Priming chemo prediction

By Chris Cain, Staff Writer

Researchers at the **Dana-Farber Cancer Institute** have developed an *in vitro* assay that predicts patient response to chemotherapy by directly measuring how prone cells are to apoptosis.[1] **Eutropics Pharmaceuticals Inc.** has exclusively licensed the assay and is pursuing it as a prognostic indicator of chemotherapy effectiveness in multiple cancers.

Chemotherapy resistance is difficult to predict because many distinct pathways lead to resistance in different cancers. One common mechanism is overexpression of members of the B cell lymphoma 2 (BCL-2; BCL2) family, which causes resistance to chemotherapy-induced apoptosis. However this family consists of approximately 20 proteins with either pro- or antiapoptotic activity, and thus it can be difficult to accurately predict how prone cells are to apoptosis by measuring levels of BCL2 protein expression.

"Systems biologists are making it their career to figure out how levels of BCL2-family proteins relate to chemotherapy sensitivity. I chose a functional approach to asking the same question," said Anthony Letai, associate professor of medicine at Dana-Farber and **Harvard Medical School**.

Letai's goal was to develop an assay that could give a more direct readout of a tumor cell's predisposition to apoptosis. He chose to focus on measuring mitochondrial membrane polarization, an indicator of a cell's physiological health—the lower the polarization across the mitochondrial membrane, the more chemosensitive a cell is. Once a critical mitochondrial membrane depolarization threshold is reached, a cell becomes committed to apoptosis.

To probe tumor cell susceptibility to apoptosis, Letai's team enlisted a peptide derived from proapoptotic members of the BCL2 family—BCL2 homology domain 3 (BH3)—to elicit depolarization. The peptide interacts with and inhibits antiapoptotic members of the BCL2 family.

The lower the levels of BH3 needed to trigger depolarization, the more sensitive the tumor cell would be to chemotherapeutic treatment. Letai calls this relationship 'mitochondrial priming' because it describes how close the cell is to an apoptotic threshold even before treatment with chemotherapeutics.

"It was very important that we develop an assay that did not require cell culture, as that has been a main stumbling block," said Letai. "We use bacterial cell culture all the time to determine bacterial sensitivity to antibiotics, but it doesn't work well for chemotherapy because tumor cells don't culture well *ex vivo* or they undergo phenotypic changes. It has certainly been tried but you don't get a good correlation to *in vivo* chemo sensitivity."

Letai had previously used a variation of the assay to determine the sensitivity of cancer cells to inhibitors of specific members of the BCL2 pathway.[2] The unanswered question was whether it could be adapted as a general method to measure cellular response to chemotherapeutics.

In samples from patients with multiple myeloma (MM), acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL) or ovarian cancer, increased mitochondrial depolarization in the assay correlated with improved response. This suggests the therapeutic index of cytotoxic chemotherapeutics may depend on increased mitochondrial priming in cancer cells.

For example, AML patients with greater mitochondrial depolarization were more likely to achieve complete remission than patients with low levels of depolarization (p=0.0027). Similarly, ALL patients with high depolarization were more likely to be relapse free (p=0.0012), and ovarian cancer patients with high depolarization had increased progression-free survival (p=0.0003).

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In total, 85 patient samples were assessed by the technique before the start of chemotherapy, of which 51 had available clinical follow-up data. Patients were treated with a range of cytotoxic chemotherapies, including DNA-damaging agents and antimitotic agents. Proteasome inhibitors including Velcade bortezomib were used in the MM patients.

Velcade induces apoptosis of MM cells, and resistance to the proteasome inhibitor has been linked to the expression of BCL2 family members. Velcade is marketed in the U.S. by **Takeda Pharmaceutical Co. Ltd.**'s **Millennium Pharmaceuticals Inc.** subsidiary to treat MM and mantle cell lymphoma.

Finally, treatment of chemoresistant tumors with a small molecule BCL2-family inhibitor increased membrane depolarization and restored sensitivity to a panel of cytotoxic cancer drugs compared with treatment using vehicle.

Taken together, the data suggest apoptotic sensitivity plays a role in determining chemotherapy efficacy.

Results were published in *Science*.

Clinical utility

Letai has exclusively licensed the assay to Eutropics, a company he cofounded in 2007 to develop the assay and small molecule inhibitors of a BCL2 family member called myeloid cell leukemia sequence 1 (MCL1).

Michael Cardone, cofounder, president and CEO of Eutropics, said the company is developing the assay both as a prognostic test for chemotherapy sensitivity and as a companion diagnostic for its preclinical MCL1 inhibitors. He said the company recently completed a contract with the **National Cancer Institute** to develop the test as a companion diagnostic for treatment of MM with Velcade.

He added, "We will demonstrate the clinical and commercial utility

of the assay for use with MM and AML therapies that are currently in use or in clinical development. As we do this we will be well prepared to use the test with our MCL1 inhibitor when it enters the clinic in the next two to three years."

Ingrid Wertz, research scientist at **Roche**'s **Genentech Inc.** unit, said the assay presents a good alternative to expression-based analysis of the BCL2 pathway. She said that, in fact, expression analysis can miss both activating and inactivating modifications to BCL2 family members.

Abbott Laboratories and Genentech are co-developing Navitoclax (ABT-263; RG7433) for solid tumors and hematological malignancies. Navitoclax is a small molecule inhibitor of BCL2, BCL-X_L and BCL2-like 2 (BCL2L2; BCLW).

Andreas Strasser, professor of the molecular genetics of cancer at **The Walter and Eliza Hall Institute of Medical Research**, said the advantage of Eutropics' assay is that it looks "more at the overall functionality of the machinery rather than the individual components, compared with western blotting of BCL2, MCL1 or other members. Unless you were to test all of them, you are only looking at some aspects of apoptosis."

Wertz added that BCL2-family proteins "are not the whole picture—proteins and pathways other than the BCL2 family also regulate cell death." She said that one next step would be to identify tumor types in which mitochondrial priming does not correlate with chemosensitivity and then try to understand why.

Eutropics said its assay is available for licensing.

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COMPANIES AND INSTITUTIONS MENTIONED

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