSmithKline plc** markets Mekinist in the U.S. to treat melanoma.

CML stem cells are known to be intrinsically resistant to tyrosine kinase inhibitors including Gleevec and are associated with disease relapse.[3–5]

Results were published in *Science Translational Medicine*.

Researchers polled by *SciBX* thought that although the results show a clear connection between Gleevec resistance in CML and PKCη activation, the open question is how widespread the effect is.

"It would be very important to see whether the described resistance mechanism is observed not only with imatinib but also with other BCR-ABL tyrosine kinase inhibitors," said Michael Deininger, a professor and chief of hematology and hematological malignancies in the Department of Internal Medicine and the **Huntsman Cancer Institute at The University of Utah**.

He added that it will be key to determine the frequency of the described BCR-ABL-independent resistance mechanism in a large number of patients with CML.

The four other marketed BCR-ABL inhibitors for CML are Bosulif bosutinib from **Pfizer Inc.**, Iclusig ponatinib from **Ariad Pharmaceuticals Inc.**, Sprycel dasatinib from **Bristol-Myers Squibb Co.** and Novartis' Tasigna nilotinib.

Although PKCη could be a new therapeutic target for combating Gleevec resistance, developing isoform-selective inhibitors of protein kinase C (PKC) family enzymes has been difficult because of the substantial homology between the isoforms.[6]

Novartis' midostaurin and sotrastaurin inhibit multiple PKC isoforms including PKCη. Midostaurin is in Phase III testing to treat acute myelogenous leukemia (AML), while sotrastaurin is in Phase I testing to treat diffuse large B cell lymphoma (DLBCL) and uveal melanoma.

Combo opportunities

A combination of Gleevec with an inhibitor of either PKCη or one of the RAF, MEK or ERK enzymes could provide a way to quash resistance in CML stem cells while maintaining the BCR-ABL blockade.

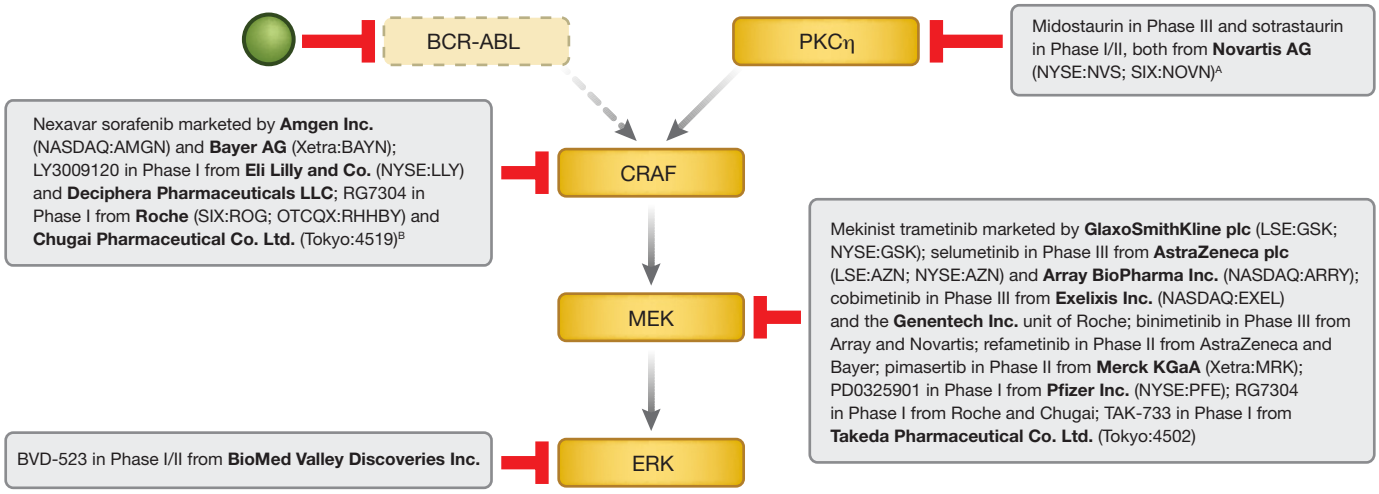

Figure 1. Circumventing BCR-ABL–independent resistance in CML. Resistance to the chronic myelogenous leukemia (CML) drug Gleevec imatinib is often caused by mutations in BCR-ABL tyrosine kinase, the protein that the drug targets, but about half of resistance cases involve that oncogene.

As reported in Ma *et al*., CML cells with BCR-ABL-independent Gleevec resistance show sustained activation of the RAF/MEK/ERK pathway despite effective suppression of BCR-ABL by the drug (green circle). This sustained activation depended on upregulation of protein kinase Cη (PRKCH; PKCη), which phosphorylates and activates CRAF (RAF1). Thus, compounds that inhibit PKCη or components of the RAF/MEK/ERK pathway might synergize with Gleevec or other BCR-ABL inhibitors to circumvent BCR-ABL-independent resistance.

Selected compounds that inhibit PKCη or components of the RAF/MEK/ERK pathway to treat cancer are shown. (Figure based on Figure 8 in ref. 1.)

[A]No companies have disclosed a PKCη-selective inhibitor. Midostaurin and sotrastaurin can inhibit multiple protein kinase C (PKC) isoforms including PKCη. [B]RG7304 has inhibitory activity toward CRAF and MEK.

Deininger noted that a potential advantage of the combination approach may be its ability to target CML stem cells, which could lead to a more durable clinical response. "What the researchers have shown is that the imatinib resistance of CML stem cells may not be so different mechanistically from BCR-ABL-independent resistance," he told *SciBX*. He added that the current generation of BCR-ABL inhibitors fail to disrupt pathways that CML stem cells depend on for survival.

Deininger added that a probable development path would be to test a combination therapy strategy in patients with CML with BCR-ABL-independent resistance who have failed second-line therapies.

However, it remains to be seen whether it would be better to combine a BCR-ABL inhibitor such as Gleevec with an inhibitor of MEK or PKCη.

A benefit of using MEK inhibitors is the availability of a marketed drug that simplifies the logistics for a clinical trial. In April, Novartis announced a deal to acquire Mekinist plus another 10 marketed cancer drugs from GlaxoSmithKline for $14.5 billion that would give the company ready access to the drugs to explore combination therapies. Novartis declined a request to comment.

In addition, Green's group found that the Gleevec and Mekinist combination had minimal toxicity in treated mice. But Deininger cautioned that MEK inhibitors in general are known to have significant toxicity in patients.

According to Green, a theoretical advantage of going after PKCη is that it could enable more direct targeting of the mechanism behind the increased RAF/MEK/ERK pathway signaling that leads to resistance.

Green said that his group is in the process of obtaining small molecule PKC inhibitors to test in CML models and is trying to further elucidate the mechanism underlying PKCη upregulation. He noted that his group also is obtaining samples from patients with CML taken prior to treatment to determine whether PKCη levels could predict which patients will become resistant to Gleevec.

The **University of Massachusetts** has filed a patent application covering methods of use for combinations of PKCη and MEK inhibitors with Gleevec. The technology is available for licensing.

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COMPANIES AND INSTITUTIONS MENTIONED

Ariad Pharmaceuticals Inc. (NASDAQ:ARIA), Cambridge, Mass.
Bristol-Myers Squibb Co. (NYSE:BMY), New York, N.Y.

GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
Howard Hughes Medical Institute, Chevy Chase, Md.
Huntsman Cancer Institute at The University of Utah, Salt Lake City, Utah
Novartis AG (NYSE:NVS;SIX:NOVN), Basel, Switzerland
Pfizer Inc. (NYSE:PFE), New York, N.Y.
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