Published in final edited form as:
Cancer Discov. 2012 January ; 2(1): 16–18. doi:10.1158/2159-8290.CD-11-0323.

Dissecting PI3Kness: The Complexity of Personalized Therapy for Ovarian Cancer

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Epithelial ovarian cancer is a chemo-responsive, but less frequently chemo-curable disease. Over the last three decades, 5-year survival has increased from 37% to 46%[1] due to more frequent application of cytoreductive surgery and the use of combination chemotherapy with platinum compounds and taxanes. Despite improvement in average survival, the overall rate of cure has not changed and remains at approximately 30%. Further, cure rates in the most common and aggressive form of ovarian cancer, high grade serous ovarian cancer, are even worse. Empirical addition of other conventional cytotoxic drugs with clinical activity against ovarian cancer has not improved outcomes.[2] Given the limitations in treatment with conventional drugs, great hope has been placed on personalized therapy with targeted agents.[3] To date, however, individual targeted agents have had only a modest impact on recurrent ovarian cancer in unselected patients. With the exception of bevacizumab, eight targeted drugs [gefitinib, imatinib, sorafenib, temsirolimus, mifepristone, enzastaurine, lapatinib and vorinostat] have produced objective response rates of less than 10% and have stabilized disease for six months in less than 25% of cases in phase II trials. It is now apparent that in high grade serous ovarian cancer, as in many other solid tumors, oncogene addiction will be rare. Combinations of targeted agents will be required to produce synthetic lethality and to achieve a significant rate of durable clinical response. Considering the number of possible targets, preclinical studies must inform the choice of agents evaluated in the clinic.

Ovarian cancers exhibit remarkable heterogeneity at a clinical, cellular and molecular level. Traditionally, ovarian cancers have been classified morphologically by histotype and grade. Different histotypes can resemble the epithelial cells found in normal gynecologic tissues including fallopian tube (serous), endometrium (endometrioid), endocervical glands (mucinous), and glycogen rich vaginal rests (clear cell). Among frankly invasive cancers, the most important morphologic distinction has been between low and high grade, prompting classification of ovarian cancers into two categories. Type I lesions constitute 10–20% of ovarian cancers and include low grade serous, mucinous, endometrioid, and clear cell histotypes. Low grade Type I cancers present in early stage (I-II), grow slowly and are relatively resistant to platinum-based chemotherapy. Type II lesions include high grade serous and undifferentiated cancers that present at late stage (III-IV) and grow more aggressively, but respond more frequently to platinum-based treatment.

The profile of genetic changes differs remarkably between Type I and Type II ovarian cancers indicating that they likely represent independent diseases or differentiation pathways and rarely, if ever, interconvert. Type I low grade cancers have near normal gene copy number, maintain wild-type p53 and appear to be driven by activating mutations of *RAS* and *PIK3CA*, inactivating mutations of *PTEN* and expression of IGFR. Type II high grade cancers have marked genomic instability and *p53* mutation in nearly all cancers.[4] Dysfunction of homologous DNA repair is mediated by mutation or silencing of *BRCA1* or *BRCA2* in >40% of high grade Type II cancers, with multiple other aberrations in the homologous repair pathway occurring at lower frequencies.[4] Additionally, changes in DNA

copy number appear to be critical drivers of malignant transformation of Type II cancers. At least 22 amplified growth stimulatory genes encode potentially druggable targets in high grade serous cancers. Whether these genes are drivers of ovarian cancer whose inhibition would impact patient outcomes remains to be determined in carefully designed clinical trials. Aside from inactivating mutations of *p53* (96%), *BRCA1* or *BRCA2* (20%), high grade type II cancers have a significant number of mutations in only 4 genes: *NF1* (4%), *RB1* (2%), *CSMD3* (6%), and *CDK12* (3%). The remaining genes are mutated in <1% of Type II ovarian cancers, based on the recent The Cancer Genome Atlas (TCGA) analysis of more than 300 clinical specimens.[4]

Abnormalities of phosphatidylinositol 3' kinase (PI3K) signaling have been detected in both Type I and Type II ovarian cancers. The PI3K pathway can be activated by mutation or amplification of its intrinsic signaling molecules or by upstream activation of *Ras* or receptor tyrosine kinases. Furthermore, loss of negative regulators such as PTEN or INPP4B can also result in pathway activation. The first indication of aberrations targeting the PIK3CA pathway in ovarian cancer was the demonstration of *AKT2* amplification.[5] This was followed by demonstration of *PIK3CA* amplification as a common event in ovarian cancer.[6] In both cases, the teams demonstrated that the aberrations were associated with pathway activation and response to PI3K pathway inhibitors.[7] Subsequent studies, and in particular the TCGA effort, have provided a more detailed understanding of the spectrum of aberrations in the PI3K pathway in ovarian cancer.

A fraction of low grade Type I ovarian cancers have activating mutations of *KRAS* (>20%) and *PIK3CA* (40%), as well as inactivating mutations of *PTEN* (3–8%). Moreover, expression of *PTEN* can be lost in 27% of Type I endometrioid ovarian cancers through multiple mechanisms including promoter methylation. Subsets of high-grade type II cancers exhibit amplification of *KRAS* (11%), *HER2* (1%), and *PIK3CA* (17%). Further, all three isoforms of AKT are highly amplified in type II cancers: *AKT1* in 3%, *AKT2* in 6%, and *AKT3* in 8% of tumors. Overall, 20% of high-grade Type II cancers have demonstrable aberrations in the PI3K pathway in terms of copy number at the DNA level and when expression is considered, 46% of patients demonstrate aberrations and thus possess the quality of "PI3Kness." However, whether amplification or deletion of pathway members, and in particular changes in expression levels, results in pathway activation and whether these changes drive tumor behavior such that targeted therapies would improve patient outcomes needs to be tested. In addition, several of these genes are located in large amplicons or deletions and may not be drivers of the amplification.

Within the PI3K signaling pathway, AKT regulates a number of cellular functions including growth, proliferation, metabolism, motility, survival and angiogenesis.[8] Serine-threonine phosphorylation of critical substrates affects glucose uptake, glycogen synthesis, protein synthesis, cell cycle control, BH3-only proteins involved in apoptosis, and transcription factors including the FOXO family and NFκB. Among the three AKT isoforms, AKT1 is widely distributed in normal tissues and is critical for cell growth and survival, AKT2 has been detected in muscle and adipocytes and is regulated by insulin, and expression of AKT3 is found primarily in normal brain and testes. All three AKT isoforms can transform cells in culture.[9] In human malignancy, amplification or overexpression of *AKT2* has been observed in colorectal, hepatic, and pancreatic as well as in ovarian cancer.[8]

In this issue of *Cancer Discovery*, Solit and colleagues have studied 17 well-characterized ovarian cancer cell lines to correlate response to AKT inhibitors with activation of the target and the presence of other genetic alterations. Activation of AKT was necessary, but not sufficient to assure growth inhibition by isoform-selective or pan-AKT inhibitors. Despite phosphorylation and activation of AKT, the majority of ovarian cancer cell lines did not

respond to AKT inhibition. In cancers that expressed AKT3, a pan-AKT inhibitor was more effective than an inhibitor with selective activity for AKT1 and AKT2. Cancer cell lines with activating mutation of *RAS* or inactivating mutations of *RB* were relatively resistant to AKT inhibition.

As each cancer cell line is derived from a single patient, use of panels is likely to have greater predictive value, particularly if their molecular abnormalities resemble precisely those found in cancer cells taken directly from patients. In the past, less than 10% of ovarian cancers obtained at surgery could be established as immortal lines in cell culture. Cell lines that could be established in culture are likely to have been highly selected for the ability to survive *ex vivo*. Many of the ovarian cancer cell lines used in the present study have been maintained in culture for many years and may have undergone genetic drift. As the authors discuss, there is only a loose correlation between the distribution of genetic lesions found in the TCGA study and the cell lines utilized in the present report. Perhaps of greatest concern is that only one of the seventeen lines had amplification of *AKT2* and none had amplification of *AKT1* or *AKT3*. Only a single cell line had amplification of *KRAS*. A number of other aberrations, including *p53* mutation, were not assessed in these cell lines. Activation of the PI3K pathway in these cell lines is largely through mutation of *PIK3CA, AKT, PTEN* and *RAS*, which are characteristic of the less common Type I cancers, but not of the prevalent high grade Type II cancers that constitute the major challenge in the clinic. Recent development of culture media that permits establishment of cell lines from >80% of ovarian cancers should facilitate development of new cell lines from Type II cancers that preserve the relevant genetic and epigenetic changes.

The finding that activation of AKT is necessary, but not sufficient to assure response to AKT inhibitors is similar to previous studies of inhibitors targeting PI3K or mTOR or both. RAS signaling abrogated sensitivity to AKT inhibitors, confirming earlier observations with other tumor types.[10] In the setting of ovarian cancer, this clearly has relevance to management of low grade Type I cancers. If *RAS* amplification has the same impact on signaling as mutation, a fraction of patients with Type II cancer should benefit from coordinate inhibition of both the RAS and PI3K pathways. Importantly, inhibition of MEK and of AKT produced greater inhibition of ovarian cancer cell growth than inhibition of AKT alone. The observation that *RB* mutation also antagonizes the functional impact of AKT inhibition is novel and suggests that RB signaling may be downstream of AKT in ovarian cancers as well as normal cells.[11] While *RB* is mutated in only 2% of Type II ovarian cancers, dysregulation of the RB pathway can occur in up to 67% of cases.[4] Consequently, careful analysis of genes involved in cell cycle regulation may be important in choosing targeted therapy to combine with AKT inhibitors.

While RAS and RB dysregulation can affect the response to AKT inhibition, one recent report suggests that selection of patients with genetic abnormalities that enhance PI3K pathway signaling can increase the fraction of patients who respond to inhibitors of the pathway.[12] In Phase I Trials at M.D. Anderson, mutational analysis of PI3K pathway members was performed on cancers from 161 patients that could be matched to PI3K pathway–targeted Phase I drugs, 131 who could not be matched, and 438 who were not tested. Although this was a heterogeneous group of patients, 29% responded when matched, 8% responded when not matched and 6% responded if not tested. At least one ovarian cancer patient was a major responder in these studies.

The present report suggests that while individual AKT inhibitors are likely to help only a small fraction of high-grade serous ovarian cancer patients, carefully chosen combinations of targeted therapy could have greater activity. Choosing the second target will require biopsy and analysis of recurrent cancer prior to and perhaps during treatment with AKT

inhibitors. Given the complexity and heterogeneity of ovarian cancer, PI3Kness must be dissected for each patient.

Acknowledgments

This work was supported by funds from the M.D. Anderson SPORE in Ovarian Cancer NCI P50 CA83639, the M.D. Anderson CCSG NCI P30 CA16672, Stand Up To Cancer Research Grant – American Association for Cancer Research Dream Team Translational Research Grant, Grant Number SU2C-AACR-DT0209, the National Foundation for Cancer Research, the Ovarian Cancer Research Fund and philanthropic support from The Zarrow Foundation and Stuart and Gaye Lynn Zarrow.

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