RESEARCH ARTICLE

Short Communication: Updates on AKT Inhibition in Estrogen Receptor Positive Breast Cancer

Haeseong Park and Cynthia X. Ma*

Authors' Affiliations:

Division of Oncology, Department of Internal Medicine, Alvin J Siteman Cancer Center, Washington University School of Medicine, St. Louis, MO

*** Corresponding author:** E-mail: cynthiaxma@wustl.edu

Abstract

Activation of phosphatidylinositol 3-kinase (PI3K)/AKT signaling is associated with endocrine resistance in estrogen receptor positive (ER+) breast cancer. AKT is an important downstream effector of the PI3K signaling pathway, regulating key cellular functions related to cancer progression and survival. Preclinical evidence supports the evaluation of AKT inhibitors as a treatment strategy for patients with ER+ breast cancer. Early phase clinical trials of AKT inhibitors provides preliminary efficacy and key toxicity profiles. Clinical trials have been focused on combining AKT inhibitors with hormonal therapy or cytotoxic chemotherapy. Here we present an update on the clinical investigation of these agents.

Inhibiting AKT in Estrogen Receptor Positive Breast Cancer: Preclinical Evidence

Phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway is one of the key pathways associated with endocrine resistance in ER+ breast cancer (1-4). Targeting mTOR, a downstream effector of the PI3K/AKT pathway, with the rapamycin analogue everolimus, has been shown to extend progression-free survival (PFS) in patients with ER+ endocrine resistant breast cancer(5). However, as rapalogues induce feedback upregulation of Akt (6, 7), which potentially limits their anti-tumor activity, direct inhibitors of AKT and PI3K are in clinical trial development for more effective PI3K pathway inhibition(8-11). This review will focus on AKT inhibitors.

AKT is a downstream effector of PI3K, which is activated by receptor tyrosine kinases either by direct or indirect interaction through phosphorylation of adaptor molecules. Activated PI3K phosphorylates a membrane phospholipid, phosphatidylinositol bisphosphate (PIP_2) to phosphatidylinositol trisphosphate (PIP_3), which binds to AKT and PDK1. PDK1 then phosphorylates AKT at T308 residue in the activating loop. In addition, mTORC2 activated by receptor tyrosine kinase activity phosphorylates AKT in the hydrophobic motif, through multisite phosphorylation of TSC2. This leads to inactivation of TSC2, then accumulation of RHEB in the GTP-bound form, and

activation of mTORC1. Several downstream effectors such as S6K1 and 4EBP1 are activated by AKT and mTORC1, leading to cell growth and proliferation among many other cellular processes(12). Other downstream effectors of AKT include MDM2, GSK3, FOXO, BAD, Casp9, eNOS, and PRAS40, thereby regulating multiple cellular functions including survival, proliferation, growth, metabolism, angiogenesis, and glucose uptake(13-16). It should be noted that both mTORC1 and S6K could attenuate the PI3K/AKT signaling by phosphorylation of serine residues at IRS1 and further degradation of IRS1. Inhibition of TORC1 may relieve the negative feedback on PI3K. Inhibition of PI3K or AKT also may result in feedback upregulation of several RTKs, leading to increased cell survival(17).

Three isoforms of AKT exist with potentially different cellular functions(18, 19). AKT1 is known to promote cellular survival and growth, but also has been shown to be associated with inhibiting cellular migration(20, 21). AKT2 overexpression is more commonly seen in human cancer, and is associated with metastatic potential(19, 22). An AKT3-deficient mouse model was shown to have defects in brain development, and potential roles in regulating the expression of ErbB2, ErbB3, and ERa has been reported(23). Upregulation of AKT3 appears to be associated with resistance to AKT1/2 specific inhibition(24). Loss of AKT1 has been shown to increase cellular invasiveness, potentially leading to increase signaling via AKT2, thus provides rationale to inhibiting all isoforms.

Several genomic alterations lead to constitutive activation of PI3K/AKT signaling. Somatic mutations in the gene coding the alpha catalytic subunit of PI3K, *PIK3CA*, occurs very frequently in up to 40% of ER + breast cancer(25, 26). Other genetic alterations that may lead to activation of PI3K pathway are frequently present, including activating AKT1 mutation in up to 4% and deleterious PTEN aberrations in up to 24% of ER+ (luminal A and B) breast cancer(26). In preclinical models, PTEN loss or activating mutations in PIK3CA/AKT1 were associated with antitumor activity when treated with AKT inhibitors(27, 28).

In addition to high frequency of molecular aberrations leading to pathway activation, several other key evidences support targeting PI3K/AKT pathway to overcome endocrine resistance in breast cancer. Molecular crosstalk between PI3K/AKT pathways and estrogen pathways leading to hormone-independent activation of estrogen receptor alpha, conferring endocrine resistance has been reported(29, 30). Breast cancer cells after long-term estrogen deprivation (LTED) showed reliance on PI3K signaling evidenced by gene expression and proteomic analyses showing increased phosphorylation of effector molecules in PI3K/AKT pathway, such as p-AKT, p-S6, p70S6K(3, 31-33). Activated AKT signaling was associated with higher risk of relapse and death in ER+ breast cancer(34). In a recent publication by Ma et al., estrogen deprivation was required for AKT inhibition to induce apoptosis in majority of cell lines tested(35). In addition, in LTED cell lines, significant synergistic effect was noted when treated with fulvestrant and an AKT inhibitor, MK-2206. These results are in line with previously reported responses to AKT inhibition in endocrine-resistant breast cancer models(36).

AKT Inhibitors in Clinical Development

MK-2206 and AZD5363 are the two most studied oral pan-AKT inhibitors in ER+ breast cancer. MK-2206 is an allosteric inhibitor of AKT, with equal potency

toward AKT1 (IC50, 5 nmol/L) and AKT2 (IC50, 12 nmol/L), and less potency against AKT3 (IC50 65nmol/L)(37). AZD5363 is an ATP competitive kinase domain inhibitor of AKT, and inhibits all three AKT isoforms with an IC50<10nmol/L(38). Both agents are being evaluated in phase I and II clinical trials in combination with various other agents. Completed and ongoing trials using these AKT inhibitors for patients with endocrine resistant breast cancer are discussed in the next section. A list of ongoing and completed clinical trials with MK-2206 and AZD5363 in various settings are shown in Table 1. Other AKT1 inhibitors, such as GSK2141795, have not been studied in ER+ breast cancer.

Table 1. Overview of completed and ongoing clinical studies of MK2206 and AZD 5363

Agent	NCT Identifier	Combination	Phase	Population	N	Results
MK2206						
	NCT01277757	Single	II	Advanced breast cancer with PIK3CA, AKT, PTEN mutations or PTEN loss; after at least one systemic therapy in the metastatic setting	30	200mg, PR 1/21(56)
	NCT01776008	Anastrozole +/- goserelin	II	Locally advanced ER+ breast cancer, PIK3CA mutated; Neoadjuvant	16	pCR=0/16, no added effect on cell proliferation or apoptosis
	NCT01344031	Anastrozole/ fulvestrant	II	Metastatic ER+ breast cancer, any number of prior lines obut must not have a history of disease progression on anastrozole (Phase IA and Phase IB), fulvestrant (Arm C), or both anastrozole and fulvestrant (Arm D)	31	RP2D MK2206= 150mg QW with prednisone prophylaxis, DLT= G3 rash, 2/30 PR, 9/30 SD>=6mo (35)
	NCT01243762	Dalotuzumab	I	Solid tumors	29 (1 breast)	MTD dalotuzumab 10mg/kg, MK2206=150mg. Common G3AE: hyperglycemia, maculopapular rash, fatigue (55)
	NCT01245205	Lapatinib	I	Solid tumors/ Breast cancer	23 (escalation), 5 breast (expansion)	MTD MK2206= 45mg QOD, lapatinib 1500mg daily; No CR/PR. DLTs included G3 fatigue, hypoNa, G2 mucositis, G4 hypoNa, G3 rash, hypocalcemia (57)
	NCT01263145	Paclitaxel	Ib	Solid tumors/ breast cancer	22 (14 breast)	RP2D paclitaxel 80mg/m2 weekly on day 1, and MK-2206 135mg weekly on day 2. No DLT. G3 AC: fatigue, rash, hyperglycemia, neutropenia. 5/21 PR, 9/21 SD (42)
	NCT01235897	Paclitaxel, trastuzumab	I	HER2 positive solid tumors	16 (12 breast)	RP2D 135mg QW, DLTs= 2 G3 rash, 1 G3 neutropenia. 3 PR, 7 PR(44)
	NCT01295632	Ridaforolimus	I	Solid tumor/breast cancer	35 (19 breast)	MTD 10 mg/d ridaforolimus 5 d/wk + 90 mg/wk MK-2206; DLT: G3 rash, 2/16 PR in breast cancer (58)

	NCT01042379	Paclitaxel, trastuzumab	II	Breast cancer, neoadjuvant	94 (MK-2206), 59 (control)	MK-2206 135mg QD? MK-2206 improves pCR rates compared to standard chemotherapy in HR- and HER2+, sufficiently for evaluation in a phase 3 neoadjuvant trial (43)
	NCT00963547	Trastuzumab or lapatinib	I	HER2 positive solid tumors	31 (27 breast)	MTD for MK2206 = 60mg QOD or 135mg QW (MTD not reached). Common toxicities: fatigue, hyperglycemia, rash. 1 CR, 1PR, 5 SD>4mo (59)
AZD5363						
	NCT01353781	Single	I	Solid tumors	41 (8 breast)	DLTs: G3 hypoxia, diarrhea, mucositis, maculopapular rash in continuous dosing; no DLT in intermittent dosing. 2/37 PR (60)
	NCT02077569	Single	II	ER+ breast cancer		Ongoing
	NCT02465060	Single	II	Akt mutated tumors		Ongoing
	NCT02299999	Single	II	Breast cancer/genomically selected		Ongoing
	NCT01226316	Single/Fulvestrant	I	Tumors with AKT1 / PIK3CA or PTEN mutation	45 (ongoing, 18 ER + breast cancer)	Preliminary results: 5/18 PR, 14 target lesion shrinkage (61)
	NCT01992952	Fulvestrant	Ib/II	ER+ breast cancer		Preliminary results: RP2D AZE5363 400mg BID. Ongoing (62)
	NCT02338622	Olaparib	I	Solid tumors		Ongoing
	NCT02423603	Paclitaxel	II	TNBC		Ongoing
	NCT01625286	Paclitaxel	I/II	ER+ breast cancer		Ongoing

Clinical Studies: Combination with Endocrine Therapy

Single agent phase I study of MK-2206 in multiple solid tumors established maximum tolerated doses (MTDs) of 60mg orally every other day or 200mg once weekly. Most common toxicities include rash, nausea, pruritus, hyperglycemia, and diarrhea; with maculopapular rash being the most common dose limiting toxicity (DLT)(39, 40). In the study by Ma et al., authors assessed the combination therapy with MK-2206 and anastrozole or fulvestrant in patients with ER + breast cancer(35). Their study showed a similar toxicity profile as with the single agent MK-2206. Grade 2 or worse rash was reported in 33% of patients treated. When combined with anastrozole or fulvestrant, the recommended phase 2 dose of MK-2206 was 150mg once weekly. A total of 30 patients were treated on this study, with 15 patients having received no prior endocrine therapy and the other 15 having received up to 5 lines of endocrine therapy in the metastatic setting. The clinical benefit rate (CBR), including partial response and stable disease for 6 months or greater, in 30 patients was 36.7% with trends towards improved responses in earlier treatment settings. Among 13 patients with measurable disease, the overall response rate was 15.4%. While disease response was not the primary objective of this study, these responses are quite modest considering the strength of preclinical evidence. As this trial did not include

patients that had disease progression on prior endocrine therapy, it is more difficult to conclude whether the antitumor effect was related to MK-2206 or the endocrine therapy in this single-arm trial. The authors suggest that one of the key reasons for lack of striking responses in this phase I study may be the dose-limiting rash related to MK-2206. Rashes were pruritic, maculopapular in nature, and responded better to prednisone rather than antihistamine therapy. Histologically, a biopsy from a single patient who had rash was consistent with hypersensitivity dermatitis. Despite adding prophylactic prednisone to MK-2206 dosing, grade 3 rash still occurred at the single agent MTD of 200mg in this study, thus limiting the combination dose to 150 mg. Other clinical trials with MK-2206 also report rash as one of the common toxicities (41-43). It is notable that in trials combining MK-2206 with paclitaxel or trastuzumab, a weekly MK-2206 dose of 135mg was used (42, 44).

A subsequent neoadjuvant Phase II trial of MK-2206 in combination with anastrozole in patients with PIK3CA mutant, clinical stage II or III ER+ HER2- breast cancer was conducted and reported recently (45). The primary endpoint of the study was pathologic complete response (pCR) to test the hypothesis of enhanced cell death with the addition of AKT inhibitor to estrogen deprivation. Patients received single agent anastrozole (goserelin was added if pre-menopausal) during cycle 0 (28 days) while waiting for the result of PIK3CA sequencing. Those positive for PIK3CA mutation was started on MK-2206 (150mg PO weekly, with 20mg daily for 3 days of prophylactic prednisone administered the day before, the day of, and the day after each MK-2206 dose) on cycle 1 day 2 (C1D2) and received a maximum of 4 28-days cycles of combination therapy before surgery. The trial was stopped at interim analysis due to the lack of pCR among the 16 patients enrolled in the first stage. Analysis of tumor biopsies obtained at baseline, following cycle 0 anastrozole monotherapy, then 2 weeks post the addition of MK-2206 failed to demonstrate an enhanced effect on cell proliferation (by Ki67 labeling index) and apoptosis (by cleaved PARP) by MK-2206 over that achieved by anastrozole alone. Although AKT phosphorylation was reduced following MK-2206 therapy, PRAS40 phosphorylation persisted. Dose limiting toxicities were common in this trial, which might have contributed to the modest effect on target inhibition. Among the 14 patients who completed for at least 1 cycle of MK-2206, 5 required at least one dose reduction due to grade 3 rash (n=4). One other patient discontinued treatment following 2 cycles of MK-2206 due to grade 4 ALT and AST elevation. Authors concluded that MK2206 with anastrozole is unlikely to be more effective than anastrozole alone in *PIK3CA* mutant endocrine naïve ER+ breast cancer.

Another phase I study reported tolerability and preliminary antitumor activity of AZD5363 in solid tumors with AKT1 E17K mutations(46). Among 18 patients with ER + breast cancer, 3 patients with confirmed partial response and 2 patients with unconfirmed partial response were reported. Two patients were treated with the combination of fulvestrant and AZD5363 after disease progression on AZD5363, and one of the two had tumor shrinkage with concordant decline in AKT1 E17K mutant allele fraction in cfDNA. In this study, 66.7% of patients experienced toxicities grade 3 or worse, with the most common being hyperglycemia, rash and diarrhea.

Based on these clinical trials, while both AKT inhibitors used in metastatic breast cancer seem to show positive signals for added efficacy, a larger proportion of patients experienced toxicities that were frequently dose-limiting.

Clinical Studies: Combination with Chemotherapy

A phase Ib study of paclitaxel in combination with MK-2206 was conducted based on the preclinical synergistic effect of the combination (27). In this study of paclitaxel and MK-2206, 14 patients with metastatic breast cancer were treated. No DLTs were reported and the MTD was determined as paclitaxel 80mg/m2 and MK-2206 200mg orally weekly; however, due to grade 2 and 3 rash reported in the dose expansion, RP2D was defined as MK-2206 135mg weekly. Four patients with metastatic breast cancer had a partial response in this study (42).

In the neoadjuvant setting, the results from the MK-2206 arm of I-SPY2 trial, an adaptive randomized trial of neoadjuvant chemotherapy, have been reported. Upon evaluating pathological complete response rates of 57 control patients and 87 MK-2206 treated patients, investigators concluded that HER2 + / hormone receptor (HR) negative, HER2 +, or HR - signatures were predictive of statistical success in a 300-patient randomized phase 3 trial in the neoadjuvant setting in combination with paclitaxel. The results from I-SPY2 indicate that MK-2206 may have more prominent activity in both HER2 + and HR - diseases, than in HR + diseases. The patients on this trial were not selected based on the presence of PI3K/AKT pathway related molecular aberrations. The endpoint of the I-SPY2 trial was pathologic complete response rate in the neoadjuvant setting. Therefore, we cannot yet infer AKT inhibitors are ineffective in ER + breast cancer based on this trial.

Interestingly, while rash was still reported as one of the common adverse events, it was not considered a DLT in either of the chemotherapy combination trials. One explanation may be the more common use of steroids as a premedication to chemotherapy in these populations that may have prevented development of severe rash.

Predicting Responses to PI3K/AKT Pathway Inhibition

Another impediment to immediate clinical application of AKT inhibitors is the unclear predictive relationship between the presence of actionable mutations in PI3K/AKT pathway and clinical responses to the drug. In the phase I study by Ma et al, 16 patients had molecular profiling done. Six patients had activating PIK3CA mutations, including one patient with concurrent AKT1 E17K and PIK3CA H1047R. Among 6 patients, one had partial response, another had stable disease for more than 6 months. The patient with concurrent AKT1 and PIK3CA mutation had stable disease for less than 6 months, and the other three patients with activating PIK3CA mutations had progressive disease. Based on this limited analysis, activating PIK3CA mutation was not associated with improved time to progression. In the neoadjuvant setting, MK-2206 in combination with anastrozole failed to improve pCR rate and induce apoptosis despite the selection of patients with PIK3CA mutant ER+ HER2- breast cancer (45). It is notable that in a basket trial of AZD5363 in patients with advanced solid tumors with AKT1 mutations, 6 of the 20 patients with ER+ breast cancer experienced partial response (4 confirmed, 2 unconfirmed). The median PFS of 5.5 months (95% CI, 2.9 to 6.9 months) for this patient population, providing the first clinical evidence that AKT1 E17K is a therapeutic target in cancer (47). Ongoing prospective studies, such as SAFIR 01(48) and NCI-MATCH (NCT02465060), include AKT inhibitors to address the potential efficacy of molecularly matched therapies.

A phase I trial of MSC2363318A, a novel dual p70S6K/Akt inhibitor is also ongoing

in patients with molecular alteration in PI3K/AKT pathway (NCT NCT01971515).

Future Directions

With only modest efficacy at tolerable doses and lack of validated predictive biomarkers, aside from mutations in AKT1 to AKT inhibition, yet with strong preclinical scientific rationale, it seems logical that the next best strategy is to identify a tolerable combination that will enhance the efficacy in treatment of endocrine resistant breast cancer.

One such strategy may be combining CDK 4/6 inhibitor with a PI3K/AKT pathway inhibitor. Both pathways play an important role in breast cancer development and progression. Inhibition of these pathways is known to enhance the effect of endocrine therapy in ER + breast cancer. Furthermore, there are no significant overlapping toxicities with neutropenia being the most common grade 3/4 toxicity by CDK4/6 inhibitors, such as palbociclib. While there are no active trials combining a CDK 4/6 inhibitor and an AKT inhibitor registered at this time, early phase trials such as ongoing TRINITI-1 (NCT02732119) attempt to evaluate the combination of mTOR and CDK 4/6 inhibition in addition to endocrine therapy.

Several other combination strategies are under investigation. Combining AKT inhibitors and MEK/ERK inhibitors in order to overcome potential kinome reprogramming related to a single pathway inhibition with AKT inhibitors appears promising(49-51). Preclinical data supporting the combination of both PI3K/Akt pathway and MAPK pathway inhibitors in ER + breast cancer have been reported(49). In preclinical models of other cancer types, blockade of autophagy with a MEK inhibitor in combination with a PI3K inhibitor, BKM120 showed synergistic cell growth suppression in lung cancer mouse xenograft model(50). An early phase clinical trial combining MK-2206 with selumetinib, a MEK 1/2 inhibitor, showed possible antitumor effect in KRAS driven non-small cell lung cancer and low grade ovarian cancer (51). A clinical trial combining trametinib and an AKT inhibitor, GSK2141795, in patients with triple negative breast cancer is ongoing (NCT01964924).

Combination strategies with RTK inhibitors, such as IGF-1R inhibitors, to address feedback upregulation of RTKs providing upstream activation of the PI3K pathway as well as the MAPK pathway can be another potential option. This concept is supported by several preclinical studies suggesting the importance crosstalk between the two pathways in ER + breast cancer (52-54). A phase I study of an anti-IGF-1 R antibody, dalotuzumab, in combination with MK2206 showed favorable safety profile(55).

Further research into the mechanism of dose limiting rash caused by AKT inhibitors may also help identify better ways to use these agents. As noted in study by Ma et al., patients at higher doses of MK-2206 appeared to have hypersensitivity dermatitis that were controlled with prophylactic prednisone. Thus, strategies such as prophylactic medication or different dosing schedules may reduce such toxicities and potentially allow administration of higher doses.

Finding an effective combination while managing anticipated dose limiting toxicities, such as hypersensitivity rash, would be critical in the further clinical development of AKT inhibitors in endocrine resistant breast cancer.

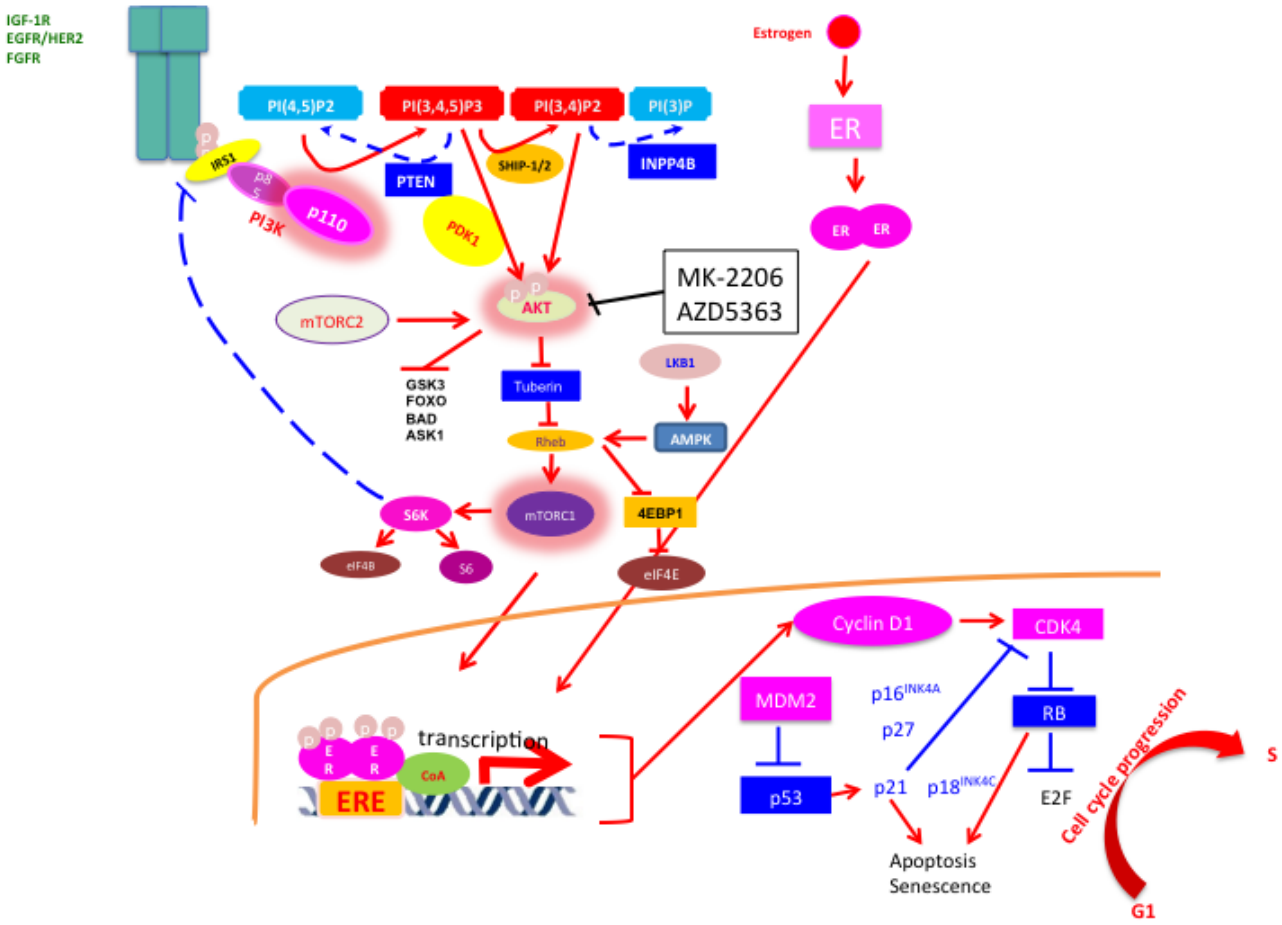

Figure 1. Conceptual diagram of PI3K pathway in breast cancer

REFERENCE:

1. Ma CX, Reinert T, Chmielewska I, Ellis MJ. Mechanisms of aromatase inhibitor resistance. Nat Rev Cancer. 2015;15(5):261-75.

2. Cantley LC. The Phosphoinositide 3-Kinase Pathway. Science. 2002;296 (5573):1655-7.

3. Ma CX, Crowder RJ, Ellis MJ. Importance of PI3-kinase pathway in response/resistance to aromatase inhibitors. Steroids. 2011;76(8):750-2.

4. Ciruelos Gil EM. Targeting the PI3K/AKT/mTOR pathway in estrogen receptor-positive breast cancer. Cancer Treat Rev. 2014;40(7):862-71.

5. Baselga J, Campone M, Piccart M, Burris HA, 3rd, Rugo HS, Sahmoud T, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med. 2012;366(6):520-9.

6. O'Reilly KE, Rojo F, She QB, Solit D, Mills GB, Smith D, et al. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer research. 2006;66(3):1500-8.

7. Wan X, Harkavy B, Shen N, Grohar P, Helman LJ. Rapamycin induces feedback activation of Akt signaling through an IGF-

1R-dependent mechanism. Oncogene. 2007;26(13):1932-40.

8. Mayer IA, Abramson VG, Isakoff SJ, Forero A, Balko JM, Kuba MG, et al. Stand up to cancer phase ib study of pan-phosphoinositide-3-kinase inhibitor buparlisib with letrozole in estrogen receptor-positive/human epidermal growth factor receptor 2-negative metastatic breast cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2014;32(12):1202-9.

9. Ma CX, Sanchez C, Gao F, Crowder R, Naughton M, Pluard T, et al. A Phase I Study of the AKT Inhibitor MK-2206 in Combination with Hormonal Therapy in Postmenopausal Women with Estrogen Receptor Positive Metastatic Breast Cancer. Clinical Cancer Research. 2016.

10. Ma CX, Luo J, Naughton M, Ademuyiwa FO, Suresh R, Griffith M, et al. A phase 1 trial of BKM120 (Buparlisib) in combination with fulvestrant in postmenopausal women with estrogen receptor positive metastatic breast cancer. Clin Cancer Res. 2015.

11. Baselga J, Im S-A, Iwata H, Clemons M, Ito Y, Awada A, et al., editors. PIK3CA status in circulating tumor DNA (ctDNA) predicts efficacy of buparlisib (BUP) plus fulvestrant (FULV) in postmenopausal women with endocrine-resistant HR+/HER2– advanced breast cancer (BC): First results from the randomized, phase III BELLE-2 trial. San Antonio Breast Cancer Symposium; 2015; San Antonio, Texas.

12. Franke TF, Kaplan DR, Cantley LC. PI3K: downstream AKTion blocks apoptosis. Cell. 1997;88(4):435-7.

13. Datta SR, Brunet A, Greenberg ME. Cellular survival: a play in three Akts. Genes & development. 1999;13(22):2905-27.

14. Staal SP. Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma. Proceedings of the National Academy of Sciences of the United States of America. 1987;84(14):5034-7.

15. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, et al. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell. 1999;96(6):857-68.

16. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell. 2007;129(7):1261-74.

17. Chandarlapaty S, Sawai A, Scaltriti M, Rodrik-Outmezguine V, Grbovic-Huezo O, Serra V, et al. AKT inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity. Cancer Cell. 2011;19(1):58-71.

18. Virtakoivu R, Pellinen T, Rantala JK, Perala M, Ivaska J. Distinct roles of AKT isoforms in regulating beta1-integrin activity, migration, and invasion in prostate cancer. Mol Biol Cell. 2012;23(17):3357-69.

19. Dillon RL, Muller WJ. Distinct biological roles for the akt family in mammary tumor progression. Cancer Res. 2010;70(11):4260-4.

20. Li CW, Xia W, Lim SO, Hsu JL, Huo L, Wu Y, et al. AKT1 Inhibits Epithelial-to-Mesenchymal Transition in Breast Cancer through Phosphorylation-Dependent Twist1 Degradation. Cancer Res. 2016;76(6):1451-62.

21. Meng Q, Xia C, Fang J, Rojanasakul Y, Jiang BH. Role of PI3K and AKT specific isoforms in ovarian cancer cell

migration, invasion and proliferation through the p70S6K1 pathway. Cell Signal. 2006;18(12):2262-71.

22. Riggio M, Perrone MC, Polo ML, Rodriguez MJ, May M, Abba M, et al. AKT1 and AKT2 isoforms play distinct roles during breast cancer progression through the regulation of specific downstream proteins. Sci Rep. 2017;7:44244.

23. Grabinski N, Mollmann K, Milde-Langosch K, Muller V, Schumacher U, Brandt B, et al. AKT3 regulates ErbB2, ErbB3 and estrogen receptor alpha expression and contributes to endocrine therapy resistance of ErbB2(+) breast tumor cells from Balb-neuT mice. Cell Signal. 2014;26(5):1021-9.

24. Stottrup C, Tsang T, Chin YR. Upregulation of AKT3 Confers Resistance to the AKT Inhibitor MK2206 in Breast Cancer. Molecular cancer therapeutics. 2016;15(8):1964-74.

25. Ellis MJ, Ding L, Shen D, Luo J, Suman VJ, Wallis JW, et al. Whole-genome analysis informs breast cancer response to aromatase inhibition. Nature. 2012;486(7403):353-60.

26. Cancer Genome Atlas N. Comprehensive molecular portraits of human breast tumours. Nature. 2012;490(7418):61-70.

27. Sangai T, Akcakanat A, Chen H, Tarco E, Wu Y, Do KA, et al. Biomarkers of response to Akt inhibitor MK-2206 in breast cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2012;18(20):5816-28.

28. Beaver JA, Gustin JP, Yi KH, Rajpurohit A, Thomas M, Gilbert SF, et al. PIK3CA and AKT1 mutations have distinct effects on sensitivity to targeted pathway inhibitors in an isogenic luminal breast cancer model system. Clinical cancer research : an official journal of the American Association for Cancer Research. 2013;19(19):5413-22.

29. Campbell RA, Bhat-Nakshatri P, Patel NM, Constantinidou D, Ali S, Nakshatri H. Phosphatidylinositol 3-kinase/AKT-mediated activation of estrogen receptor alpha: a new model for anti-estrogen resistance. J Biol Chem. 2001;276(13):9817-24.

30. Sun M, Paciga JE, Feldman RI, Yuan Z, Coppola D, Lu YY, et al. Phosphatidylinositol-3-OH Kinase (PI3K)/AKT2, activated in breast cancer, regulates and is induced by estrogen receptor alpha (ERalpha) via interaction between ERalpha and PI3K. Cancer Res. 2001;61(16):5985-91.

31. Miller TW, Hennessy BT, Gonzalez-Angulo AM, Fox EM, Mills GB, Chen H, et al. Hyperactivation of phosphatidylinositol-3 kinase promotes escape from hormone dependence in estrogen receptor-positive human breast cancer. J Clin Invest. 2010;120(7):2406-13.

32. Creighton CJ, Hilger AM, Murthy S, Rae JM, Chinnaiyan AM, El-Ashry D. Activation of mitogen-activated protein kinase in estrogen receptor alpha-positive breast cancer cells in vitro induces an in vivo molecular phenotype of estrogen receptor alpha-negative human breast tumors. Cancer Res. 2006;66(7):3903-11.

33. Creighton CJ, Fu X, Hennessy BT, Casa AJ, Zhang Y, Gonzalez-Angulo AM, et al. Proteomic and transcriptomic profiling reveals a link between the PI3K pathway and lower estrogen-receptor (ER) levels and activity in ER+ breast cancer. Breast Cancer Res. 2010;12(3):R40.

34. Kirkegaard T, Witton CJ, McGlynn LM, Tovey SM, Dunne B, Lyon A, et al. AKT activation predicts outcome in breast cancer patients treated with tamoxifen. J Pathol. 2005;207(2):139-46.

35. Ma CX, Sanchez C, Gao F, Crowder R, Naughton M, Pluard T, et al. A Phase I Study of the AKT Inhibitor MK-2206 in Combination with Hormonal Therapy in Postmenopausal Women with Estrogen Receptor-Positive Metastatic Breast Cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2016;22(11):2650-8.

36. Ribas R, Pancholi S, Guest SK, Marangoni E, Gao Q, Thuleau A, et al. AKT Antagonist AZD5363 Influences Estrogen Receptor Function in Endocrine-Resistant Breast Cancer and Synergizes with Fulvestrant (ICI182780) In Vivo. Molecular cancer therapeutics. 2015;14(9):2035-48.

37. Hirai H, Sootome H, Nakatsuru Y, Miyama K, Taguchi S, Tsujioka K, et al. MK-2206, an allosteric Akt inhibitor, enhances antitumor efficacy by standard chemotherapeutic agents or molecular targeted drugs in vitro and in vivo. Molecular cancer therapeutics. 2010;9(7):1956-67.

38. Davies BR, Greenwood H, Dudley P, Crafter C, Yu DH, Zhang J, et al. Preclinical pharmacology of AZD5363, an inhibitor of AKT: pharmacodynamics, antitumor activity, and correlation of monotherapy activity with genetic background. Molecular cancer therapeutics. 2012;11(4):873-87.

39. Yap TA, Yan L, Patnaik A, Fearen I, Olmos D, Papadopoulos K, et al. First-in-man clinical trial of the oral pan-AKT inhibitor MK-2206 in patients with advanced solid tumors. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2011;29(35):4688-95.

40. Yap TA, Yan L, Patnaik A, Tunariu N, Biondo A, Fearen I, et al. Interrogating two schedules of the AKT inhibitor MK-2206 in patients with advanced solid tumors incorporating novel pharmacodynamic and functional imaging biomarkers. Clinical cancer research : an official journal of the American Association for Cancer Research. 2014;20(22):5672-85.

41. Doi T, Tamura K, Tanabe Y, Yonemori K, Yoshino T, Fuse N, et al. Phase 1 pharmacokinetic study of the oral pan-AKT inhibitor MK-2206 in Japanese patients with advanced solid tumors. Cancer Chemother Pharmacol. 2015;76(2):409-16.

42. Gonzalez-Angulo AM, Krop I, Akcakanat A, Chen H, Liu S, Li Y, et al. SU2C phase Ib study of paclitaxel and MK-2206 in advanced solid tumors and metastatic breast cancer. J Natl Cancer Inst. 2015;107(3).

43. Tripathy D, Chien AJ, Hylton N, Buxton MB, Ewing CA, Wallace AM, et al. Adaptively randomized trial of neoadjuvant chemotherapy with or without the Akt inhibitor MK-2206: Graduation results from the I-SPY 2 Trial. [Abstract]. J Clin Oncol 33, (suppl; abstr 524). 2015.

44. Chien AJ, Cockerill A, Fancourt C, Schmidt E, Moasser MM, Rugo HS, et al. A phase 1b study of the Akt-inhibitor MK-2206 in combination with weekly paclitaxel and trastuzumab in patients with advanced HER2-amplified solid tumor malignancies. Breast cancer research and treatment. 2016;155(3):521-30.

45. Ma CX, Suman V, Goetz MP, Northfelt DW, Burkard ME, Ademuyiwa F, et al. A Phase II trial of neoadjuvant

MK2206, an AKT inhibitor, with anastrozole in clinical stage 2 or 3 PIK3CA mutant ER positive and HER2 negative breast cancer. Clinical Cancer Research. 2017.

46. Hyman DM, Smyth L, Bedard PL, Oza A, Dean E, Armstrong A, et al. AZD5363, a catalytic pan-Akt inhibitor, in Akt1 E17K mutation positive advanced solid tumors. [Abstract]. In: Proceedings of the 2015 AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics; 2015 Nov 5-9; Boston, Massachusetts Philadelphia (PA): AACR. 2015.

47. Hyman DM, Smyth LM, Donoghue MTA, Westin SN, Bedard PL, Dean EJ, et al. AKT Inhibition in Solid Tumors With AKT1 Mutations. Journal of Clinical Oncology. 2017;35(20):2251-9.

48. Andre F, Bachelot T, Commo F, Campone M, Arnedos M, Dieras V, et al. Comparative genomic hybridisation array and DNA sequencing to direct treatment of metastatic breast cancer: a multicentre, prospective trial (SAFIR01/UNICANCER). Lancet Oncol. 2014;15(3):267-74.

49. Fu X, Creighton CJ, Biswal NC, Kumar V, Shea M, Herrera S, et al. Overcoming endocrine resistance due to reduced PTEN levels in estrogen receptor-positive breast cancer by co-targeting mammalian target of rapamycin, protein kinase B, or mitogen-activated protein kinase kinase. Breast Cancer Res. 2014;16(5):430.

50. Ren H, Guo H, Thakur A, Zhang S, Wang T, Liang Y, et al. Blockade efficacy of MEK/ERK-dependent autophagy enhances PI3K/Akt inhibitor NVP-BKM120's therapeutic effectiveness in lung cancer cells. Oncotarget. 2016.

51. Tolcher AW, Khan K, Ong M, Banerji U, Papadimitrakopoulou V, Gandara DR, et al. Antitumor activity in RAS-driven tumors by blocking AKT and MEK. Clinical cancer research : an official journal of the American Association for Cancer Research. 2015;21(4):739-48.

52. Luey BC, May FE. Insulin-like growth factors are essential to prevent anoikis in oestrogen-responsive breast cancer cells: importance of the type I IGF receptor and PI3-kinase/Akt pathway. Mol Cancer. 2016;15:8.

53. Mata R, Palladino C, Nicolosi ML, Lo Presti AR, Malaguarnera R, Ragusa M, et al. IGF-I induces upregulation of DDR1 collagen receptor in breast cancer cells by suppressing MIR-199a-5p through the PI3K/AKT pathway. Oncotarget. 2016;7(7):7683-700.

54. Fox EM, Kuba MG, Miller TW, Davies BR, Arteaga CL. Autocrine IGF-I/insulin receptor axis compensates for inhibition of AKT in ER-positive breast cancer cells with resistance to estrogen deprivation. Breast Cancer Res. 2013;15(4):R55.

55. Brana I, Berger R, Golan T, Haluska P, Edenfield J, Fiorica J, et al. A parallel-arm phase I trial of the humanised anti-IGF-1R antibody dalotuzumab in combination with the AKT inhibitor MK-2206, the mTOR inhibitor ridaforolimus, or the NOTCH inhibitor MK-0752, in patients with advanced solid tumours. Br J Cancer. 2014;111(10):1932-44.

56. Akt Inhibitor MK2206 in Treating Patients With Advanced Breast Cancer, https://clinicaltrials.gov/ct2/show/results/NCT01277757 [Accessed March 22, 2017] [

57. Wisinski KB, Tevaarwerk AJ, Burkard ME, Rampurwala M, Eickhoff J,

Bell MC, et al. Phase I Study of an AKT Inhibitor (MK-2206) Combined with Lapatinib in Adult Solid Tumors Followed by Dose Expansion in Advanced HER2+ Breast Cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2016;22(11):2659-67.

58. Gupta S, Argiles G, Munster PN, Hollebecque A, Dajani O, Cheng JD, et al. A Phase I Trial of Combined Ridaforolimus and MK-2206 in Patients with Advanced Malignancies. Clinical cancer research : an official journal of the American Association for Cancer Research. 2015;21(23):5235-44.

59. Hudis C, Swanton C, Janjigian YY, Lee R, Sutherland S, Lehman R, et al. A phase 1 study evaluating the combination of an allosteric AKT inhibitor (MK-2206) and trastuzumab in patients with HER2-positive solid tumors. Breast Cancer Res. 2013;15(6):R110.

60. Tamura K, Hashimoto J, Tanabe Y, Kodaira M, Yonemori K, Seto T, et al. Safety and tolerability of AZD5363 in Japanese patients with advanced solid tumors. Cancer Chemother Pharmacol. 2016;77(4):787-95.

61. DM Hyman, L Smyth, PL Bedard, A Oza, E Dean, A Armstrong, et al. AZD5363, a catalytic pan-AKT inhibitor, in AKT1 E17K mutation positive advanced solid tumors. Presented at at the AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics, 2015 [Abstract].

62. Jones R, Howell S, Rugman P, Casbard A, Bongard E. FAKTION; Phase 1b/2 randomised placebo controlled trial of fulvestrant +/- AZD5363 in postmenopausal women with advanced breast cancer previously treated with a 3rd generation aromatase inhibibitor. National Cancer Research Institute Cancer Conference 2016 [Abstract].

