

IN THE SPOTLIGHT

The Promise of Combining Inhibition of PI3K and PARP as Cancer Therapy

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Summary: Analyses of *in vitro* and patient-derived *in vivo* models of breast cancer reveal that a combination of inhibitors of the enzymes PARP and phosphoinositide 3-kinase (PI3K) is a potentially effective treatment regimen for breast cancer tumors with elevated activity of the PI3K pathway. *Cancer Discov*; 2(11); 982-4. ©2012 AACR.

Commentary on Ibrahim et al., p. 1036 (9).

Commentary on Juvekar et al., p. 1048 (10).

PARP inhibitors are a class of developmental anticancer agents that exert their cytotoxic effect by modulating the repair of DNA damage (1). Although originally developed as chemosensitizers, PARP inhibitors are also able to elicit synthetic lethality in tumor cells carrying loss-of-function mutations in tumor suppressor genes such as *BRCA1* and *BRCA2* (1). Loss of these genes causes a deficiency in the homologous recombination (HR) DNA double-strand break (DSB) repair pathway; it is thought that the loss of HR restricts the ability of cells to repair DNA lesions caused by PARP inhibition (1). In this issue of *Cancer Discovery*, two articles report the novel observation that inhibition of phosphoinositide 3-kinase (PI3K) signaling can exacerbate the effects of PARP inhibitors in killing cancer cells.

In the clinic, phase I and II clinical trials have shown that the PARP inhibitor olaparib can elicit significant and sustained antitumor responses as a single agent in cancer patients with *BRCA* mutant tumors while having a favorable toxicity profile (1, 2). Based on promising data from these trials, there was considerable interest in expanding the use of PARP inhibitors beyond patients with germline *BRCA* mutations using two main strategies. First, clinical studies were expanded to include populations that should theoretically harbor a high proportion of “BRCAness” (3) phenotypes. These included high-grade serous ovarian cancer, a disease in which tumors are characterized by a relatively high frequency of HR gene mutations (4), and triple-negative breast cancer, in which tumors lack *ERBB2* amplification as well as estrogen receptor (ER) and progesterone receptor (PR) expression but share a number of characteristics with *BRCA1* mutant breast cancer. Although a phase II study in high-grade serous ovarian cancer showed that olaparib can reduce the risk of recurrence when used as maintenance therapy after chemotherapy (5), small studies of olaparib as a single agent in triple-negative breast cancer have been relatively disappointing (6).

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Second, based on preclinical studies showing additive or synergistic effects of combining PARP inhibitors with a number of chemotherapeutic agents, clinical trials assessing PARP inhibitor drug combination regimens have been conducted. However, combinations with temozolomide, platinum salts, paclitaxel, or gemcitabine have generally shown exacerbation of toxicity, most often myelosuppression, requiring dose reductions (1). This suggests that, although there may be an additive or even synergistic chemopotentiality with these drug combinations in various models, there is not a wide therapeutic index between normal and tumor tissues in humans and therefore delivering these might be problematic.

These factors have brought the clinical development of PARP inhibitors to a crossroads. The early results in familial *BRCA*-mutant patients still strongly support the case for registration trials in this genetically defined subgroup, but the jury is still out regarding optimal combination approaches and predictive biomarkers in nonfamilial subtypes (1) and therefore, further insights into this area are to be welcomed.

The two recently published articles discuss the combination of PARP inhibitors with PI3K inhibitors as a therapeutic strategy in different breast cancer populations. Activation of the PI3K pathway is a common feature of many tumor types and leads to the stimulation of cell growth, mobility, survival, and metabolism (7), and also sensing of DSBs during DNA repair (8).

In the first article, Ibrahim and colleagues (9) report on their assessment of the potential of combining PI3K and PARP inhibition in triple-negative breast cancers, a tumor type often characterized by aberrant PI3K pathway activation. On siRNA silencing of *PIK3CA* in a *BRCA* wild type, *PTEN* mutant triple-negative cell line, the authors observed an accumulation of phosphorylated histone-2AX (γ -H2AX), a marker of DNA damage, inferring that the loss of *PIK3CA* results in genomic instability, a phenomenon enhanced by the addition of olaparib. siRNA silencing of *PIK3CA* or pharmacologic inhibition with a pan-PI3K inhibitor (BKM120) was accompanied by a concomitant reduction in the expression of *BRCA1* and *BRCA2* and a reduction in the cellular capacity to conduct HR, hinting at a mechanistic explanation

for the observations. Consistent with the reduction in BRCA1/2 expression, the authors also show that *in vitro* sensitivity to olaparib and an additional clinical PARP inhibitor, ABT888, can be achieved by PI3K pathway blockade.

To validate these observations *in vivo*, 3 patient-derived triple-negative breast cancer xenografts were assessed for sensitivity to PI3K inhibitor and PARP inhibitor combinations. Each of these tumors was PTEN deficient, with one also mutant in *PIK3CA*. This analysis confirmed elevated γ -H2AX and decreased BRCA1 expression following treatment with a PI3K inhibitor in two of the three models. Furthermore, the decrease in BRCA1 expression correlated with an enhanced response to the combination of PARP inhibitor and PI3K inhibitor when compared with either drug alone. Intriguingly, the tumor xenograft that did not respond favorably to the drug combination did not exhibit an increase in extracellular signal-regulated kinase (ERK) phosphorylation following PI3K blockade, leading the authors to investigate whether downregulation of BRCA1/2 was mediated by ERK activation, which seemed to be the case.

In silico analysis of ERK-regulated transcription factors for potential *BRCA1/2* promoter binding sites revealed that the ERK-activated transcription factor, ETS1, might regulate *BRCA1/2* expression. Supporting this, the activation of ERK seen after PI3K inhibition was associated with a concomitant increase in phosphorylated/activated ETS1. Furthermore, siRNA knockdown of ETS1 was sufficient to increase *BRCA1/2* expression and could block the sensitivity of MDA-MB-231 cells to dual treatment with a PI3K inhibitor and a PARP inhibitor suggesting a critical role for this transcription factor (Fig. 1).

In the companion article from Juvekar and colleagues (10), a PI3K/PARP inhibitor combination was assessed in the context of *BRCA1* deficiency. The study is built on the observation that the PI3K pathway is overexpressed in the *MMTV-CreBrca1^{fl/p53}* mouse, a genetically engineered model of spontaneous breast cancer in which tumors carry a hypomorphic *BRCA1* allele. Both AKT and ERK phosphorylation were

increased and PTEN and INPP4B expression were absent in the tumors generated in these mice. FDG-positron emission tomographic (PET) imaging was used to determine the effects of PI3K inhibition, illustrating that the initially avid FDG uptake in tumors was reduced by a median of 46.7% within 48 hours of treatment with BKM120 (a PI3K inhibitor), a biomarker effect sustained over a period of 2 weeks with continued treatment. This response was accompanied by a decrease in the levels of phosphorylated AKT and a reduction in the vascularity of tumors. Compensatory activation of the EGF receptor (EGFR)/mitogen-activated protein kinase (MAPK) pathways in *BRCA1* mutant cell lines in response to treatment with a PI3K inhibitor (BKM120) was noted, but this activation was prevented by concomitant administration of a PARP inhibitor (olaparib). Consistent with the findings of Ibrahim and colleagues (9), PI3K inhibition led to an increase in γ -H2AX accumulation and a reduction in AKT phosphorylation, which was exacerbated by the addition of a PARP inhibitor. This seems to be via an ATM-independent mechanism, possibly mediated by DNA-protein kinase (PK).

The localization of the DNA recombinase RAD51 to distinct nuclear foci (probably sites of active DNA repair by HR) represents one of the critical events in HR. Juvekar and colleagues show that RAD51 foci formation and by inference residual baseline HR function in the *BRCA1* hypomorphic model was completely blocked by treatment with a PI3K inhibitor, a result supported by the use of PI3K siRNA. Testing with the combination of a PARP inhibitor and a PI3K inhibitor *in vitro* and *in vivo* revealed that, *in vivo* only, a significant suppression of tumor growth was observed. A similar result was seen in two patient-derived xenografts with sustained responses to the combination of a PI3K inhibitor and a PARP inhibitor.

These two independent articles show that in some situations where the PI3K pathway is activated, PI3K inhibition is associated with reduction of HR capability and sensitization to PARP inhibitors. The mechanism by which *BRCA* gene transcription is modulated via ETS, proposed by Ibrahim and colleagues, is compelling (Fig. 1) and could perhaps also explain the PARP inhibitor sensitivity of prostate cancer models carrying *ETS* gene translocations observed by others (11). How this mechanism observed in *BRCA* wild-type triple-negative breast cancer fits with the observation by Juvekar and colleagues in a *BRCA1* hypomorphic model is less clear, and alternative mechanisms including the modulation of β -NAD⁺ levels (the critical cofactor used by PARP superfamily enzymes) were proposed.

Although there can be little doubt that there is an enhanced effect of the combination of these two targeted treatments, what is less clear is whether this exploits a true therapeutic window in these tumors or, when tested clinically, if this combination will run into problems with toxicity *in vivo* as have previous PARP inhibitor combinations? Juvekar and colleagues (10) did not observe overt signs of excess toxicity in mice treated with the combination, even over prolonged periods of treatment and perhaps the relatively mild side-effect profiles of olaparib and BKM120 will allow either for a therapeutic window or the delivery of sufficient doses to elicit an antitumor effect with manageable toxicity.

Another issue is the choice of biomarker for patient selection; options include *BRCA* mutation, *PTEN*, or *INPP4B* mutation

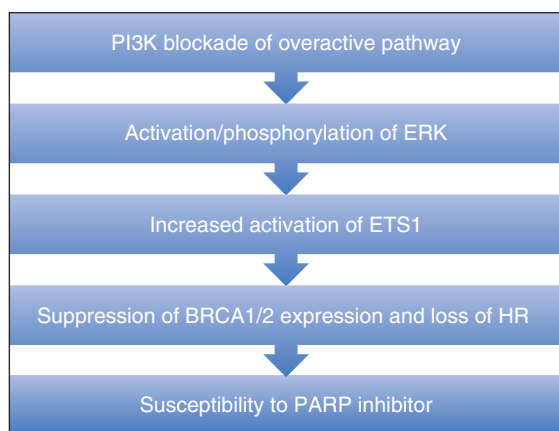


Figure 1. Mechanistic rationale for PARP inhibitor sensitization caused by PI3K pathway inhibition proposed by Ibrahim and colleagues (9). Blockade of hyperactive PI3K signaling drives ERK-dependent activation of the ETS transcription factor. This, in turn, suppresses *BRCA* gene transcription, causing a deficiency of HR and PARP inhibitor sensitivity.

or expression loss, functional loss of HR, or increased phospho-AKT, among others. Furthermore, is it correct to extrapolate the findings to, for example, all triple-negative breast cancer? Several lines of evidence suggest that triple-negative breast cancer shows considerable heterogeneity.

One final concern is, as with all targeted therapies, the emergence of resistance. Juvekar and colleagues (10) allude to this when they identify a drug resistant “pushing margin” that continues to grow during treatment with a PI3K inhibitor despite maintaining target inhibition as evidenced by continued suppression of AKT phosphorylation.

Despite these caveats and uncertainties, this combination certainly warrants testing in clinical trials and may provide a way forward for the clinical use of PARP inhibitors in a wider population of patients.

Disclosure of Potential Conflicts of Interest

A. Ashworth and C.J. Lord are inventors on patents describing the use of PARP inhibitors and may benefit as part of the ICR Rewards to Inventors Scheme. C.J. Lord receives honoraria for service on the speakers' bureau for AstraZeneca.

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