Overcoming ibrutinib resistance

By Lauren Martz, Staff Writer

Imbruvica ibrutinib's unprecedented response rate in mantle cell lymphoma is generating excitement around the new drug, but as with many targeted cancer therapies, tumors can ultimately become resistant. A recent study from **Weill Cornell Medical College** suggests that another therapeutic about to hit the market—the cyclin dependent kinase 4 inhibitor palbociclib—could prevent or overcome resistance when used in combination with Imbruvica or a phosphoinositide 3-kinase inhibitor.[1]

The researchers are now beginning clinical trials to test the different combinations in patients resistant to Imbruvica.

Mantle cell lymphoma (MCL) is a form of non-Hodgkin's lymphoma (NHL) that is highly proliferative and thus particularly prone to drug resistance. Imbruvica received accelerated approval from the **FDA** last year for recurrent MCL and has grabbed attention from physicians and investors alike for the unusually long response period it provides before the disease recurs.

Imbruvica is marketed by **Pharmacyclics Inc.** and **Johnson & Johnson** for recurrent MCL and chronic lymphocytic leukemia (CLL). It is also in Phase III trials for B cell lymphoma and NHL and Phase II testing for other lymphomas and multiple myeloma.

About one-third of patients show primary resistance to the drug, which means they do not respond when it is first added to their treatment regimen. In addition, many patients who initially respond stop doing so after taking the drug for a period of time—termed acquired resistance.

Mark Roschewski, staff clinician in the Lymphoma Therapeutics Section of the **NIH**'s Center for Cancer Research, told *SciBX*, "Imbruvica has remarkably high response rates in MCL but is not likely curative, and it is anticipated that the majority of patients, if not all, will eventually develop resistance."

Imbruvica targets the B cell receptor pathway enzyme Bruton's tyrosine kinase (BTK) and indirectly inhibits protein kinase B (PKB; PKBA; AKT; AKT1). However, partly because it is so new, little is known about its resistance mechanism.

Now, the Weill Cornell group, led by Selina Chen-Kiang, has investigated Imbruvica's resistance mechanism and found that BTK is mutated in acquired but not primary resistance and that combinations of cyclin dependent kinase 4 (CDK4) inhibitors with phosphoinositide 3-kinase (PI3K) inhibitors can overcome the acquired resistance. Although they did not find a specific BTK mutation responsible for primary resistance, they did find that CDK4 inhibitors also sensitize the resistant cancers to other therapeutics including Imbruvica and PI3K inhibitors.

Chen-Kiang is a professor of pathology and of immunobiology and microbial pathogens at Weill Cornell.

There are at least four other BTK inhibitors in earlier stages of development for various cancers (*see* **Table 1, "CDK4 and CDK6 or BTK inhibitors in development for cancer"**).

Resisting arrest

Because no specific mutations had been associated with ibrutinib resistance in MCL, Chen-Kiang's team performed whole-exome and whole-transcriptome sequencing on serial biopsy samples from patients with drug-resistant MCL.

Table 1. CDK4 and CDK6 or BTK inhibitors in development for cancer. At least five cyclin dependent kinase 4 (CDK4) and CDK6 inhibitors and five Bruton's tyrosine kinase (BTK) inhibitors are in development from preclinical to marketed for various cancers. The table shows the most advanced cancer indication for each product.

Source: BCIQ: BioCentury Online Intelligence

Company	Product	Target	Indication	Phase of development
Amgen Inc. (NASDAQ:AMGN); **Pfizer Inc.** (NYSE:PFE)	Palbociclib	CDK4; CDK6	Breast cancer	Registration
Novartis AG (NYSE:NVS; SIX:NOVN); **Otsuka Pharmaceutical Co. Ltd.**	LEE011	CDK4; CDK6	Breast cancer	Phase III
Eli Lilly and Co. (NYSE:LLY)	Abemaciclib	CDK4; CDK6	Mantle cell lymphoma (MCL)	Phase II
MetaMax LLC	MM-D37K	CDK4; CDK6	Solid tumors	Phase I/II
Onconova Therapeutics Inc. (NASDAQ:ONTX)	ON 1233000	CDK4; CDK6; NUAK family SNF-like kinase 1 (NUAK1; ARK5)	Multiple myeloma (MM) and MCL	Preclinical
Pharmacyclics Inc. (NASDAQ:PCYC); **Johnson & Johnson** (NYSE:JNJ)	Imbruvica ibrutinib	BTK	Chronic lymphocytic leukemia (CLL) and MCL	Marketed
Celgene Corp. (NASDAQ:CELG)	CC-292	BTK	B cell lymphoma; CLL	Phase I
Ono Pharmaceutical Co. Ltd. (Tokyo:4528)	ONO-4059	BTK	B cell lymphoma	Phase I
Biogen Idec Inc. (NASDAQ: BIIB); **Sunesis Pharmaceuticals Inc.** (NASDAQ: SNSS)	SNS-062	BTK	Cancer	Preclinical
Tolero Pharmaceuticals Inc.	TP-4207	BTK	CLL; MM; non-Hodgkin's lymphoma (NHL)	Preclinical

The researchers' first discovery was a missense cysteine-to-serine mutation in the Imbruvica-binding site of *BTK* (C481S) in samples from a patient with MCL who had a partial response to the drug for 14 months before relapsing. The mutation, which prevented binding of Imbruvica to its target, was present in samples taken during the relapse but absent in biopsies taken before treatment and germline samples from a cheek swab.

Targeted sequencing found the same mutation in a second patient at relapse who had a partial response to Imbruvica for 30 months before relapsing. The mutation was absent in six patients with either primary resistance to Imbruvica or a shorter, transient response to the drug. Thus, the C481S mutation appeared to be associated with acquired but not primary or transient resistance to Imbruvica in MCL.

"Acquired resistance to Imbruvica will be a clinical problem to avoid. It is also possible that maintenance strategies with Imbruvica will contribute to this resistance and that treatment strategies that avoid maintenance will be the most effective at avoiding such resistance."

—Mark Roschewski, National Institutes of Health

The data correlated with a study from **The Ohio State University** earlier this year that identified the same *BTK* C481S mutation in patients with CLL who relapsed after responding to Imbruvica. That team also showed that BTK C481S has about 25-fold lower affinity for Imbruvica than the wild-type protein.[2] The work was led by Jennifer Woyach, an assistant professor of internal medicine at Ohio State.

In the Weill Cornell study, Imbruvica bound and inhibited BTK in MCL cells from patients with primary or transient resistance, which suggested that its resistance mechanism operated through a BTK-independent pathway. However, AKT was not suppressed by Imbruvica and the enzyme remained active, which suggested that the PI3K pathway might be involved in primary resistance to the drug (*see* **Figure 1**, "**Blocking signaling to overcome Imbruvica resistance in MCL cells**").

AKT lies downstream of PI3K and is influenced by cell cycle control enzymes. In addition, relapsed MCL is highly proliferative and often shows upregulation of cell cycle genes including *CDK4*.

Thus Chen-Kiang and colleagues hypothesized that an overactive cell cycle may contribute to primary—and possibly also acquired—resistance. They tested CDK4 inhibition because it induces prolonged early G1

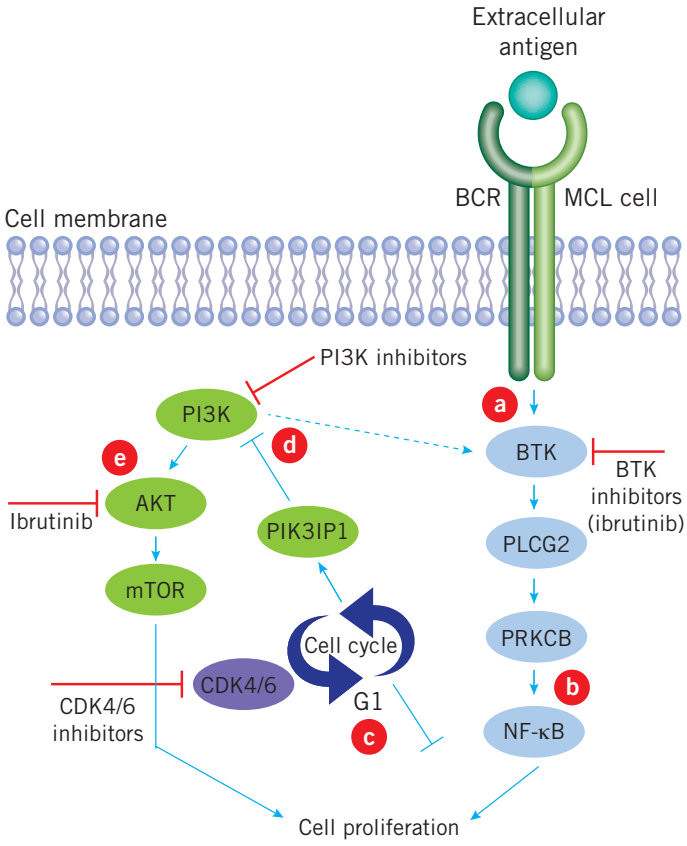

Figure 1. Blocking signaling to overcome Imbruvica resistance in MCL cells. [**a**] B cell receptor (BCR) stimulation by an extracellular antigen induces Bruton's tyrosine kinase (BTK) activation in mantle cell lymphoma (MCL) cells. Activation is higher in MCL cells than normal B cells. BTK inhibitors such as Imbruvica ibrutinib act by directly binding BTK to block downstream signaling. In addition, Imbruvica indirectly inhibits protein kinase B (PKB; PKBA; AKT; AKT1).

Acquired resistance develops when the Imbruvica-binding site on BTK contains the C481S mutation, which blocks inhibition of BTK and allows downstream signaling.

[**b**] BTK signaling ultimately leads to NF-κB activation through a pathway that involves phospholipase $C_{\gamma 2}$ (phosphatidylinositol-specific) (PLCG2) and protein kinase Cβ (PRKCB), which promotes cell proliferation and survival. Inhibiting BTK blocks this source of NF-κB activation.

[**c**] In a separate pathway, inhibition of cyclin dependent kinase 4 (CDK4) stalls and prolongs the MCL cell cycle in the G1 phase. Prolonging G1 also inhibits NF-κB activation. Therefore, halting the cell cycle at G1 with CDK4 and CDK6 inhibitors can help block cell proliferation caused by ibrutinib resistance.

[**d**] Prolonging G1 also activates the phosphoinositide 3-kinase (PI3K) negative regulator phosphoinositide 3-kinase interacting protein 1 (PIK3IP1; HGFL) to inhibit activation of the PI3K pathway.

[**e**] PI3K also activates AKT in MCL cells and leads to cell proliferation and survival through mammalian target of rapamycin (mTOR; FRAP; RAFT1) activation. Imbruvica indirectly inhibits AKT and blocks signaling through this pathway. In addition, PI3K inhibitors prevent downstream activation of AKT and block another prosurvival pathway. MCL cells with primary resistance to Imbruvica proliferate despite inhibition of BTK by the drug. Resistance may be caused by persistent activation of the PI3K-AKT pathway. The CDK4 and CDK6 inhibitor palbociclib can restore Imbruvica sensitivity in cell models of acquired resistance. Combinations of palbociclib with different PI3K inhibitors can overcome Imbruvica resistance in cell models of acquired or primary resistance.

arrest that stops cell replication and sensitizes cancer cells to various chemotherapeutics.[3,4]

In three MCL cell lines with primary Imbruvica resistance, inhibition of CDK4 by palbociclib prevented cell proliferation and sensitized cells to Imbruvica-induced apoptosis. Combined treatment with Imbruvica plus palbociclib inhibited activation of both AKT and BTK. These studies suggest that CDK4 inhibition may overcome primary Imbruvica resistance. Although palbociclib also inhibits CDK6, that enzyme is not expressed in MCL.

Chen-Kiang said that combining Imbruvica with palbociclib did not affect MCL cells with acquired resistance.

Pfizer Inc. and **Amgen Inc.** have palbociclib in registration for breast cancer, and it has received breakthrough designation from the FDA for that indication. At least five other CDK4 and CDK6 inhibitors are in clinical or preclinical testing for cancers (*see* **Table 1, "CDK4 and CDK6 or BTK inhibitors in development for cancer"**).

Finally, the researchers wanted to find a therapeutic combination that could overcome both primary and acquired resistance to Imbruvica. They expressed either wild-type or *BTK* C481S in chicken lymphoma cells that lack endogenous *BTK* expression and tested palbociclib in combination with various PI3K inhibitors rather than Imbruvica. The CDK4 inhibitor sensitized cells expressing both mutant and wild-type BTK to the PI3K inhibitors Zydelig idelalisib, pictilisib or copanlisib.

Idelalisib from **Gilead Sciences Inc.** is approved for CLL and NHL. **Roche**'s **Genentech Inc.** unit has pictilisib (GDC-0941) in Phase II testing for breast cancer and non–small cell lung cancer (NSCLC). **Bayer AG** has copanlisib (BAY 80-6946) in Phase II testing for NHL and solid tumors.

There are at least four other PI3K inhibitors including PI3Kα- and PI3Kδ-specific inhibitors, which are the isoforms expressed in MCL, in Phase III testing for cancers.

The team concluded that treatment with CDK4 inhibitors might sensitize cells to Imbruvica in patients with primary resistance, whereas combining CDK4 inhibitors with PI3K inhibitors might overcome both primary and acquired resistance to Imbruvica in MCL.

Data were published in *Cancer Discovery*.

First-line advantage

Despite the need for clinical validation, the findings offer potential for combination therapies in MCL that can counteract Imbruvica resistance. Indeed, some researchers suggested to *SciBX* that such combinations could become first-line therapy if the results hold up in the clinic. Imbruvica is used as a single agent in patients with MCL after they have tried other therapeutic options, although it is in Phase III testing with other combination regimens for first-line use.

Roschewski told *SciBX*, "I think that Imbruvica will soon be in first-line combinations. Most major advances in lymphoma therapy have come from employing the best agents up front and not waiting until resistance occurs."

He added, "Acquired resistance to Imbruvica will be a clinical problem to avoid. It is also possible that maintenance strategies with Imbruvica will contribute to this resistance and that treatment strategies that avoid maintenance will be the most effective at avoiding such resistance."

Darrin Beaupre said that Pharmacyclics is actively reviewing the idea of combining Imbruvica with cell-cycle modulators such as CDK4 inhibitors. "We have potential interest in the combination and are currently looking at all options," he said. Beaupre is VP of clinical medicine and early development at Pharmacyclics.

He added, "Any therapy works best in the first-line setting, and we are considering options in first-line and in relapsed patients. Imbruvica is a particularly exciting drug because even patients with relapsed disease have high response rates with a favorable safety profile."

According to the paper, combining Imbruvica with palbociclib should not be the only approach to try to overcome resistance. Combining PI3K inhibitors with palbociclib—without Imbruvica—could have anticancer effects for a broader group of patients resistant to Imbruvica.

"The combination of PI3K inhibitors and CDK4 inhibitors still needs to be formally tested. Unfortunately, there are many cases in cancer where something looks in the laboratory like it should work great but then doesn't work as well in the clinic," said Woyach. "Even if this combination ends up being very promising in the clinic, there will probably be situations where patients don't respond to this combination, don't tolerate it or develop resistance, so there will continue to be a need for Imbruvica as well as a need to continue to develop new therapies."

Chen-Kiang added that the implications of this work could go beyond Imbruvica resistance in MCL. CDK4 dysregulation, she said, is common in other human cancers such as breast cancer, NSCLC and glioblastoma. Thus, combination therapy involving palbociclib could overcome resistance to a variety of cancer therapeutics, she said.

Chen-Kiang told *SciBX* that Weill Cornell is enrolling patients in a trial to evaluate Imbruvica and palbociclib that is open to all patients with MCL who have failed one or more therapies.

"Most patients would have already been treated with Imbruvica and become resistant after a durable or transient response or are not responding at all," she said. The trial is supported by the **National Cancer Institute** and the NCI's Cancer Therapy Evaluation Program. She added that a second trial evaluating palbociclib with idelalisib is in the final stages of planning.

The patent status is unavailable. The Cornell team is discussing licensing options with undisclosed parties.

Martz, L. *SciBX* 7(33); doi:10.1038/scibx.2014.971
Published online Aug. 28, 2014

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COMPANIES AND INSTITUTIONS MENTIONED

Amgen Inc. (NASDAQ:AMGN), Thousand Oaks, Calif.
Bayer AG (Xetra:BAYN), Leverkusen, Germany
Food and Drug Administration, Silver Spring, Md.
Genentech Inc., South San Francisco, Calif.
Gilead Sciences Inc. (NASDAQ:GILD), Foster City, Calif.
Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.
National Cancer Institute, Bethesda, Md.
National Institutes of Health, Bethesda, Md.
The Ohio State University, Columbus, Ohio
Pfizer Inc. (NYSE:PFE), New York, N.Y.
Pharmacyclics Inc. (NASDAQ:PCYC), Sunnyvale, Calif.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
Weill Cornell Medical College, New York, N.Y.