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## **Nexus between PI3K/AKT and Estrogen Receptor Signaling in Breast Cancer**

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**Abstract:** Signaling from estrogen receptor alpha ( $ER\alpha$ ) and its ligand estradiol (E2) is critical for growth of ~70% of breast cancers. Therefore, several drugs that inhibit  $ER\alpha$  functions are in clinical use for decades and new classes of anti-estrogens are continuously being developed. Although a significant number of  $ER\alpha+$  breast cancers respond to anti-estrogen therapy, ~30% of these breast cancers recur, sometimes even after 20 years of initial diagnosis. Mechanism of resistance to anti-estrogens is one of the intensely studied disciplines in breast cancer. Several mechanisms have been proposed including mutations in *ESR1*, crosstalk between growth factor and  $ER\alpha$  signaling, and interplay between cell cycle machinery and  $ER\alpha$  signaling. *ESR1* mutations as well as crosstalk with other signaling networks lead to ligand independent activation of  $ER\alpha$  thus rendering anti-estrogens ineffective, particularly when treatment involved anti-estrogens that do not degrade  $ER\alpha$ . As a result of these studies, several therapies that combine anti-estrogens that degrade  $ER\alpha$  with PI3K/AKT/mTOR inhibitors targeting growth factor signaling or CDK4/6 inhibitors targeting cell cycle machinery are used clinically to treat recurrent  $ER\alpha+$  breast cancers. In this review, we discuss nexus between  $ER\alpha$ -PI3K/AKT/mTOR pathways and how understanding of this nexus has helped to develop combination therapies.

## Main Text

Breast cancer is one of the leading causes of death in women. According to the American Cancer Society report of 2019, 79% of total breast cancer cases are estrogen receptor alpha (ER $\alpha$ )-positive. The five-year survival rate of women with ER $\alpha$ + breast cancer is around 90% [1]. With recent advancements, survival rate of breast cancer patients has improved significantly. However, a major challenge is the development of resistance to available therapies. For example, previously responsive ER $\alpha$ + tumors show eventual resistance to the anti-estrogen tamoxifen [2]. Therefore, the study of resistance mechanisms to endocrine therapy requires an understanding of ER structure, molecular pathways, and interaction with components of other signaling cascades.

**Biology of ERs:** Estrogen receptors belong to the nuclear receptor superfamily [3]. The two different forms of ER – ER $\alpha$  and ER $\beta$  are coded by two distinct genes *ESR1* and *ESR2*, which are located on chromosomes 6 and 14, respectively [4]. ERs are comprised of six functional domains, similar to other members of the nuclear receptor family [3]. The N-terminal A/B domain bears the ligand independent activation function 1 (AF1) to which various transcription coregulators and activators bind. The DNA binding domain (DBD), which is also referred as C domain is important for interaction of ER with the genome. The DBD of ER binds to cis-regulatory element termed Estrogen Response Element (ERE) and activates estrogen response genes such as pS2/TFF1, GREB1, and IGFBP4 [5]. Other domains include the D-domain, also known as the hinge domain, which contains nuclear localization signal; E domain or ligand binding domain (LBD) to which ligands bind and the non-conserved F domain [6, 7]. The DBD of ER $\alpha$  and ER $\beta$  are 97% identical, whereas the LBD shares 56%

homology. Hence, individual ligand-receptor interactions activate distinct pathways through recruitment of different co-activator/co-repressor molecules, thereby, altering the transcriptional profile. Crystal structures have revealed the DBD-LBD form a distinct L-shaped boot structure [8]. This spatial organization has been reported to be critical for receptor function. This structure can be perturbed by small molecules and formed the basis for developing many clinically used anti-estrogens [8].

ERs are randomly distributed in the cell and are maintained in the inactive state by the co-repressor proteins including NCoR family of proteins such as NCoR1, SMRT [9]. At least 19 proteins with co-repressor activity have been described and these co-repressors recruit histone deacetylases (HDACs) to mediate the actions of anti-estrogens [9]. To activate ER-dependent transcription, ER-co-repressor complexes need to dissociate and be replaced by co-activator complexes. More than 400 co-activators have been described in the literature and several of them can bind to ER $\alpha$  in a ligand-dependent manner [10]. As estrogen diffuses through cell membrane, the ER encounters the ligand and binding occurs. This binding leads to conformational changes in ER and dissociation of ER-co-repressor complex. The dissociation of inhibitory proteins activates the receptor, which then leads to homo- or heterodimerization. ER can also undergo such conformational changes through post-translational modifications including phosphorylation (described below). Phosphorylated receptor dimers are then transported into the nucleus for the transcription of ER target genes. Activated ER binds to ERE and recruits co-regulatory molecules to initiate transcription. Binding of ER to chromatin and subsequent activation of gene expression is additionally controlled by a distinct group of transcription factors called pioneer factors [11].

Although there is still some debate on whether pioneer factors facilitate ER binding or ER facilitates pioneer factor binding, we recently reported chromatin accessibility changes in estradiol treated cells and observed a modest but significant enrichment of pioneer factor binding sites in gene regulatory regions of genes induced by E2 suggesting that ER facilitates pioneer factor binding in at least a subset of genes [12]. Since many excellent reviews on relationship between pioneer factors and ERs have been published [11, 13], ER-pioneer factor interactions are not discussed further.

Estrogens regulate activity of ERs by serving as ligands. Estrone (E1), Estradiol (E2) and Estriol (E3) are three major forms of estrogens. E2 is the most important ligand of ERs. Androgens are converted into estrogens by the enzyme aromatase through a process called aromatization. Synthesis of estrogens takes place mainly in the ovaries and the reaction is stimulated by Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH). Therefore, prophylactic oophorectomy reduces the risk of recurrence and is advisable in many breast cancer patients. [14, 15].

As described above, the majority of ER $\alpha$ -E2 actions is within in the nucleus. However, depending on the cell type, a small fraction of ER $\alpha$  is involved in non-genomic action by tethering to the plasma membrane [16]. Plasma membrane bound ER $\alpha$  may interact with growth factor receptors upon E2 binding, which subsequently leads to activation of non-receptor tyrosine kinases such as Src. This in turn leads to phosphorylation of aromatase, increase in aromatase activity and a resulting induction of E2 and aromatase autocrine feedforward loop and integration of genomic and non-genomic actions of ER $\alpha$  [17] (**Figure 1**). Varieties of transcription-independent signal transduction pathways are activated through

non-genomic actions of ER $\alpha$  through SRC. Nuclear and membrane ER $\alpha$  exist in 9:1 ratio, although ratio varies between cell types [18]. This 10% of membrane ER $\alpha$  manipulates the transcription profile of the cell. Modifications such as palmitoylation (Cys447) and myristoylation aid in tethering of the ER $\alpha$  to the plasma membrane [19] [20]. Similar to nuclear ER $\alpha$ , membrane bound ER $\alpha$  exists mainly as homodimers [21]. The E2-activated membrane ER $\alpha$  undergoes depalmitoylation, dissociates from the membrane and then interacts with signaling molecules such as PI3K [22]. Consequently signals downstream of PI3K including AKT are activated, which promote cell proliferation and survival. Other signaling pathways activated by the membrane ER $\alpha$  are Mitogen-activated protein kinase (MAPK/Erk1/2) [23-25]. In cancer, activation of this cascade leads to tumor growth and progression. **Figure 1** provides a summary of multiple mechanisms of ER $\alpha$  activation and actions.

**Anti-estrogen therapies for breast cancer:** Clinical, pathologic and genomic scoring parameters determine whether anti-estrogen therapy is advisable either as a single agent or in combination with chemotherapies. Clinical and pathologic features include ER $\alpha$  and progesterone receptor positivity, involvement of lymph nodes, whereas genomic score includes 21-gene recurrence score [26]. Anti-estrogen therapy involves small molecules that induce conformational changes in ER $\alpha$  that prevent E2 binding, cause ER $\alpha$  degradation, or block E2 synthesis by inhibiting the activity of aromatase. Based on these mechanisms of action, anti-estrogen therapies can be broadly divided into three groups; selective estrogen receptor modulators (SERMs), selective estrogen receptor down regulators/degraders (SERDs), and aromatase inhibitors (AIs) [27, 28].

**SERMs:** SERMs are the most preferred type of treatment for ER $\alpha$ -positive breast cancer and they act by binding to ER $\alpha$  and suppress E2-regulated gene expression by enhancing co-repressor instead of co-activator recruitment to ER $\alpha$  [29]. Examples of SERMs include tamoxifen, raloxifene, lasofoxifene, arzoxifene, bazedoxifene, toremifene, acolbifene, ospemifene, titanocetyl dichloride analogues of tamoxifen, CHF4227, 4'-Methoxy derivative, 2,3,4-triarylbenzopyran derivatives, acetic acid 4-{4-[4-(2-diisopropylaminoethoxy)-phenyl]-3-phenylchroman-2-yl}phenyl ester, 4-{3-Phenyl-4-[4-(2-pyrrolidin-1-yl-ethoxy) phenyl]chroman-2-yl}phenol, 4-{4-[4-(2-Diisopropylaminoethoxy)-phenyl]-3-phenyl-chroman-2-yl}-phenol, acetic acid 4-{4-[4-(3-dimethylaminopropoxy)phenyl]-3-phenylchroman-2-yl}phenyl ester, tetrahydroisoquinoline derivatives, 3-oxygenated benzothiophene, benzoxepin analogues and m-Carborane [27, 30-34]. Tamoxifen is the most frequently used SERM to treat breast cancer.

**SERDs:** SERDs are known as pure ER antagonists. Binding of SERDs to ER $\alpha$  disrupts dimerization, DNA binding and premature proteosomal degradation of the receptor [35]. Examples of SERDs include fulvestrant, etacstil, steroidal RU58668, (E/Z)-4-(1-{4-[2-(dodecylamino)ethoxy]phenyl}-2-phenylbut-1-en-1-yl)phenol, GW5638, GW7604, (E)-3-(3,5-Difluoro-4-((1R,3R)-2-(2-fluoro-2-methylpropyl)-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylic acid (AZD9496), 5,6-Bis(4-hydroxyphenyl)-N-phenyl-7-oxabicyclo[2.2.1]hept-5-ene-2-sulfonamide, {4-[2-(Decylamino)ethoxy]phenyl}[6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thioph-en-3-yl]methanone, (4-(2-(Dodecylamino) ethoxy)-phenyl)(6-hydroxy-2-(4-hydroxy-

phenyl)benzo[b]thiophen-3-yl) methanone, GDC-0810 or ARN-810, and 3,20-Dihydroxy-13 $\alpha$ -19-norpregna-1,3,5(10)-triene derivatives [30, 36-40]. Fulvestrant can be a choice either in first line hormone therapy setting or after tamoxifen and AI failure [41, 42].

**AIs:** Aromatase, encoded by *CYP19A1* gene, is an enzyme of cytochrome P450 family which is involved in biosynthesis of estrogens from androgen precursors. Aromatase is expressed in several estrogen-producing tissues including ovaries, breast, placenta, adrenal glands, testicles, adipose tissue, bone, liver, muscles, and brain. Systemic inhibition of estrogen biosynthesis by aromatase inhibitors (AIs) block ER signaling and consequently reduces the circulating estrogen levels by more than 90% [43]. AIs are typically given under postmenopausal setting as aromatization of androgens is the main source of E2 at this stage. The long-term deprivation of estrogen, however, causes osteoporosis and hypersensitivity to the low level of estrogen. AIs are normally classified into two subtypes according to their chemical structure; steroidal (type I inhibitors) that includes testolactone, exemestane, formestane and non-steroidal (type II inhibitors) that includes letrozole, anastrozole, YM<sub>511</sub>, aminoglutethimide and fadrozole [44, 45]. Steroidal AIs, also known as suicidal inhibitors, first bind to the natural substrate binding site of the aromatase and become a reactive intermediate that covalently binds to aromatase resulting in irreversible inhibition. By contrast, non-steroidal AIs bind non-covalently to heme moiety of aromatase and saturate its active site that result in reversible inhibition. Breast cancers can acquire resistance to AIs after prolonged suppression of estrogen production by mechanisms other than those caused by fulvestrant or tamoxifen (described below). Because of this reason, tumors that have



acquired resistance to AIs respond to other anti-estrogen therapies. Development of resistance to AIs is a major clinical concern in the breast cancer and it is an area of great research focus [46].

**PI3K-AKT-mTOR signaling axis in breast cancer:** AKT, also referred to as Protein Kinase B (PKB), is part of the critical PI3K-AKT-mTOR pathway deregulated in multiple cancers [47]. There are three functional AKTs encoded by distinct genes, AKT1, AKT2 and AKT3 (also called PKB $\alpha$ , PKB $\beta$ , PKB $\gamma$ ) [48, 49]. The AKT3 isoform can be alternatively spliced, resulting in different expression and activation patterns, which further complicates expression/activity landscape of AKTs [50]. Structurally, AKTs consist of a central serine/threonine kinase domain, an N-terminal Pleckstrin Homology (PH) domain, and a hydrophobic C-terminal tail. The major pathway of AKT activation is through the class I phosphatidylinositol 3-kinase (PI3K) family [49]. Receptor tyrosine kinases (RTKs) engage extracellular growth factors and respond by activating PI3K at the cell membrane. PI3K converts phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) to phosphatidylinositol (3,4,5)-triphosphate (PIP<sub>3</sub>), which interacts with PH domain of AKT and transfers AKTs to the cell membrane along with phosphoinositide-dependent protein kinase 1 (PDK1). This confers conformational changes in AKTs that expose T308 and S473 which are phosphorylation sites in the kinase-domain and the regulatory C-terminal domains of AKTs, respectively [51, 52]. At the membrane, AKT is phosphorylated by PDK at T308 in a PIP<sub>3</sub>-dependent manner. Further PIP<sub>3</sub>-dependent phosphorylation by mTORC2 at S473 is required for full AKT

activation [49]. Facchinetti and colleagues described mTORC2-dependent phosphorylation of T450, which is responsible for AKT folding and stability [53].

The tumor suppressor protein PTEN is a negative regulator of AKT as it converts PIP3 back to PIP2, limiting the duration of activation of the cascade. Predictably, inactivating PTEN mutations have been shown to be oncogenic mutations and important risk factors of breast cancer [54]. Other mechanisms also contribute to AKT activation. For example, EGF activation of AKT in breast cancer is mediated by calmodulin [55]. Furthermore, earlier studies have demonstrated the importance of GTP-bound Ras-GAP in the activation of PI3K downstream of platelet-derived growth factor (PDGF) signaling, which highlights crosstalk between PI3K-AKT-mTOR and Ras-Raf-MEK-ERK pathways [56]. Further demonstrating this interconnectedness is a report suggesting that AKT upregulates macrophage inhibitory cytokine-1 (MIC-1) expression, which in turn increases activation of ERK1 [57]. Additional pathways that regulate AKTs include mRNA methylation of upstream AKT regulators, aberration of normal miRNA control over AKT and its regulators, changes in ubiquitination of the PH domain causing failure to localize to the membrane, and changes in regulation by lncRNAs [47].

Current literature on isoform-specific roles of AKT in cancer progression is full of contradictions. Despite lack of total consensus, literature favors the possibility that AKT1 is involved in increased proliferation and tumor growth as well as decreased apoptosis, whereas AKT2 is associated with increased migration, invasion and metastasis. AKT3 appears to play a role in increasing both proliferation and metastasis [58]. Inhibition of AKT1 in MMTV-ErbB2/neu and MMTV-PyMT-induced mouse mammary tumors results in diminished tumor

development due to lower expression of Ki-67 and cyclin D and increased apoptosis [59]. Pro-tumorigenic role AKT1 is evident from a study on miR-409-3p. miR-409-3p reduced proliferation, decreased invasion and migration of breast cancer cells *in vitro* by downregulating AKT1 [60]. Few studies have described tumor suppressor role of AKT1 in breast cancer. For example, reduced activity of AKT1 has been associated with a dysregulation of p53 and DNA-damage induced transcription [61]. In one study, AKT1 was observed to be central to the reduction of breast cancer invasiveness by TIS21, which is a tumor suppressor. Specifically, TIS21 impacts motility and metastasis by reducing the assembly of the cytoskeleton. This TIS21-mediated decrease in cancer cell motility involves AKT1-dependent downregulation of diaphanous-related formin and decreased Nox4-mediated ROS formation [62]. Additional support for anti-metastatic activity of AKT1 came from studies that examined the role of CXCR2 in metastasis. CXCR2 increased breast cancer metastasis which correlated with lower AKT1 expression [63]. Interestingly, we reported distinct prognostic significance of AKT in breast cancer based on subcellular localization; nuclear localization of activated AKT is associated with better prognosis [64]. Few of the discrepancy noted in the literature could, therefore, be due to lack of consideration to subcellular distribution of AKT in experimental models.

Like AKT1, the role of the AKT2 isoform in breast cancer is complex and there are conflicting reports in the literature. Many studies have implicated AKT2 in proliferation and metastasis of various cancers. In a lung cancer cell line, for example, knockdown of AKT2 resulted in lower proliferation and invasiveness, which correlated with reduced retinoblastoma (Rb) phosphorylation and COX2 expression [65]. In PTEN-deficient prostate

tumors, AKT2 is necessary for growth and survival [66]. In breast cancer, AKT2 may increase metastatic potential via several mechanisms. For example, upregulation of AKT2 causes  $\beta$ 1-integrin-mediated increase in adhesion and invasion via collagen IV. In this regard, AKT2 was found to localize specifically to collagen IV matrix during cell attachment [67]. Furthermore, AKT2 increases expression of the actin-bundling protein, palladin, which is associated with invasive breast cancer [68]. These reports collectively provide evidence for the role of AKT2 in breast cancer metastasis. There are also evidence for tumor suppressor function of AKT2. For example, AKT2 ablation was shown to result in an acceleration of tumor induction in MMTV-ErbB2/neu and MMTV-PyMT transgenic mice [59]. A consensus can be drawn in that while AKT1-mediated signals are associated with cell proliferation and survival, AKT2-mediated signals are associated with metastatic progression with limited or growth inhibitory actions on the primary tumor.

AKT3 has also been linked to breast cancer proliferation and survival. AKT3 is expressed in animal models of ErbB2+ tumors and contributes to proliferation [69]. Interestingly, there are reports that AKT3 is of a particular significance in Triple Negative Breast Cancer (TNBC). *AKT3* amplification or translocation with *MAGI3* gene leading to constitutive AKT3 activity is reported in breast cancer [70]. Another study has shown that inhibition of AKT3 but not AKT1/2 leads to decreased mammosphere formation [71]. Predictably, AKT3 has been identified as a potential therapeutic target for the treatment of cancer. Treatment with miRNA-29b, which targets AKT3, caused reduced vascularization via modulation of VEGF and c-Myc levels, and reduced growth *in vivo*. Furthermore,

overexpression of AKT3 leads to lower expression levels of p53, p21 and p27, and increased expression of cyclin-D1, Bcl2 and XIAP [72].

*PIK3CA* and *AKT* isoforms are frequent targets of mutations/amplification in breast cancer. Almost 50% of breast cancers show genomic aberrations associated with these genes (**Figure 2**). It is interesting that at least 50% of tumors with *ESR1* mutation/amplification contain mutations/amplifications in the components of PI3K/AKT/mTOR pathway. The following sections describe how genomic aberrations in PI3K/AKT pathway may contribute to resistance to anti-estrogens by modulating ER $\alpha$  functions.

#### **Mechanisms of resistance to anti-estrogens:**

**Ligand-independent activation of ER $\alpha$ :** Sluyser and Mester (1985) proposed that mutations in ER $\alpha$  lead to ligand independent activation and mutated receptor may deregulate cell proliferation [73]. Earlier sequencing studies of primary and metastatic tumors with and without tamoxifen treatment revealed low frequency *ESR1* mutations [74]. Similar results were observed when *ESR1* was sequenced in ER $\alpha$ + and ER $\alpha$ - tumors. Interestingly, about 1% of mutation frequency was observed in these early studies. In 1997, another group identified three missense mutations (Ser47Thr, Lys531Glu, and Tyr537Asn) in the *ESR1* gene [75] and the resulting mutant proteins displayed hyperactivity in the absence of ligand [76]. Later, a clinical sequencing program confirmed earlier published results and added new point mutations (Leu536Gln, Tyr537Ser, Tyr537Cys, Tyr537Asn and Asp538Gly) to the list [77]. Interestingly, these mutations were acquired upon anti-estrogen treatment, observed mostly in metastatic tumors, show constitutive activity at variable magnitude and

differentially responsive to the SERD fulvestrant [78]. Another study with 625 postmenopausal and 328 premenopausal ER $\alpha$ + tumors revealed that the ER $\alpha$ + tumors are highly heterogeneous and concluded that more comprehensive studies are required to explore whether *ESR1* mutations occur in primary tumors [79].

The second mechanism of ligand-independent activation involves receptor phosphorylation. At least 16 different amino acids in ER $\alpha$  have been suggested to undergo phosphorylation [80]. These residues include S46/47, Y52, S102/4/6, S118, S154, S167, S212, Y219, S236, S282, S294, S305, T311, Y537, S554, and S559. Interestingly, few of the *ESR1* mutations observed in breast cancer metastatic samples corresponds to phosphorylatable residues (Y537, for example) suggesting relevance of these phosphorylations in ER $\alpha$  function. Kinases involved in these phosphorylations include PKC, c-Abl, GSK-3, ERK1/2, CDK2, CDK7, IKK $\alpha$ , mTOR/p70S6K, p90RSK, AKT, CK2, and SRC.

Other post-translational modifications (PTMs) can influence ER $\alpha$  activity and potentially impact response to anti-estrogens. For example, PRMT1 methylates Arginine 260 within ER $\alpha$  DBD. This methylation is required for interaction with PI3K and Src [81]. Furthermore, p300 acetylates ER $\alpha$  at Lysine 266 and 268 [82]. These acetylations enhance DNA binding and transactivation function of the receptor. Other modifications such as ubiquitination, SUMOylation and palmitoylation have been shown to affect ER $\alpha$  stability, function and localization [83]. Therefore, these PTMs can be potential prognostic or predictive biomarkers for tumor evaluation and response to anti-estrogens [83].

***Interplay between PI3K/AKT and ER $\alpha$  signaling to overcome the effects of anti-estrogens:***

Our lab considered a nexus between PI3K/AKT and ER signaling and the role of this axis in anti-estrogen resistance in late 1990s, even before genomic revolution revealing enrichment of genomic aberrations of PI3K/AKT pathway genes in ER $\alpha$ + breast cancers. Scientific premise for studies was based on the presence of consensus sequence (R-X-R-X-X-S/T) for AKT phosphorylation in ER $\alpha$  surrounding the amino acid S167 (RERLAST) [84]. Others subsequently reproduced crosstalk between ER $\alpha$  and AKT signaling [85, 86]. A cBioportal [87] analysis shows ~50% of breast cancers with genomic aberrations in *PIK3CA*, *AKT1*, *AKT2*, *AKT3* and/or *ESR1* suggesting relevance of this signaling axis in breast cancer (**Figure 2**). Below we summarize our studies describing specific effects of AKT on ER $\alpha$  signaling and complement our studies with other reports in the literature.

***AKT influences genome-wide binding of ER $\alpha$  and E2-mediated gene expression:*** Post-genomic era witnessed significant advances in our understanding of transcription initiation process, particularly binding of transcription factors to chromatin. Various groups used chromatin immunoprecipitation assay followed by microarray hybridization (ChIP-on-Chip) or sequencing (ChIP-seq) to map binding patterns of ER $\alpha$  to chromatin with and without E2 treatment [88, 89]. Depending on the study, >3000 ER $\alpha$  binding regions, many of them enriched for EREs, were observed in E2-treated ER $\alpha$ + cell line MCF-7. These types of studies also revealed the role of pioneer factors such as FOXA1 in binding of ER $\alpha$  to the genome. We used the ChIP-on-Chip assay of parental MCF-7 cells and MCF-7 cells overexpressing constitutively active AKT to determine the influence of AKT on genome wide DNA binding of ER $\alpha$  *in vivo* [90]. We coupled ChIP-on-Chip data with RNA

microarray to correlate ER $\alpha$  binding to genome with gene expression changes in E2  $\pm$  AKT-dependent manner. We observed ~40% changes in ER $\alpha$  binding patterns in cells with constitutively active AKT compared to parental cells and AKT caused an increase in the expression of E2-regulated genes that are enriched for the TGF- $\beta$ , NF- $\kappa$ B/TNF, retinoic acid and E2F pathways. Consequently, the AKT-overexpressing MCF-7 cells were resistant to TGF- $\beta$ -induced growth inhibition compared to the parental MCF-7 cells. Furthermore, we reported a secondary role for overexpressed AKT that involved changes in the E2-regulated expression of E2F2 and E2F6 and secondary E2-response. AKT also altered E2-regulated expression of both oncogenic and tumor-suppressor microRNAs [91]. In a subsequent study, following the observation of a differential role of AKT1 and AKT2 in E2-regulated gene expression and the absence of an effect of individual AKT isoforms on E2 response in BT-474 cells, we postulated that the effects of PI3K/AKT signaling on the genomic activity of the ER $\alpha$  is dependent on cell type [92].

Further complexity in AKT-ER $\alpha$  crosstalk emerged during studies related to understanding the mechanisms of resistance to PI3K/AKT inhibitors. Toska and colleagues [93] observed that KMT2D, a histone methyltransferase, is central to activation of ER $\alpha$  by PI3K/AKT signaling. Inhibition of PI3K activity caused an impairment in AKT-mediated phosphorylation and subsequent inactivation of KMT2D. In cells treated with PI3K/AKT inhibitors, a compensatory pathway activated ER $\alpha$  through unphosphorylated KMT2D. KMT2D opened chromatin state at ER $\alpha$  binding sites that allowed recruitment of pioneer factors like FOXA1 and PBX1 and ER $\alpha$ -mediated transcription (**Figure 3**). Further studies identified another negative feedback system that involved SGK1. Authors observed that



PI3K inhibition, which induced KMT2D activity with enhanced ER $\alpha$  transcriptional activity, also led to increased expression of SGK1. SGK1 subsequently phosphorylated KMT2D and impaired the ability of KMT2D to stimulate the transcriptional activity of the ER $\alpha$  in a negative feedback mechanism [94]. However, it is unknown whether genome wide binding patterns and transcriptional targets of ER $\alpha$  differ when it is activated directly through phosphorylation by AKT or gain access to the genome under conditions with elevated KMT2D activity but lower AKT activity (**Figure 3**). Nonetheless, these results provide an explanation as to why PI3K/AKT-mediated resistance to anti-estrogens cannot be therapeutically overcome with PI3K/AKT inhibitors alone and at least a combination of PI3K/AKT inhibitors and SERDs may be required.

***ER $\alpha$ -mediated alternative splicing and influence of AKT:*** Alternative splicing is important for generation of complex and diverse proteomes that mediate cellular processes such as apoptosis, growth, motility, differentiation, and stem cell maintenance in response to various extracellular factors [95-97]. Genomic alterations that impact few of these cellular processes contribute to etiology and progression of cancer [98]. E2, via ERs, promotes alternative splicing of specific genes that affect breast cancer cell behavior. We demonstrated that AKT alters E2-mediated splicing of genes [99]. To identify endogenous targets of E2-ER $\alpha$  mediated alternative splicing and potential roles of AKT in splicing, we had previously utilized exon-specific microarray technique to evaluate patterns of alternative splicing in parental and AKT-overexpressing MCF-7 cells, with or without E2 treatment. This was preceded by a CD44 minigene splicing experiment that indicated a significant effect of AKT on E2-mediated alternative splicing. AKT specifically altered E2-mediated splicing of

FAS/CD95, FGFR2 and AXIN-1 genes with consequent effects on FAS-mediated apoptosis and response to Keratinocyte Growth Factor (KGF), a FGFR2 ligand [99]. Since FGFR2-mediated signaling counteracts the effects of tamoxifen [100], AKT-mediated resistance to anti-estrogens could involve its effects on E2-mediated FGFR2 splicing/signaling.

***AKT is a bridge between growth factor and ER $\alpha$  Signaling:*** Switching of ER $\alpha$ + cancer cells from dependency on E2-mediated proliferative signals to growth factor-dependent signals is a major mechanism of resistance to anti-estrogens [101]. Because of intra-tumor heterogeneity, it is difficult to exclude the possibility that anti-estrogen resistance in some cases is due to clonal selection of *de novo* anti-estrogen resistant ER $\alpha$ + cancer cells with inherently enhanced growth factor signaling capacity. In either way, the PI3K/AKT signaling axis, which is downstream of multiple extracellular growth factors including Epidermal Growth Factor (EGF), Platelet-Derived Growth Factor (PDGF) and Insulin-like Growth Factor, could serve as bridge between growth factors and ER $\alpha$  [102-105]. This interaction between ER $\alpha$  and growth factor signaling pathways impacts transcriptional activity of the ER $\alpha$ , both in the presence and absence of E2, considerably affecting response to breast cancer therapy. For example, Lupien and colleagues [106] showed the EGF can induce genome-wide binding of ER $\alpha$  and the genomic targets of ER $\alpha$  following induction by EGF are distinct from E2-induced genomic targets. These EGF-induced genomic targets of ER $\alpha$  overlapped with genes overexpressed in HER2-positive breast cancers. We propose that AKT is one of the mediators EGF-dependent ER $\alpha$  binding to the genome. Since EGF mediated genome-wide binding of ER $\alpha$  is independent of E2, EGF-induced genome-wide binding of ER $\alpha$  cannot be restrained by tamoxifen or AIs. Consistent with these findings, an increased levels

of pAKT and AKT kinase activity was observed in four out of six hormone resistant cell lines, with a concordant increase in sensitivity of the cell lines to hormonal therapy following inhibition of AKT phosphorylation by PI3K and AKT inhibitors [107]. Moreover, a retrospective study by Bostner et al., [85] showed an association between the activity of phosphorylated P13K, AKT and mTOR and resistance to Tamoxifen therapy.

Few studies have proposed an alternative mechanism to PI3K/AKT-mediated resistance to anti-estrogens. For example, an inverse correlation between PI3K activation scores and ER expression levels in ER $\alpha$ + breast cancer has been described [108]. Increased PI3K activity was reported to be associated with a decrease in ER $\alpha$  expression and a concurrent development of resistance to hormonal therapy. This was observed through an analysis of proteomic and transcriptomic signatures of PI3K in ER $\alpha$ + Luminal B breast tumors [108]. This report is consistent with a previous molecular-pathology study which revealed that loss of PTEN activity is associated with a decrease in ER $\alpha$  and progesterone receptor (PR) expression [109], a phenomenon that is likely to be due to uncontrolled PI3K activation and subsequent induction of AKT activity. Taken together, these reports provide evidence for an alternative mechanism by which PI3K and AKT wean cancer cells away from E2-ER $\alpha$  and alter cancer cell properties with consequent effects on their response to hormonal therapy. Therefore, the PI3K/AKT signaling axis is being evaluated as a probable target for the mitigation of resistance to endocrine therapy in breast cancer cells [13, 110]. However, the potential for hyperactivity of feedback loops of the PI3K/AKT signaling cascade upon targeting this axis confounds the effectiveness of PI3K/AKT inhibitors as evident from clinical experience described below [86, 111] [112].

**Current clinical strategies to treat anti-estrogen resistant breast cancers:**

Many years of preclinical and translational research has enabled cataloging of the following signaling axis in resistance to endocrine therapies: genomic abnormalities in *ESR1*, *CCND1*-*CDK4/6*-*RB*, and *PI3K*-*AKT*-*mTOR* signaling pathways.

**Targeting mutant *ER* $\alpha$  through new class of *SERDs*:** *ESR1* mutations are commonly acquired as a result of selective pressure of endocrine therapy that forces *ER* $\alpha$  to acquire ligand-independent signaling capabilities [113, 114]. Inhibition of activities of these mutants by rationally-designed novel therapeutic strategies have the potential to substantially improve outcomes. Fulvestrant has shown some efficacy in *ESR1*-altered *ER* $\alpha$ -positive breast cancers previously treated with *SERMs* and continues to be the treatment of choice at present [78]. However there are other *SERDs* under development, which can degrade both wild type and mutant *ER* $\alpha$  to a similar degree. These include *GDC0927*, *AZD9496* and *RAD1901*, which are in phase I, and *GDC0810*, which is in phase II clinical trials [115].

**Inhibition of *CCND1*-*CDK4/6*-*RB* pathway:** The *CCND1*-*CDK4/6*-*RB* pathway is involved in cell cycle progression [116]. This pathway controls whether a cell arrests or advances at G1-S phase of the cell cycle. At this checkpoint, cyclin-D binds with *CDK4/6* to promote progression of cell cycle via inhibition of tumor suppressor retinoblastoma (*RB*) protein. Approximately 35% of *ER* $\alpha$ + breast cancers demonstrated amplification of *CCND1* gene (encoding cyclin-D1), and about 16% demonstrated amplification of gene that encodes *CDK4* [117] [118]. Moreover, loss of endogenous negative regulators of *CDK4/6*, *CDKN2A* and *CDKN2C*, results in hyperactivity of *CDK4/6* in *ER* $\alpha$ + breast cancers [119]. This suggests the therapeutic utility of chemical inhibitors of *CDK4/6* in *ER* $\alpha$ + breast cancers

[119] [120]. The CDK4/6 inhibitors, palbociclib (PD-0332991), ribociclib and abemaciclib, in combination with endocrine therapy are frequently used to treat recurrent ER $\alpha$ + breast cancer and have improved progression free survival (PFS) [121, 122]. In the PALOMA trial, combination of CDK4/6 inhibitors with the aromatase inhibitor letrozole exhibited improved PFS compared to letrozole alone [123]. But the findings from PALOMA-1 trial demonstrated that genetic aberrations of CCND1-CDK4/6 axis are not predictive for clinical efficacy of palbociclib treatment [124]. In the phase 3 MONALEESA-2 trial, ribociclib plus letrozole or tamoxifen significantly prolonged PFS in postmenopausal ER $\alpha$ + breast cancers previously untreated with systemic therapy [125, 126]. In the MONARCH-3 trial, abemaciclib with a nonsteroidal AI was used in postmenopausal ER $\alpha$ + breast cancer and this treatment regime was associated with significantly increased median PFS [127]. CDK4/6 inhibitors with Fulvestrant were approved by FDA as a line of treatment for endocrine therapy-resistant metastatic disease. Although CDK4/6 inhibitors are effective in improving PFS, eventual resistance to these inhibitors is an issue. Resistance mechanisms to these inhibitors are one of the intensely explored current research topics [116].

***Inhibition of PI3K-AKT-mTOR pathway:*** As noted above, mutations in PI3K-AKT-mTOR pathway genes are frequently observed in ER $\alpha$ + breast cancers and at least 50% of breast cancers with *ESR1* mutation/amplifications displayed genomic aberrations of this pathway (**Figure 2**). Based on these observations as well as significant amount of preclinical data described above, it is logical to conduct clinical studies combining inhibitors of PI3K-AKT-mTOR pathways with anti-estrogens. Indeed, targeting the PI3K-AKT-mTOR pathway has

been demonstrated to be beneficial in both neoadjuvant and advanced settings in ER $\alpha$ + breast cancers [128] [129]. Everolimus (Afinitor), a mTORC1 inhibitor, is a frontline drug that interrupts the PI3K-mediated signaling. It has been approved in combination with hormonal therapies to treat advanced postmenopausal ER $\alpha$ + breast cancer [128]. In the BOLERO-2 trial, combination of everolimus with exemestane showed improved median PFS of 10.6 months; however, tumors with *PIK3CA* mutations were not responsive [105, 128, 130]. In the same trial, addition of everolimus to standard endocrine therapy demonstrated a potential predictive efficacy in patients with circulating *ESR1* mutations [131, 132].

Several other PI3K-AKT pathway-targeted therapies have been examined clinically. *PIK3CA* mutations-containing tumors, which progressed after treatment with AI, showed improved PFS when treated with PIK3CA $\alpha$  alpha isoform specific alpelisib and fulvestrant combination [133]. The alpelisib plus letrozole combination revealed a clinical benefit along with higher tolerable toxicity profile [134]. Many phase II and III trials are still in progress with PIK3CA $\alpha$ -specific inhibitors to further determine the predictive therapeutic target value of *PIK3CA* mutations [135, 136]. In the earlier trials, combination of fulvestrant with pan-isoform PI3K inhibitors, i.e. buparlisib and pictilisib or  $\beta$  isoform-sparing PI3K inhibitor taselisib were evaluated, which indicated limited clinical benefits [137, 138]. In the phase III BELLE-2 trial, initial results showed that patients with circulating DNA with *PIK3CA* mutations benefited from the combined treatment of PI3K inhibitor BKM120 and fulvestrant [139]. Despite some clinical efficacy, this combination therapy is not being pursued further due to toxicity profile.

Breast cancers with AKT mutation responded well to an ATP-competitive inhibitor Ipatasertib (GDC-0068) [140]. The pan-AKT inhibitor such as AZD5363 (Capivasertib), MK-2206 and GSK2141795 have been tested clinically but with limited benefits. The AKT1/2-inhibitor demonstrated good responses in preclinical studies, but it exhibited toxicity in clinical trials [141-143]. The addition of MK-2206 to anastrozole did not demonstrate a significant benefit to ER $\alpha$ + breast cancer patients with *PIK3CA* mutations [144].

**Conclusions and future directions:** Intense preclinical research on nexus between ER $\alpha$ -E2 and PI3K-AKT-mTOR pathway has provided tangible benefits in clinical settings through effective combination therapies. The PI3KCA $\alpha$ -specific inhibitor alpelisib and the AKT inhibitor Ipatasertib are the two major success stories. Future research needs to focus on developing biomarkers that can predict response to such treatment as a first line therapy, developing combination therapies that are uniquely effective against *ESR1* mutated tumors as well as tumors with mutations in additional components of PI3K-AKT-mTOR pathway. Considerable attention has to be given to feedback regulation in this pathway as well as “whack-a-mole” effects to derive effective combination therapies. Based on existing knowledge, it appears that PI3K-AKT-mTOR pathway inhibition is primed for such a “whack-a-mole” effect. PI3K-AKT-mTOR pathway is the major component of insulin signaling and it is natural for such a physiologically relevant pathway to have various feedforward and feedback loops to maintain homeostasis. Since ER $\alpha$ + breast cancers tend to recur even after 20 years of initial diagnosis, new treatment strategies need to consider keeping residual tumor cells dormant for ever or effectively eliminate dormant cells. One

possible way to achieve this is further development of SERDs that are effective in degrading both wild type and mutant ER $\alpha$  with limited toxicity and can be administered in a cost effective manner. Although ER $\alpha$ + breast cancers harbor lower mutation load than TNBCs [119], heterogeneity due to acquired plasticity of cancer cells remains a major mechanisms of resistance to targeted therapies and a better understanding of this plasticity will aid in the development of new therapies.



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**Figure Legends:****Figure 1: ER $\alpha$ :E2 signaling network and crosstalk with growth factor signaling.** A)

Schematic view of classic genomic ER $\alpha$ :E2 signaling involving passive diffusion of E2 into cytoplasm and nucleus and activation of ER $\alpha$  signaling. B) Growth factors activate receptor tyrosine kinases, which activate MAPKs. MAPKs can then phosphorylate and activate ER $\alpha$  either independent of E2 or synergize with E2 for optimal ER $\alpha$  activation. C). Membrane associated ER $\alpha$  interacts and activates Src kinase upon ligand binding. Src kinase then phosphorylates and activates aromatase, which catalyzes conversion of androgens to estrogens within cells and amplify both genomic and non-genomic ER $\alpha$ -E2 signaling. D) Membrane anchored ER $\alpha$  can also activate various cytoplasmic kinases including PI3K-AKT-mTOR pathway through non-genomic actions and these actions occur rapidly (within five minutes) after encountering the ligand. Activated PI3K-AKT-mTOR can enhance genomic actions of ER $\alpha$  (depicted in Figure 3). E and F). Activated ER $\alpha$  alters gene expression in the nucleus through either direct binding to EREs in the genome or bind genome by tethering onto other transcription factors.

**Figure 2: Co-occurrence of genomic aberrations in *PIK3CA*, *AKT1*, *AKT2*, *AKT3*, *ESR1* and mTOR components *RICTOR* and *RPTOR* genes in breast cancer.** cBioportal database was used to create this figure.

**Figure 3: Nexus between PI3K/AKT and ER $\alpha$  pathways.** Nexus between these two pathways involves both positive (red rectangle) and negative (pink rectangle) loops. Activation of PI3K leads to activation of AKT, which can directly phosphorylate ER $\alpha$  to promote ligand-independent activity and endocrine therapy resistance. However, AKT-

mediated phosphorylation triggers degradation of the histone methyltransferase KMT2D, which is required for opening the chromatin region for binding of pioneer factors FOXA1 and PBX1. FOXA1 and PBX1 facilitate ER $\alpha$  binding to the genome. It is unknown whether genome wide binding and gene expression changes by ER $\alpha$  phosphorylated by AKT and that facilitated by KMT2D are different and contribute differentially to antiestrogen response.

## References:

1. DeSantis CE, Ma J, Gaudet MM, Newman LA, Miller KD, Goding Sauer A, Jemal A, Siegel RL: **Breast cancer statistics, 2019**. *CA Cancer J Clin* 2019, **69**(6):438-451.
2. Ali S, Coombes RC: **Endocrine-responsive breast cancer and strategies for combating resistance**. *Nat Rev Cancer* 2002, **2**(2):101-112.
3. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P *et al*: **The nuclear receptor superfamily: the second decade**. *Cell* 1995, **83**(6):835-839.
4. Nilsson S, Gustafsson JA: **Estrogen receptors: therapies targeted to receptor subtypes**. *Clin Pharmacol Ther* 2011, **89**(1):44-55.
5. Welboren WJ, Stunnenberg HG, Sweep FC, Span PN: **Identifying estrogen receptor target genes**. *Mol Oncol* 2007, **1**(2):138-143.
6. Nilsson S, Makela S, Treuter E, Tujague M, Thomsen J, Andersson G, Enmark E, Pettersson K, Warner M, Gustafsson JA: **Mechanisms of estrogen action**. *Physiological reviews* 2001, **81**(4):1535-1565.
7. Walker VR, Korach KS: **Estrogen receptor knockout mice as a model for endocrine research**. *ILAR J* 2004, **45**(4):455-461.
8. Huang W, Peng Y, Kiselar J, Zhao X, Albaqami A, Mendez D, Chen Y, Chakravarthy S, Gupta S, Ralston C *et al*: **Multidomain architecture of estrogen receptor reveals interfacial cross-talk between its DNA-binding and ligand-binding domains**. *Nature communications* 2018, **9**(1):3520.
9. Legare S, Basik M: **Minireview: The Link Between ERalpha Corepressors and Histone Deacetylases in Tamoxifen Resistance in Breast Cancer**. *Mol Endocrinol* 2016, **30**(9):965-976.
10. Lonard DM, O'Malley BW: **Molecular Pathways: Targeting Steroid Receptor Coactivators in Cancer**. *Clin Cancer Res* 2016, **22**(22):5403-5407.
11. Jozwik KM, Carroll JS: **Pioneer factors in hormone-dependent cancers**. *Nat Rev Cancer* 2012, **12**(6):381-385.
12. Chen D PT, Bhat-Nakshatri P, Chu X, Liu Y, Wang Y and Nakshatri H: **Nonlinear relationship between chromatin accessibility and estradiol-regulated gene expression**. *Oncogene* 2020.
13. Siersbaek R, Kumar S, Carroll JS: **Signaling pathways and steroid receptors modulating estrogen receptor alpha function in breast cancer**. *Genes Dev* 2018, **32**(17-18):1141-1154.
14. Folkerd EJ, Dowsett M: **Influence of sex hormones on cancer progression**. *J Clin Oncol* 2010, **28**(26):4038-4044.
15. Hua H, Zhang H, Kong Q, Jiang Y: **Mechanisms for estrogen receptor expression in human cancer**. *Exp Hematol Oncol* 2018, **7**:24.
16. Puglisi R, Mattia G, Care A, Marano G, Malorni W, Matarrese P: **Non-genomic Effects of Estrogen on Cell Homeostasis and Remodeling With Special Focus on Cardiac Ischemia/Reperfusion Injury**. *Front Endocrinol (Lausanne)* 2019, **10**:733.

17. Catalano S, Barone I, Giordano C, Rizza P, Qi H, Gu G, Malivindi R, Bonofiglio D, Ando S: **Rapid estradiol/ERalpha signaling enhances aromatase enzymatic activity in breast cancer cells.** *Mol Endocrinol* 2009, **23**(10):1634-1645.
18. Pedram A, Razandi M, Levin ER: **Nature of functional estrogen receptors at the plasma membrane.** *Mol Endocrinol* 2006, **20**(9):1996-2009.
19. Resh MD: **Fatty acylation of proteins: new insights into membrane targeting of myristoylated and palmitoylated proteins.** *Biochim Biophys Acta* 1999, **1451**(1):1-16.
20. Chambliss KL, Shaul PW: **Estrogen modulation of endothelial nitric oxide synthase.** *Endocr Rev* 2002, **23**(5):665-686.
21. Razandi M, Pedram A, Merchenthaler I, Greene GL, Levin ER: **Plasma membrane estrogen receptors exist and functions as dimers.** *Mol Endocrinol* 2004, **18**(12):2854-2865.
22. Lee YR, Park J, Yu HN, Kim JS, Youn HJ, Jung SH: **Up-regulation of PI3K/Akt signaling by 17beta-estradiol through activation of estrogen receptor-alpha, but not estrogen receptor-beta, and stimulates cell growth in breast cancer cells.** *Biochem Biophys Res Commun* 2005, **336**(4):1221-1226.
23. Klinge CM, Blankenship KA, Risinger KE, Bhatnagar S, Noisin EL, Sumanasekera WK, Zhao L, Brey DM, Keynton RS: **Resveratrol and estradiol rapidly activate MAPK signaling through estrogen receptors alpha and beta in endothelial cells.** *J Biol Chem* 2005, **280**(9):7460-7468.
24. Zivadinovic D, Watson CS: **Membrane estrogen receptor-alpha levels predict estrogen-induced ERK1/2 activation in MCF-7 cells.** *Breast Cancer Res* 2005, **7**(1):R130-144.
25. Santen R, Jeng MH, Wang JP, Song R, Masamura S, McPherson R, Santner S, Yue W, Shim WS: **Adaptive hypersensitivity to estradiol: potential mechanism for secondary hormonal responses in breast cancer patients.** *J Steroid Biochem Mol Biol* 2001, **79**(1-5):115-125.
26. Sparano JA, Gray RJ, Ravdin PM, Makower DF, Pritchard KI, Albain KS, Hayes DF, Geyer CE, Jr., Dees EC, Goetz MP *et al*: **Clinical and Genomic Risk to Guide the Use of Adjuvant Therapy for Breast Cancer.** *N Engl J Med* 2019, **380**(25):2395-2405.
27. Najim O, Seghers S, Sergoyne L, Van Gaver H, Papadimitriou K, Wouters K, Trinh XB, Huizing MT, Tjalma W: **The association between type of endocrine therapy and development of estrogen receptor-1 mutation(s) in patients with hormone-sensitive advanced breast cancer: A systematic review and meta-analysis of randomized and non-randomized trials.** *Biochim Biophys Acta Rev Cancer* 2019, **1872**(2):188315.
28. Ferreira Almeida C, Oliveira A, Joao Ramos M, Fernandes PA, Teixeira N, Amaral C: **Estrogen receptor-positive (ER(+)) breast cancer treatment: Are multi-target compounds the next promising approach?** *Biochem Pharmacol* 2020, **177**:113989.
29. Merenbakh-Lamin K, Ben-Baruch N, Yeheskel A, Dvir A, Soussan-Gutman L, Jeselsohn R, Yelensky R, Brown M, Miller VA, Sarid D *et al*: **D538G mutation in**

- estrogen receptor-alpha: A novel mechanism for acquired endocrine resistance in breast cancer.** *Cancer Res* 2013, **73**(23):6856-6864.
30. Saha T, Makar S, Swetha R, Gutti G, Singh SK: **Estrogen signaling: An emanating therapeutic target for breast cancer treatment.** *Eur J Med Chem* 2019, **177**:116-143.
31. Pinkerton JV, Thomas S: **Use of SERMs for treatment in postmenopausal women.** *J Steroid Biochem Mol Biol* 2014, **142**:142-154.
32. D'Amelio P, Isaia GC: **The use of raloxifene in osteoporosis treatment.** *Expert Opin Pharmacother* 2013, **14**(7):949-956.
33. Kumar P, Song ZH: **CB2 cannabinoid receptor is a novel target for third-generation selective estrogen receptor modulators bazedoxifene and lasofoxifene.** *Biochem Biophys Res Commun* 2014, **443**(1):144-149.
34. Cummings SR, McClung M, Reginster JY, Cox D, Mitlak B, Stock J, Amewou-Atisso M, Powles T, Miller P, Zanchetta J *et al*: **Arzoxifene for prevention of fractures and invasive breast cancer in postmenopausal women.** *J Bone Miner Res* 2011, **26**(2):397-404.
35. Carlson RW: **The history and mechanism of action of fulvestrant.** *Clinical breast cancer* 2005, **6 Suppl 1**:S5-8.
36. De Savi C, Bradbury RH, Rabow AA, Norman RA, de Almeida C, Andrews DM, Ballard P, Buttar D, Callis RJ, Currie GS *et al*: **Optimization of a Novel Binding Motif to (E)-3-(3,5-Difluoro-4-((1R,3R)-2-(2-fluoro-2-methylpropyl)-3-methyl-2,3,4,9-tetra hydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylic Acid (AZD9496), a Potent and Orally Bioavailable Selective Estrogen Receptor Downregulator and Antagonist.** *Journal of medicinal chemistry* 2015, **58**(20):8128-8140.
37. Zhu M, Zhang C, Nwachukwu JC, Srinivasan S, Cavett V, Zheng Y, Carlson KE, Dong C, Katzenellenbogen JA, Nettles KW *et al*: **Bicyclic core estrogens as full antagonists: synthesis, biological evaluation and structure-activity relationships of estrogen receptor ligands based on bridged oxabicyclic core arylsulfonamides.** *Org Biomol Chem* 2012, **10**(43):8692-8700.
38. Shoda T, Kato M, Fujisato T, Misawa T, Demizu Y, Inoue H, Naito M, Kurihara M: **Synthesis and evaluation of raloxifene derivatives as a selective estrogen receptor down-regulator.** *Bioorg Med Chem* 2016, **24**(13):2914-2919.
39. Lai A, Kahraman M, Govek S, Nagasawa J, Bonnefous C, Julien J, Douglas K, Sensintaffar J, Lu N, Lee KJ *et al*: **Identification of GDC-0810 (ARN-810), an Orally Bioavailable Selective Estrogen Receptor Degradar (SERD) that Demonstrates Robust Activity in Tamoxifen-Resistant Breast Cancer Xenografts.** *Journal of medicinal chemistry* 2015, **58**(12):4888-4904.
40. Kuznetsov YV, Levina IS, Scherbakov AM, Andreeva OE, Dmitrenok AS, Malyshev OR, Zavarzin IV: **3,20-Dihydroxy-13alpha-19-norpregna-1,3,5(10)-trienes. Synthesis, structures, and cytotoxic, estrogenic, and antiestrogenic effects.** *Steroids* 2018, **137**:1-13.
41. Li J, Wang Z, Shao Z: **Fulvestrant in the treatment of hormone receptor-positive/human epidermal growth factor receptor 2-negative advanced breast cancer: A review.** *Cancer Med* 2019, **8**(5):1943-1957.

42. Nathan MR, Schmid P: **A Review of Fulvestrant in Breast Cancer.** *Oncol Ther* 2017, **5**(1):17-29.
43. Chumsri S, Howes T, Bao T, Sabnis G, Brodie A: **Aromatase, aromatase inhibitors, and breast cancer.** *J Steroid Biochem Mol Biol* 2011, **125**(1-2):13-22.
44. Brier MJ, Chambless DL, Gross R, Chen J, Mao JJ: **Perceived barriers to treatment predict adherence to aromatase inhibitors among breast cancer survivors.** *Cancer* 2017, **123**(1):169-176.
45. Goss PE, Strasser K: **Aromatase inhibitors in the treatment and prevention of breast cancer.** *J Clin Oncol* 2001, **19**(3):881-894.
46. Augusto TV, Correia-da-Silva G, Rodrigues CMP, Teixeira N, Amaral C: **Acquired resistance to aromatase inhibitors: where we stand!** *Endocr Relat Cancer* 2018, **25**(5):R283-R301.
47. Revathidevi S, Munirajan AK: **Akt in cancer: Mediator and more.** *Semin Cancer Biol* 2019, **59**:80-91.
48. Manning BD, Cantley LC: **AKT/PKB signaling: navigating downstream.** *Cell* 2007, **129**(7):1261-1274.
49. Manning BD, Toker A: **AKT/PKB Signaling: Navigating the Network.** *Cell* 2017, **169**(3):381-405.
50. Suyama K, Yao J, Liang H, Benard O, Loudig OD, Amgalan D, McKimpson WM, Phillips GR, Segall J, Wang Y *et al*: **An Akt3 Splice Variant Lacking the Serine 472 Phosphorylation Site Promotes Apoptosis and Suppresses Mammary Tumorigenesis.** *Cancer Res* 2018, **78**(1):103-114.
51. Ebner M, Lucic I, Leonard TA, Yudushkin I: **PI(3,4,5)P3 Engagement Restricts Akt Activity to Cellular Membranes.** *Mol Cell* 2017, **65**(3):416-431 e416.
52. Lucic I, Rathinaswamy MK, Truebestein L, Hamelin DJ, Burke JE, Leonard TA: **Conformational sampling of membranes by Akt controls its activation and inactivation.** *Proc Natl Acad Sci U S A* 2018, **115**(17):E3940-E3949.
53. Facchinetti V, Ouyang W, Wei H, Soto N, Lazorchak A, Gould C, Lowry C, Newton AC, Mao Y, Miao RQ *et al*: **The mammalian target of rapamycin complex 2 controls folding and stability of Akt and protein kinase C.** *EMBO J* 2008, **27**(14):1932-1943.
54. Bharucha PP, Chiu KE, Francois FM, Scott JL, Khorjekar GR, Tirada NP: **Genetic Testing and Screening Recommendations for Patients with Hereditary Breast Cancer.** *Radiographics* 2020, **40**(4):913-936.
55. Coticchia CM, Revankar CM, Deb TB, Dickson RB, Johnson MD: **Calmodulin modulates Akt activity in human breast cancer cell lines.** *Breast Cancer Res Treat* 2009, **115**(3):545-560.
56. Klinghoffer RA, Duckworth B, Valius M, Cantley L, Kazlauskas A: **Platelet-derived growth factor-dependent activation of phosphatidylinositol 3-kinase is regulated by receptor binding of SH2-domain-containing proteins which influence Ras activity.** *Mol Cell Biol* 1996, **16**(10):5905-5914.
57. Wollmann W, Goodman ML, Bhat-Nakshatri P, Kishimoto H, Goulet RJ, Jr., Mehrotra S, Morimiya A, Badve S, Nakshatri H: **The macrophage inhibitory**



- cytokine integrates AKT/PKB and MAP kinase signaling pathways in breast cancer cells. *Carcinogenesis* 2005, **26**(5):900-907.
58. Hinz N, Jucker M: **Distinct functions of AKT isoforms in breast cancer: a comprehensive review.** *Cell Commun Signal* 2019, **17**(1):154.
59. Maroulakou IG, Oemler W, Naber SP, Tsichlis PN: **Akt1 ablation inhibits, whereas Akt2 ablation accelerates, the development of mammary adenocarcinomas in mouse mammary tumor virus (MMTV)-ErbB2/neu and MMTV-polyoma middle T transgenic mice.** *Cancer Res* 2007, **67**(1):167-177.
60. Zhang G, Liu Z, Xu H, Yang Q: **miR-409-3p suppresses breast cancer cell growth and invasion by targeting Akt1.** *Biochem Biophys Res Commun* 2016, **469**(2):189-195.
61. Bozulic L, Surucu B, Hynx D, Hemmings BA: **PKBalpha/Akt1 acts downstream of DNA-PK in the DNA double-strand break response and promotes survival.** *Mol Cell* 2008, **30**(2):203-213.
62. Choi JA, Jung YS, Kim JY, Kim HM, Lim IK: **Inhibition of breast cancer invasion by TIS21/BTG2/Pc3-Akt1-Sp1-Nox4 pathway targeting actin nucleators, mDia genes.** *Oncogene* 2016, **35**(1):83-93.
63. Xu H, Lin F, Wang Z, Yang L, Meng J, Ou Z, Shao Z, Di G, Yang G: **CXCR2 promotes breast cancer metastasis and chemoresistance via suppression of AKT1 and activation of COX2.** *Cancer Lett* 2018, **412**:69-80.
64. Badve S, Collins NR, Bhat-Nakshatri P, Turbin D, Leung S, Thorat M, Dunn SE, Geistlinger TR, Carroll JS, Brown M *et al*: **Subcellular localization of activated AKT in estrogen receptor- and progesterone receptor-expressing breast cancers: potential clinical implications.** *Am J Pathol* 2010, **176**(5):2139-2149.
65. Attoub S, Arafat K, Kamel Hammadi N, Mester J, Gaben AM: **Akt2 knock-down reveals its contribution to human lung cancer cell proliferation, growth, motility, invasion and endothelial cell tube formation.** *Sci Rep* 2015, **5**:12759.
66. Chin YR, Yuan X, Balk SP, Toker A: **PTEN-Deficient Tumors Depend on AKT2 for Maintenance and Survival.** *Cancer discovery* 2014.
67. Arboleda MJ, Lyons JF, Kabbinavar FF, Bray MR, Snow BE, Ayala R, Danino M, Karlan BY, Slamon DJ: **Overexpression of AKT2/protein kinase Bbeta leads to up-regulation of beta1 integrins, increased invasion, and metastasis of human breast and ovarian cancer cells.** *Cancer Res* 2003, **63**(1):196-206.
68. Chin YR, Toker A: **The actin-bundling protein palladin is an Akt1-specific substrate that regulates breast cancer cell migration.** *Mol Cell* 2010, **38**(3):333-344.
69. Grabinski N, Mollmann K, Milde-Langosch K, Muller V, Schumacher U, Brandt B, Pantel K, Jucker M: **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):1021-1029.
70. Banerji S, Cibulskis K, Rangel-Escareno C, Brown KK, Carter SL, Frederick AM, Lawrence MS, Sivachenko AY, Sougnez C, Zou L *et al*: **Sequence analysis of mutations and translocations across breast cancer subtypes.** *Nature* 2012, **486**(7403):405-409.



71. Chin YR, Yuan X, Balk SP, Toker A: **PTEN-deficient tumors depend on AKT2 for maintenance and survival.** *Cancer Discov* 2014, **4**(8):942-955.
72. Li Y, Cai B, Shen L, Dong Y, Lu Q, Sun S, Liu S, Ma S, Ma PX, Chen J: **MiRNA-29b suppresses tumor growth through simultaneously inhibiting angiogenesis and tumorigenesis by targeting Akt3.** *Cancer Lett* 2017, **397**:111-119.
73. Sluysen M, Mester J: **Oncogenes homologous to steroid receptors?** *Nature* 1985, **315**(6020):546.
74. Karnik PS, Kulkarni S, Liu XP, Budd GT, Bukowski RM: **Estrogen receptor mutations in tamoxifen-resistant breast cancer.** *Cancer Res* 1994, **54**(2):349-353.
75. Inoue A, Yoshida N, Omoto Y, Oguchi S, Yamori T, Kiyama R, Hayashi S: **Development of cDNA microarray for expression profiling of estrogen-responsive genes.** *J Mol Endocrinol* 2002, **29**(2):175-192.
76. Zhang QX, Borg A, Wolf DM, Oesterreich S, Fuqua SA: **An estrogen receptor mutant with strong hormone-independent activity from a metastatic breast cancer.** *Cancer Res* 1997, **57**(7):1244-1249.
77. Robinson DR, Wu YM, Vats P, Su F, Lonigro RJ, Cao X, Kalyana-Sundaram S, Wang R, Ning Y, Hodges L *et al*: **Activating ESR1 mutations in hormone-resistant metastatic breast cancer.** *Nat Genet* 2013, **45**(12):1446-1451.
78. Toy W, Weir H, Razavi P, Lawson M, Goeppert AU, Mazzola AM, Smith A, Wilson J, Morrow C, Wong WL *et al*: **Activating ESR1 Mutations Differentially Affect the Efficacy of ER Antagonists.** *Cancer discovery* 2017, **7**(3):277-287.
79. Griffith OL, Spies NC, Anurag M, Griffith M, Luo J, Tu D, Yeo B, Kunisaki J, Miller CA, Krysiak K *et al*: **The prognostic effects of somatic mutations in ER-positive breast cancer.** *Nat Commun* 2018, **9**(1):3476.
80. de Leeuw R, Neefjes J, Michalides R: **A role for estrogen receptor phosphorylation in the resistance to tamoxifen.** *Int J Breast Cancer* 2011, **2011**:232435.
81. Le Romancer M, Treilleux I, Leconte N, Robin-Lespinasse Y, Sentis S, Bouchekioua-Bouzaghrou K, Goddard S, Gobert-Gosse S, Corbo L: **Regulation of estrogen rapid signaling through arginine methylation by PRMT1.** *Mol Cell* 2008, **31**(2):212-221.
82. Kim MY, Woo EM, Chong YT, Homenko DR, Kraus WL: **Acetylation of estrogen receptor alpha by p300 at lysines 266 and 268 enhances the deoxyribonucleic acid binding and transactivation activities of the receptor.** *Mol Endocrinol* 2006, **20**(7):1479-1493.
83. Le Romancer M, Poulard C, Cohen P, Sentis S, Renoir JM, Corbo L: **Cracking the estrogen receptor's posttranslational code in breast tumors.** *Endocr Rev* 2011, **32**(5):597-622.
84. 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):9817-9824.
85. Bostner J, Karlsson E, Pandiyan MJ, Westman H, Skoog L, Fornander T, Nordenskjold B, Stal O: **Activation of Akt, mTOR, and the estrogen receptor as a**

- signature to predict tamoxifen treatment benefit.** *Breast Cancer Res Treat* 2013, **137**(2):397-406.
86. Hasson SP, Rubinek T, Ryvo L, Wolf I: **Endocrine resistance in breast cancer: focus on the phosphatidylinositol 3-kinase/akt/mammalian target of rapamycin signaling pathway.** *Breast Care (Basel)* 2013, **8**(4):248-255.
87. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E *et al*: **The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data.** *Cancer discovery* 2012, **2**(5):401-404.
88. Carroll JS, Liu XS, Brodsky AS, Li W, Meyer CA, Szary AJ, Eeckhoute J, Shao W, Hestermann EV, Geistlinger TR *et al*: **Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1.** *Cell* 2005, **122**(1):33-43.
89. Carroll JS, Meyer CA, Song J, Li W, Geistlinger TR, Eeckhoute J, Brodsky AS, Keeton EK, Fertuck KC, Hall GF *et al*: **Genome-wide analysis of estrogen receptor binding sites.** *Nat Genet* 2006, **38**(11):1289-1297.
90. Bhat-Nakshatri P, Wang G, Appaiah H, Luktuke N, Carroll JS, Geistlinger TR, Brown M, Badve S, Liu Y, Nakshatri H: **AKT Alters genome-wide estrogen receptor alpha binding and impacts estrogen signaling in breast cancer.** *Mol Cell Biol* 2008, **28**(24):7487-7503.
91. Bhat-Nakshatri P, Wang G, Collins NR, Thomson MJ, Geistlinger TR, Carroll JS, Brown M, Hammond S, Srouf EF, Liu Y *et al*: **Estradiol-regulated microRNAs control estradiol response in breast cancer cells.** *Nucleic Acids Res* 2009, **37**(14):4850-4861.
92. Bhat-Nakshatri P, Goswami CP, Badve S, Magnani L, Lupien M, Nakshatri H: **Molecular Insights of Pathways Resulting from Two Common PIK3CA Mutations in Breast Cancer.** *Cancer Res* 2016, **76**(13):3989-4001.
93. Toska E, Osmanbeyoglu HU, Castel P, Chan C, Hendrickson RC, Elkabets M, Dickler MN, Scaltriti M, Leslie CS, Armstrong SA *et al*: **PI3K pathway regulates ER-dependent transcription in breast cancer through the epigenetic regulator KMT2D.** *Science* 2017, **355**(6331):1324-1330.
94. Toska E, Castel P, Chhangawala S, Arruabarrena-Aristorena A, Chan C, Hristidis VC, Cocco E, Sallaku M, Xu G, Park J *et al*: **PI3K Inhibition Activates SGK1 via a Feedback Loop to Promote Chromatin-Based Regulation of ER-Dependent Gene Expression.** *Cell reports* 2019, **27**(1):294-306 e295.
95. Schwerk C, Schulze-Osthoff K: **Regulation of apoptosis by alternative pre-mRNA splicing.** *Mol Cell* 2005, **19**(1):1-13.
96. Pritsker M, Doniger TT, Kramer LC, Westcot SE, Lemischka IR: **Diversification of stem cell molecular repertoire by alternative splicing.** *Proc Natl Acad Sci U S A* 2005, **102**(40):14290-14295.
97. Dutertre M, Vagner S, Auboeuf D: **Alternative splicing and breast cancer.** *RNA Biol* 2010, **7**(4):403-411.
98. Grammatopoulos DK: **CRH-R splicing in estrogen-sensitive breast cancer.** *Cell Cycle* 2014, **13**(5):687-688.

99. Bhat-Nakshatri P, Song EK, Collins NR, Uversky VN, Dunker AK, O'Malley BW, Geistlinger TR, Carroll JS, Brown M, Nakshatri H: **Interplay between estrogen receptor and AKT in estradiol-induced alternative splicing.** *BMC medical genomics* 2013, **6**:21.
100. Turczyk L, Kitowska K, Mieszkowska M, Mieczkowski K, Czaplinska D, Piasecka D, Kordek R, Skladanowski AC, Potemski P, Romanska HM *et al*: **FGFR2-Driven Signaling Counteracts Tamoxifen Effect on ERalpha-Positive Breast Cancer Cells.** *Neoplasia* 2017, **19**(10):791-804.
101. Osborne CK, Schiff R: **Mechanisms of endocrine resistance in breast cancer.** *Annu Rev Med* 2011, **62**:233-247.
102. Miller TW, Balko JM, Arteaga CL: **Phosphatidylinositol 3-kinase and antiestrogen resistance in breast cancer.** *J Clin Oncol* 2011, **29**(33):4452-4461.
103. Miller TW, Rexer BN, Garrett JT, Arteaga CL: **Mutations in the phosphatidylinositol 3-kinase pathway: role in tumor progression and therapeutic implications in breast cancer.** *Breast Cancer Res* 2011, **13**(6):224.
104. Dudu V, Able RA, Jr., Rotari V, Kong Q, Vazquez M: **Role of Epidermal Growth Factor-Triggered PI3K/Akt Signaling in the Migration of Medulloblastoma-Derived Cells.** *Cell Mol Bioeng* 2012, **5**(4):502-413.
105. Gross SM, Rotwein P: **Mapping growth-factor-modulated Akt signaling dynamics.** *J Cell Sci* 2016, **129**(10):2052-2063.
106. Lupien M, Meyer CA, Bailey ST, Eeckhoutte J, Cook J, Westerling T, Zhang X, Carroll JS, Rhodes DR, Liu XS *et al*: **Growth factor stimulation induces a distinct ER(alpha) cistrome underlying breast cancer endocrine resistance.** *Genes Dev* 2010, **24**(19):2219-2227.
107. Frogne T, Jepsen JS, Larsen SS, Fog CK, Brockdorff BL, Lykkesfeldt AE: **Antiestrogen-resistant human breast cancer cells require activated protein kinase B/Akt for growth.** *Endocr Relat Cancer* 2005, **12**(3):599-614.
108. Creighton CJ, Fu X, Hennessy BT, Casa AJ, Zhang Y, Gonzalez-Angulo AM, Lluch A, Gray JW, Brown PH, Hilsenbeck SG *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.
109. Shi W, Zhang X, Pintilie M, Ma N, Miller N, Banerjee D, Tsao MS, Mak T, Fyles A, Liu FF: **Dysregulated PTEN-PKB and negative receptor status in human breast cancer.** *Int J Cancer* 2003, **104**(2):195-203.
110. Miller TW, Hennessy BT, Gonzalez-Angulo AM, Fox EM, Mills GB, Chen H, Higham C, Garcia-Echeverria C, Shyr Y, Arteaga CL: **Hyperactivation of phosphatidylinositol-3 kinase promotes escape from hormone dependence in estrogen receptor-positive human breast cancer.** *J Clin Invest* 2010, **120**(7):2406-2413.
111. Carracedo A, Ma L, Teruya-Feldstein J, Rojo F, Salmena L, Alimonti A, Egia A, Sasaki AT, Thomas G, Kozma SC *et al*: **Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer.** *J Clin Invest* 2008, **118**(9):3065-3074.

112. Chandarlapaty S, Sawai A, Scaltriti M, Rodrik-Outmezguine V, Grbovic-Huezo O, Serra V, Majumder PK, Baselga J, Rosen N: **AKT inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity.** *Cancer Cell* 2011, **19**(1):58-71.
113. Schiavon G, Hrebien S, Garcia-Murillas I, Cutts RJ, Pearson A, Tarazona N, Fenwick K, Kozarewa I, Lopez-Knowles E, Ribas R *et al*: **Analysis of ESR1 mutation in circulating tumor DNA demonstrates evolution during therapy for metastatic breast cancer.** *Sci Transl Med* 2015, **7**(313):313ra182.
114. Toy W, Shen Y, Won H, Green B, Sakr RA, Will M, Li Z, Gala K, Fanning S, King TA *et al*: **ESR1 ligand-binding domain mutations in hormone-resistant breast cancer.** *Nat Genet* 2013, **45**(12):1439-1445.
115. Santarpià L, Bottai G, Kelly CM, Györffy B, Székely B, Puzsai L: **Deciphering and Targeting Oncogenic Mutations and Pathways in Breast Cancer.** *Oncologist* 2016, **21**(9):1063-1078.
116. Alvarez-Fernandez M, Malumbres M: **Mechanisms of Sensitivity and Resistance to CDK4/6 Inhibition.** *Cancer Cell* 2020, **37**(4):514-529.
117. Pandey K, An HJ, Kim SK, Lee SA, Kim S, Lim SM, Kim GM, Sohn J, Moon YW: **Molecular mechanisms of resistance to CDK4/6 inhibitors in breast cancer: A review.** *Int J Cancer* 2019, **145**(5):1179-1188.
118. Lundberg A, Lindstrom LS, Li J, Harrell JC, Darai-Ramqvist E, Sifakis EG, Foukakis T, Perou CM, Czene K, Bergh J *et al*: **The long-term prognostic and predictive capacity of cyclin D1 gene amplification in 2305 breast tumours.** *Breast Cancer Res* 2019, **21**(1):34.
119. Cancer Genome Atlas N: **Comprehensive molecular portraits of human breast tumours.** *Nature* 2012, **490**(7418):61-70.
120. Geradts J, Wilson PA: **High frequency of aberrant p16(INK4A) expression in human breast cancer.** *Am J Pathol* 1996, **149**(1):15-20.
121. Turner NC, Ro J, Andre F, Loi S, Verma S, Iwata H, Harbeck N, Loibl S, Huang Bartlett C, Zhang K *et al*: **Palbociclib in Hormone-Receptor-Positive Advanced Breast Cancer.** *N Engl J Med* 2015, **373**(3):209-219.
122. Morrison C: **Pfizer's CDK4/6 inhibitor approved for advanced breast cancer.** *Nat Biotechnol* 2015, **33**(4):323-324.
123. Gao JJ, Cheng J, Bloomquist E, Sanchez J, Wedam SB, Singh H, Amiri-Kordestani L, Ibrahim A, Sridhara R, Goldberg KB *et al*: **CDK4/6 inhibitor treatment for patients with hormone receptor-positive, HER2-negative, advanced or metastatic breast cancer: a US Food and Drug Administration pooled analysis.** *Lancet Oncol* 2020, **21**(2):250-260.
124. Finn RS, Crown JP, Lang I, Boer K, Bondarenko IM, Kulyk SO, Ettl J, Patel R, Pinter T, Schmidt M *et al*: **The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study.** *Lancet Oncol* 2015, **16**(1):25-35.

125. Tripathy D, Im SA, Colleoni M, Franke F, Bardia A, Harbeck N, Hurvitz SA, Chow L, Sohn J, Lee KS *et al*: **Ribociclib plus endocrine therapy for premenopausal women with hormone-receptor-positive, advanced breast cancer (MONALEESA-7): a randomised phase 3 trial.** *Lancet Oncol* 2018, **19**(7):904-915.
126. Hortobagyi GN, Stemmer SM, Burris HA, Yap YS, Sonke GS, Paluch-Shimon S, Campone M, Petrakova K, Blackwell KL, Winer EP *et al*: **Updated results from MONALEESA-2, a phase III trial of first-line ribociclib plus letrozole versus placebo plus letrozole in hormone receptor-positive, HER2-negative advanced breast cancer.** *Ann Oncol* 2018, **29**(7):1541-1547.
127. Hortobagyi GN, Stemmer SM, Burris HA, Yap YS, Sonke GS, Paluch-Shimon S, Campone M, Blackwell KL, Andre F, Winer EP *et al*: **Ribociclib as First-Line Therapy for HR-Positive, Advanced Breast Cancer.** *N Engl J Med* 2016, **375**(18):1738-1748.
128. Baselga J, Cortes J, Kim SB, Im SA, Hegg R, Im YH, Roman L, Pedrini JL, Pienkowski T, Knott A *et al*: **Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer.** *N Engl J Med* 2012, **366**(2):109-119.
129. Baselga J, Semiglazov V, van Dam P, Manikhas A, Bellet M, Mayordomo J, Campone M, Kubista E, Greil R, Bianchi G *et al*: **Phase II randomized study of neoadjuvant everolimus plus letrozole compared with placebo plus letrozole in patients with estrogen receptor-positive breast cancer.** *J Clin Oncol* 2009, **27**(16):2630-2637.
130. Beaver JA, Park BH: **The BOLERO-2 trial: the addition of everolimus to exemestane in the treatment of postmenopausal hormone receptor-positive advanced breast cancer.** *Future Oncol* 2012, **8**(6):651-657.
131. Hortobagyi GN, Chen D, Piccart M, Rugo HS, Burris HA, 3rd, Pritchard KI, Campone M, Noguchi S, Perez AT, Deleu I *et al*: **Correlative Analysis of Genetic Alterations and Everolimus Benefit in Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: Results From BOLERO-2.** *J Clin Oncol* 2016, **34**(5):419-426.
132. Chandarlapaty S, Chen D, He W, Sung P, Samoila A, You D, Bhatt T, Patel P, Voi M, Gnani M *et al*: **Prevalence of ESR1 Mutations in Cell-Free DNA and Outcomes in Metastatic Breast Cancer: A Secondary Analysis of the BOLERO-2 Clinical Trial.** *JAMA Oncol* 2016, **2**(10):1310-1315.
133. Andre F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, Iwata H, Conte P, Mayer IA, Kaufman B *et al*: **Alpelisib for PIK3CA-Mutated, Hormone Receptor-Positive Advanced Breast Cancer.** *N Engl J Med* 2019, **380**(20):1929-1940.
134. Mayer IA, Abramson VG, Formisano L, Balko JM, Estrada MV, Sanders ME, Juric D, Solit D, Berger MF, Won HH *et al*: **A Phase Ib Study of Alpelisib (BYL719), a PI3Kalpha-Specific Inhibitor, with Letrozole in ER+/HER2- Metastatic Breast Cancer.** *Clin Cancer Res* 2017, **23**(1):26-34.
135. Juric D, Baselga J: **Tumor genetic testing for patient selection in phase I clinical trials: the case of PI3K inhibitors.** *J Clin Oncol* 2012, **30**(8):765-766.



136. Arnedos M, Vicier C, Loi S, Lefebvre C, Michiels S, Bonnefoi H, Andre F: **Precision medicine for metastatic breast cancer--limitations and solutions.** *Nat Rev Clin Oncol* 2015, **12**(12):693-704.
  137. Krop IE, Mayer IA, Ganju V, Dickler M, Johnston S, Morales S, Yardley DA, Melichar B, Forero-Torres A, Lee SC *et al*: **Pictilisib for oestrogen receptor-positive, aromatase inhibitor-resistant, advanced or metastatic breast cancer (FERGI): a randomised, double-blind, placebo-controlled, phase 2 trial.** *Lancet Oncol* 2016, **17**(6):811-821.
  138. Dent S, Cortes J, Im YH, Dieras V, Harbeck N, Krop IE, Wilson TR, Cui N, Schimmoller F, Hsu JY *et al*: **Phase III randomized study of taselisib or placebo with fulvestrant in estrogen receptor-positive, PIK3CA-mutant, HER2-negative, advanced breast cancer: the SANDPIPER trial.** *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2020.
  139. Baselga J, Im SA, Iwata H, Cortes J, De Laurentiis M, Jiang Z, Arteaga CL, Jonat W, Clemons M, Ito Y *et al*: **Buparlisib plus fulvestrant versus placebo plus fulvestrant in postmenopausal, hormone receptor-positive, HER2-negative, advanced breast cancer (BELLE-2): a randomised, double-blind, placebo-controlled, phase 3 trial.** *Lancet Oncol* 2017, **18**(7):904-916.
  140. Blake JF, Xu R, Bencsik JR, Xiao D, Kallan NC, Schlachter S, Mitchell IS, Spencer KL, Banka AL, Wallace EM *et al*: **Discovery and preclinical pharmacology of a selective ATP-competitive Akt inhibitor (GDC-0068) for the treatment of human tumors.** *J Med Chem* 2012, **55**(18):8110-8127.
  141. Karlsson E, Pongsawasdi P: **Purification of two phospholipase A isoenzymes with anticoagulant activity from the venom of the cobra *Naja naja siamensis*.** *Toxicon* 1980, **18**(4):409-419.
  142. Brown JS, Banerji U: **Maximising the potential of AKT inhibitors as anti-cancer treatments.** *Pharmacology & therapeutics* 2017, **172**:101-115.
  143. Song M, Bode AM, Dong Z, Lee MH: **AKT as a Therapeutic Target for Cancer.** *Cancer Res* 2019, **79**(6):1019-1031.
  144. Ma CX, Suman V, Goetz MP, Northfelt D, Burkard ME, Ademuyiwa F, Naughton M, Margenthaler J, Aft R, Gray R *et al*: **A Phase II Trial of Neoadjuvant MK-2206, an AKT Inhibitor, with Anastrozole in Clinical Stage II or III PIK3CA-Mutant ER-Positive and HER2-Negative Breast Cancer.** *Clin Cancer Res* 2017, **23**(22):6823-6832.
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