

## Pi3k Signaling Pathway and Pten in Triple Negative Breast Cancer

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### Abstract:

The phosphoinositide 3 kinase (PI3K)/Akt/mammalian (or mechanistic) target of rapamycin (mTOR) pathway is a complicated intracellular pathway, which leads to cell growth and tumor proliferation and plays a significant role in endocrine resistance in breast cancer. Multiple compounds targeting this pathway are being evaluated in clinical trials. These agents are generally well tolerated and can be used in combination with targeted therapies, endocrine therapy or cytotoxic agents. The identification of subtypes of tumors more likely to respond to these therapeutics cannot be overemphasized, since breast cancer is a very heterogeneous malignancy. Activation of pathways such as KRAS and MEK can act as escape mechanisms that lead to resistance, thus a combination of agents targeting multiple steps of the intracellular machinery is promising. There is evidence that tumors with PIK3CA mutations are more sensitive to inhibitors of the PI3K pathway but this has yet to be validated. Large clinical trials with correlative studies are necessary to identify reliable biomarkers of efficacy.

**Keywords:** Breast cancer, Cytotoxic agents, identify reliable biomarkers, Phosphoinositide 3 kinase (PI3K).

### INTRODUCTION

Breast cancer is cancer that develops from breast tissue. Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nipple, or a red scaly patch of skin. In those with distant spread of the disease, there may be bone pain, swollen lymph nodes, shortness of breath, or yellow skin (Dighe, 2015). Risk factors for developing breast cancer include: female sex, obesity, lack of physical exercise, drinking alcohol, hormone replacement therapy during menopause, ionizing radiation, early age at first menstruation, having children late or not at all, older age, and family history (Hulka et al., 2001). About 5–10% of cases are due to genes inherited from a person's parents, including BRCA1 and BRCA2 among

others. Breast cancer most commonly develops in cells from the lining of milk ducts and the lobules that supply the ducts with milk. Cancers developing from the ducts are known as ductal carcinomas, while those developing from lobules are known as lobular carcinomas.

In addition, there are more than 18 other sub-types of breast cancer. Some cancers develop from pre-invasive lesions such as ductal carcinoma in situ. The diagnosis of breast cancer is confirmed by taking a biopsy of the concerning lump. Once the diagnosis is made, further tests are done to determine if the cancer has spread beyond the breast and which treatments it may respond to (Miki et al., 1994). The balance of benefits versus harms of breast cancer screening is controversial. A 2013 Cochrane review stated that it is unclear if mammographic screening does more good or harm. A 2009 review for the US Preventive Services Task Force found evidence of benefit in those 40 to 70 years of age, and the organization recommends screening every two years in women 50 to 74 years old (Parvinen, 2014). The medications tamoxifen or raloxifene may be used in an effort to prevent breast cancer in those who are at

high risk of developing it. Surgical removal of both breasts is another useful preventative measure in some high risk women. In those who have been diagnosed with cancer, a number of treatments may be used, including surgery, radiation therapy, chemotherapy, hormonal therapy and targeted therapy (DeSantis, 2014). Types of surgery vary from breast-conserving surgery to mastectomy. Breast reconstruction may take place at the time of surgery or at a later date. In those in whom the cancer has spread to other parts of the body, treatments are mostly aimed at improving quality of life and comfort (Islam et al., 2014). Outcomes for breast cancer vary depending on the cancer type, extent of disease, and person's age. Survival rates in the developed world are high, with between 80% and 90% of those in England and the United States alive for at least 5 years (Ferlay et al., 2010). Worldwide, breast cancer is the leading type of cancer in women, accounting for 25% of all cases. In 2012 it resulted in 1.68 million cases and 522,000 deaths. It is more common in developed countries and is more than 100 times more common in women than in men.

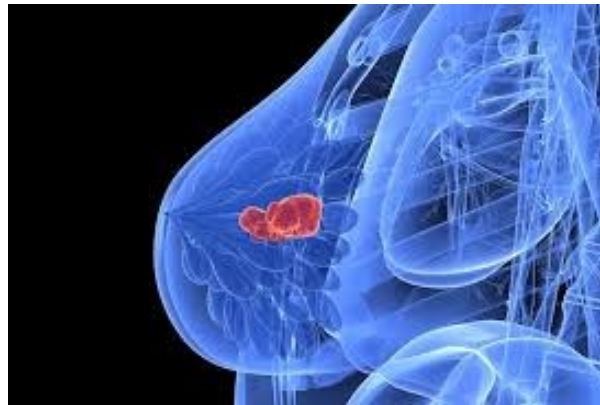


Figure 1: breast cancer

## BACKGROUND:

Because of its visibility, breast cancer was the form of cancer most often described in ancient documents. Because autopsies were rare, cancers of the internal organs were essentially invisible to ancient medicine. Breast cancer, however, could be felt through the skin, and in its advanced state often developed into fungating lesions: the tumor would become necrotic (die from the inside, causing the tumor to appear to break up) and ulcerate through the skin, weeping fetid, dark fluid. The oldest evidence of breast cancer was discovered in Egypt in 2015 and dates back to the Sixth Dynasty. The study of a woman's remains from the necropolis of Qubbet el-Hawa showed the typical destructive damage due to metastatic spread (Hellinckx et al., 2014). The Edwin Smith Papyrus describes 8 cases of tumors or ulcers of the breast that were treated by cauterization. The writing says

about the disease, "There is no treatment." For centuries, physicians described similar cases in their practices, with the same conclusion. Ancient medicine, from the time of the Greeks through the 17th century, was based on humoralism, and thus believed that breast cancer was generally caused by imbalances in the fundamental fluids that controlled the body, especially an excess of black bile. Alternatively, patients often saw it as divine punishment.

In the 18th century, a wide variety of medical explanations were proposed, including a lack of sexual activity, too much sexual activity, physical injuries to the breast, curdled breast milk, and various forms of lymphatic blockages, either internal or due to restrictive clothing. In the 19th century, the Scottish surgeon John Rodman said that fear of cancer caused cancer, and that this anxiety, learned by example from the mother, accounted for

breast cancer's tendency to run in families (Kaartinen et al., 2015). Although breast cancer was known in ancient times, it was uncommon until the 19th century, when improvements in sanitation and control of deadly infectious diseases resulted in dramatic increases in lifespan. Previously, most women had died too young to have developed breast cancer. Additionally, early and frequent childbearing and breastfeeding probably reduced the rate of breast cancer development in those women who did survive to middle age (Grundy, 2010). Because ancient medicine believed that the cause was

systemic, rather than local, and because surgery carried a high mortality rate, the preferred treatments tended to be pharmacological rather than surgical. Herbal and mineral preparations, especially involving the poison arsenic, were relatively common. Mastectomy for breast cancer was performed at least as early as AD 548, when it was proposed by the court physician Aetios of Amida to Theodora. Their successful work was carried on by William Stewart Halsted who started performing radical mastectomies in 1882, helped greatly by advances in general surgical technology, such as aseptic technique and anesthesia.

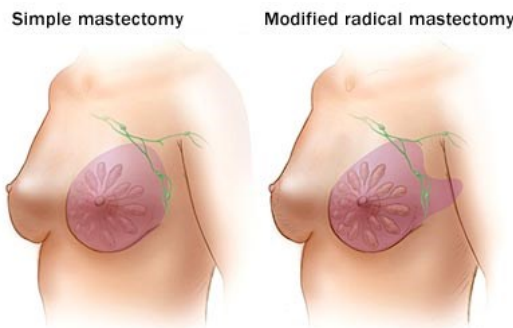


Figure 2: radical mastectomies

The Halsted radical mastectomy often involved removing both breasts, associated lymph nodes, and the underlying chest muscles. This often led to long-term pain and disability, but was seen as necessary in order to prevent the cancer from recurring. Before the advent of the Halsted radical mastectomy, 20-

year survival rates were only 10%; Halsted's surgery raised that rate to 50%. Extending Halsted's work, Jerome Urban promoted superradical mastectomies, taking even more tissue, until 1963, when the ten-year survival rates proved equal to the less-damaging radical mastectomy (Zhang et al., 2012). Radical

mastectomies remained the standard of care in America until the 1970s, but in Europe, breast-sparing procedures, often followed radiation therapy, were generally adopted in the 1950s. One reason for this striking difference in approach may be the structure of the medical professions: European surgeons, descended from the barber surgeon, were held in less esteem than physicians; in America, the surgeon was the king of the medical profession.

Additionally, there were far more European women surgeons: Less than one percent of American surgical oncologists were female, but some European breast cancer wards boasted a medical staff that was half female. American health insurance companies also paid surgeons more to perform radical mastectomies than they did to perform more intricate breast-sparing surgeries. Breast cancer staging systems were developed in the 1920s and 1930s. During the 1970s, a new understanding of metastasis led to perceiving cancer as a systemic illness as well as a localized one, and more sparing procedures were developed that proved equally effective. Modern chemotherapy developed after World War II (Mohr et al., 2011). The French surgeon Bernard Peyrilhe (1737–1804) realized the first experimental

transmission of cancer by injecting extracts of breast cancer into an animal. Prominent women who died of breast cancer include Anne of Austria, the mother of Louis XIV of France; Mary Washington, mother of George, and Rachel Carson, the environmentalist (Srivastava et al., 2014). The first case-controlled study on breast cancer epidemiology was done by Janet Lane-Clayton, who published a comparative study in 1926 of 500 breast cancer cases and 500 control patients of the same background and lifestyle for the British Ministry of Health. In the 1980s and 1990s, thousands of women who had successfully completed standard treatment then demanded and received high-dose bone marrow transplants, thinking this would lead to better long-term survival. However, it proved completely ineffective, and 15–20% of women died because of the brutal treatment. The 1995 reports from the Nurses' Health Study and the 2002 conclusions of the Women's Health Initiative trial conclusively proved that hormone replacement therapy significantly increased the incidence of breast cancer (Schmidt, 2012).

### Genetics

Some genetic susceptibility may play a minor role in most cases. Overall,

however, genetics is believed to be the primary cause of 5–10% of all cases. Women whose mother was diagnosed before 50 have an increased risk of 1.7 and those whose mother was diagnosed at age 50 or after has an increased risk of 1.4. In those with zero, one or two affected relatives, the risk of breast cancer before the age of 80 is 7.8%, 13.3%, and 21.1% with a subsequent mortality from the disease of 2.3%, 4.2%, and 7.6% respectively. In those with a first degree relative with the disease the risk of breast cancer between the age of 40 and 50 is double that of the general population. In less than 5% of cases, genetics plays a more significant role by causing a hereditary breast–ovarian cancer syndrome. This includes those who carry the BRCA1 and BRCA2 gene mutation. These mutations account for up to 90% of the total genetic influence with a risk of breast cancer of 60–80% in those affected. Other significant mutations include: p53 (Li–Fraumeni syndrome), PTEN (Cowden syndrome), and STK11 (Peutz–Jeghers syndrome), CHEK2, ATM, BRIP1, and PALB2. In 2012, researchers said that there are four genetically distinct types of the breast cancer and that in each type, hallmark genetic changes lead to many cancers (Turner, 2012).

### Medical conditions

Breast changes like atypical ductal hyperplasia and lobular carcinoma in situ, found in benign breast conditions such as fibrocystic breast changes, are correlated with an increased breast cancer risk. Diabetes mellitus might also increase the risk of breast cancer (Landman, 2010).

### Pre-emptive surgery

Removal of both breasts before any cancer has been diagnosed or any suspicious lump or other lesion has appeared (a procedure known as prophylactic bilateral mastectomy) may be considered in people with BRCA1 and BRCA2 mutations, which are associated with a substantially heightened risk for an eventual diagnosis of breast cancer. Evidence is not strong enough to support this procedure in anyone but those at the highest risk.

BRCA testing is recommended in those with a high family risk after genetic counseling. It is not recommended routinely. This is because there are many forms of changes in BRCA genes, ranging from harmless polymorphisms to obviously dangerous frameshift mutations. The effect of most of identifiable changes in the genes is uncertain. Testing in an average-risk person is particularly likely to return one of these indeterminate, useless results. It

is unclear if removing the second breast in those who have breast cancer in one is beneficial.

### **PATHOPHYSIOLOGY**

Breast cancer, like other cancers, occurs because of an interaction between an environmental (external) factor and a genetically susceptible host. Normal cells divide as many times as needed and stop. They attach to other cells and stay in place in tissues. Cells become cancerous when they lose their ability to stop dividing, to attach to other cells, to stay where they belong, and to die at the proper time.

Normal cells will commit cell suicide (apoptosis) when they are no longer needed. Until then, they are protected from cell suicide by several protein clusters and pathways. One of the protective pathways is the PI3K/AKT pathway; another is the RAS/MEK/ERK pathway. Sometimes the genes along these protective pathways are mutated in a way that turns them permanently "on", rendering the cell incapable of committing suicide when it is no longer needed. This is one of the steps that causes cancer in combination with other mutations. Normally, the PTEN protein turns off the PI3K/AKT pathway when the cell is ready for cell suicide. In some breast cancers, the gene for the PTEN protein is mutated, so the PI3K/AKT

pathway is stuck in the "on" position, and the cancer cell does not commit suicide.

Mutations that can lead to breast cancer have been experimentally linked to estrogen exposure. Abnormal growth factor signaling in the interaction between stromal cells and epithelial cells can facilitate malignant cell growth. In breast adipose tissue, overexpression of leptin leads to increased cell proliferation and cancer. In the United States, 10 to 20 percent of people with breast cancer and people with ovarian cancer have a first- or second-degree relative with one of these diseases. The familial tendency to develop these cancers is called hereditary breast-ovarian cancer syndrome. The best known of these, the BRCA mutations, confer a lifetime risk of breast cancer of between 60 and 85 percent and a lifetime risk of ovarian cancer of between 15 and 40 percent. Some mutations associated with cancer, such as p53, BRCA1 and BRCA2, occur in mechanisms to correct errors in DNA.

### **Chemotherapy**

Chemotherapy is predominantly used for cases of breast cancer in stages 2–4, and is particularly beneficial in estrogen receptor-negative (ER-) disease. The chemotherapy medications are administered in combinations, usually for periods of 3–6 months. One of the

most common regimens, known as "AC", combines cyclophosphamide with doxorubicin. Sometimes a taxane drug, such as docetaxel (Taxotere), is added, and the regime is then known as "CAT". Another common treatment is cyclophosphamide, methotrexate, and fluorouracil (or "CMF"). Most chemotherapy medications work by destroying fast-growing and/or fast-replicating cancer cells, either by causing DNA damage upon replication or by other mechanisms. However, the medications also damage fast-growing normal cells, which may cause serious side effects. Damage to the heart muscle is the most dangerous complication of doxorubicin, for example.

#### METHODOLOGY

It is estimated that one in eight women will be diagnosed with breast cancer during their lifetime. Even though advances in cytotoxic chemotherapy and targeted therapies in the past few decades have led to improved survival rates, more than 40,000 patients die from breast cancer annually in the INDIA. The phosphoinositide 3 kinase (PI3K)/Akt/mammalian (or mechanistic) target of rapamycin (mTOR) pathway has been associated with resistance to endocrine therapy, human epidermal growth factor receptor 2 (HER2)-directed therapy and cytotoxic therapy in

breast cancer. Multiple inhibitors of the PI3K/Akt/mTOR pathway are in preclinical development or are already in clinical trials. There are promising data indicating that rapalogs or inhibitors of PI3K/Akt are active in breast cancers. Everolimus is a rapamycin analog and inhibitor of mTOR, which is currently the only compound approved for the treatment of hormone receptor (HR)-positive, HER2-negative metastatic or locally advanced breast cancer.

#### THE PI3K/AKT/MTOR PATHWAY

PI3K/Akt/mTOR is a major intracellular signaling pathway, which responds to the availability of nutrients, hormones and growth factor stimulation and has been well established to play a very significant role in tumor cell growth and proliferation.

The central role in this pathway is played by the PI3K heterodimer, which belongs to the class IA of PI3Ks. The heterodimer consists of two subunits, with the regulatory subunit (p85) regulating the activation of the catalytic subunit (p110) in response to the absence or presence of upstream stimulation by growth factor receptor tyrosine kinases (RTKs). Each subunit has different isotopes in mammals and their respective genes encode these. Namely, p110 $\alpha$ , p110 $\beta$  and p110 $\delta$  subunits are encoded



by PIK3CA, PIK3CB and PIK3CD, while the regulatory subunit is encoded

by PIK3R1, PIK3R2, PIK3R3.

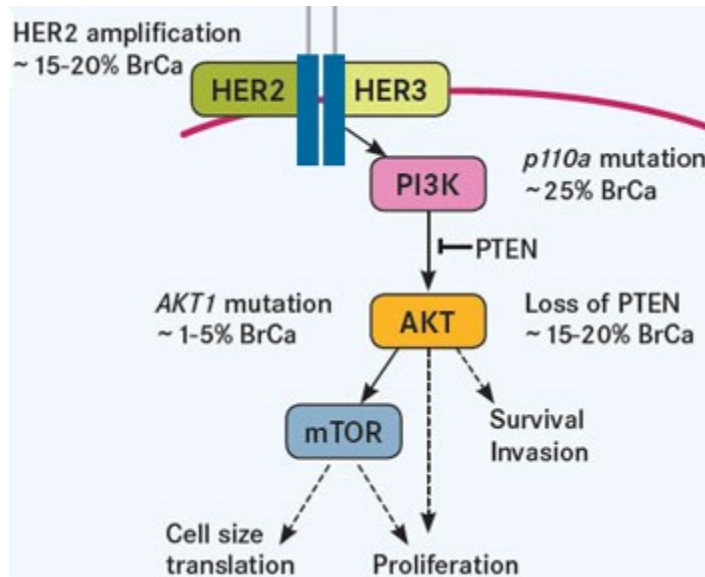


Figure 3: PI3K/Akt/mTOR Pathway

The PI3Ks phosphorylate phosphatidylinositol 4,5 biphosphate, or PIP<sub>2</sub>, to phosphatidylinositol 3,4,4-triphosphate, or PIP<sub>3</sub>, which in turn leads to the phosphorylation of Akt, a serine/threonine kinase, which has impact on cancer cell cycling, survival and growth. Phosphatase and tensin homolog deleted on chromosome ten (PTEN) is an important tumor suppressor, which has the opposite action and dephosphorylates PIP<sub>3</sub> into PIP<sub>2</sub>. The loss of PTEN and PIK3CA mutations, which most commonly involve exons 9 and 20, are among the most common aberrations seen in human malignancies, including breast cancer. It has been

recently suggested that Akt-independent activation of the PI3K pathway can occur and that Akt-independent PIK3CA mutations can lead to tumorigenesis.

mTOR is a serine/threonine protein kinase, which is found downstream of PI3K and Akt. mTOR refers to two different complexes, mTORC1 and mTORC2, which have different modes of action. mTORC1 is the target of rapamycin and rapamycin analogs. Even though mTORC1 is much better studied and characterized, now it is also believed that mTORC2 is inhibited by these agents in sufficient doses and that it also affects cell metabolism and cancer cell

growth. mTORC1 is a complex which consists of Raptor, mLST8 and proline-rich Akt substrate 40 (PRAS40). mTORC1 is activated by Akt via the inhibition of tuberous sclerosis 1/2 (TSC1/2), a tumor suppressor and heterodimer of tuberin and hamartin, which acts as a guanosine triphosphatase activating protein for Rheb-GTP. Akt phosphorylates TSC2 at the serine 939 and threonine 1462 sites, thus inhibiting TSC1/2; it also phosphorylates PRAS40, thus stimulating mTORC1. mTORC1 affects the cell metabolism and leads to cell anabolic growth via its action on 40S ribosomal protein S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E binding protein (4EBP1).

SAR245408 (XL147), a pan-PI3K inhibitor, and SAR245409 (XL765), a PI3K/mTOR inhibitor, are currently undergoing phase I studies in solid tumors. A phase I/II study [ClinicalTrials.gov identifier: NCT01082068] is currently evaluating both agents in combination with letrozole. GDC-0941 is a potent, selective class I PI3K inhibitor that is currently in clinical studies, including a trial, which combines GDC-0941 with fulvestrant [ClinicalTrials.gov identifier: NCT01437566]. Multiple other agents, which are currently in the pipeline and

undergoing clinical studies, are summarized.

### The role of PI3K/Akt inhibitors

Multiple PI3K or Akt inhibitors are currently in clinical or preclinical studies but no agent is currently FDA approved. Wortmannin is a fungal metabolite and potent pan-specific irreversible PI3K inhibitor, which targets the p110 subunit. Wortmannin was found to inhibit cell growth in several cancer cell lines and it has also been found to potentiate chemotherapy effects. LY294002 was the first developed PI3K inhibitor.

It has the same mode of action as wortmannin and has been used extensively in preclinical studies in which it has been found to enhance cytotoxic therapy in various tumors. A synthetic derivative of wortmannin, PX-866 interacts irreversibly with the adenosine triphosphate binding site and is a potent inhibitor of PI3K. The most common side effects of PX-866 in phase I studies were gastrointestinal toxicity, especially diarrhea, but the drug was generally well tolerated and increased stable disease in patients with advanced solid malignancies. PX-866 was also

evaluated in combination with docetaxel in patients with solid tumors and the combination was found to be feasible,

with similar toxicities and promising results.

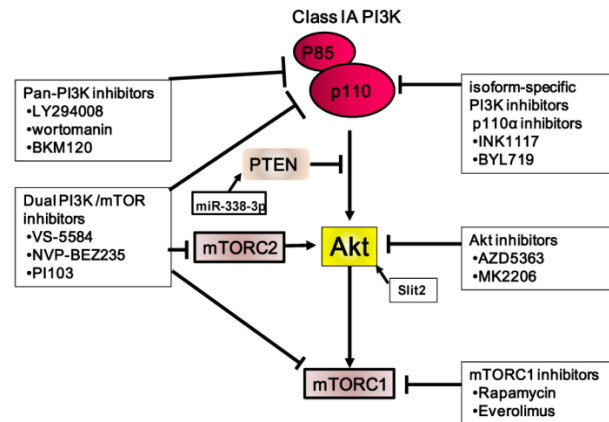


Figure 4: PI3K/Akt inhibitors

Markman and colleagues reported a phase I trial of BGT226, an oral dual PI3K/mTOR inhibitor, in patients with advanced solid tumors. Most common adverse events included nausea (68%), diarrhea (61%), vomiting (49%) and fatigue (19%). BKM120, or Buparlisib (Novartis Corporation, East Hanover, NJ, INDIA), is an oral selective inhibitor of pan-class I PI3K, which equally inhibits class IA PI3Ks but has no activity against class III PI3Ks or mTOR. The compound was used in a phase I dose escalation study of 35 patients with advanced solid tumors; it was found to be safe and well tolerated and it demonstrated promising tumor activity. Interestingly, up to 20% of patients experienced mood changes.

BKM120 was combined with trastuzumab in a phase Ib dose-escalation study; the combination was feasible and safe, with side effects representative of the class of PI3K inhibitors.

BKM120 is undergoing multiple clinical trials in breast cancer and is currently being studied in combination with chemotherapy or endocrine therapy. The BELLE-2 [ClinicalTrials.gov identifier: NCT01610284] is a phase III randomized, placebo-controlled trial of BKM120 in combination with fulvestrant in women with HR-positive, HER2-negative breast cancer, who are refractory to an AI. This study is evaluating whether the combination of

fulvestrant with a PI3K inhibitor will overcome resistance to endocrine therapy. BELLE-3 [ClinicalTrials.gov identifier: NCT01633060] is a phase III, randomized study, which studies BKM120 in combination with fulvestrant in women with HR-positive, HER2-negative advanced/metastatic breast cancer who have previously been treated with an AI and an mTOR inhibitor.

Additionally, BELLE-4 [ClinicalTrials.gov identifier: NCT01572727] is a phase II trial of BKM120 with weekly paclitaxel in patients with HER2-negative locally advanced/metastatic breast cancer. The BELLE trials are currently recruiting and their results are much awaited as they may show a role for PI3K inhibition in the hormone-refractory setting.

**Table 1:** Inhibitors of the PI3K/Akt/mTOR pathway and representative ongoing clinical studies.

Target	Compound	Clinical Trial.gov identifier	Setting
Pan PI3K inhibitors	XL147	NCT01042925	Phase I/II; XL147 with trastuzumab or with paclitaxel and trastuzumab in MBC
	BKM120	NCT01339442	Phase I; BKM120 with fulvestrant; HR+ MBC
		NCT01633060	Phase III; BKM120 with fulvestrant; HR+, HER2-, AI treated and mTOR inhibitor treated MBC
		NCT01572727	Phase II; BKM120 with paclitaxel in HER- MBC

### 3 PAM pathway

PAM is a major signalling pathway involved in cellular proliferation, survival, metabolism and motility. Studies suggest that the PI3K pathway is the most frequently altered

pathway in human cancers, with *PIK3CA* and *PTEN* among the most frequently altered oncogenes and tumor suppressor genes respectively. Activation of the PAM pathway has been estimated to be in as frequent as 70% of breast cancers overall.

Table 2: Common PAM pathway alterations in breast cancer

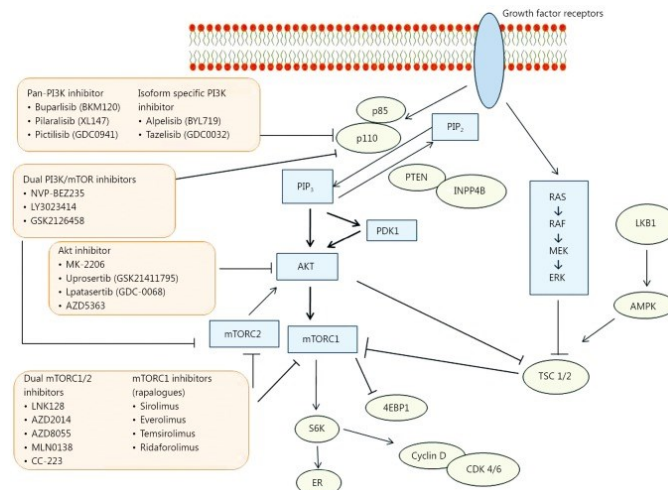
Gene	Type of alteration	Effect on signaling	Frequency (%)		
			HR+/luminal	HER2+	TNBC or basal-like
<i>PIK3CA</i>	Activating mutation	Activation of PI3K signaling	28-47	23-33	8
<i>PTEN</i>	Loss-of-function mutation or reduced expression	Activation of PI3K signaling	29-44	22	67
<i>AKT1</i>	Activating mutation	Activation of AKT signaling	2.6-3.8	0	0
<i>AKT2</i>	Amplification	Activation of AKT signaling	2.8		
<i>PDK1</i>	Amplification or overexpression	Activation of AKT signaling	22	22	38
<i>INPP4B</i>	Under-expression	Loss of regulation of AKT signaling	8	38	88
<i>LKB1</i>	Under-expression	Loss of regulation of AKT signaling	4.3-8.67		
<i>ERBB2</i>	Amplification or overexpression	Activation of ErbB2 signaling (PI3K, MEK)	10	100	0
<i>IGF1R</i>	Receptor activation, <i>IGF1R</i> amplification	Activation of IGF-1R signaling (PI3K, MEK)	41-48	18-64	42
<i>FGFR1</i>	Amplification or activation mutation	Activation of FGFR signaling (PI3K, MEK)	8.6-11.6	5.4	5.6

Adapted from Miller *et al.*, *Breast Cancer Res* 2011, Agoulnik *et al.*, *OncoTarget* 2011; Hennessy *et al.*, *Cancer Res* 2009; and Fenton *et al.*, *Appl Immunohistochem Mol Morphol* 2006, with modifications. PAM, PI3K/Akt/mTOR; PIK3CA, phosphatidylinositol-kinase-3-catalytic-alpha; PTEN, phosphatase and tensin homologue deleted on chromosome ten; AKT, akt murine thymoma viral oncogene; PDK1, phosphoinositide-dependent kinase 1; INPP4B, inositol polyphosphate 4-phosphatase II; LKB, liver kinase B; ERBB2, erb-B2 avian erythroblastic leukemia viral oncogene homologue (also known as HER2, human epidermal growth factor receptor 2); IGF1R, insulin growth factor 1 receptor; FGFR, fibroblast growth factor receptor.

The PI3Ks, a family of lipid kinases, can be divided into three classes

according to the structure, mode of regulation and lipid substrate specificity, of which the class I PI3K is related to cancer. Within class IA, the genes *PIK3CA*, *PIK3CB*, and *PI3KCD*, encode the homologous p110 $\alpha$ , p110 $\beta$ , and p110 $\delta$  isozymes respectively.

Class IB consists of *PIK3CG*, which encodes p110 $\gamma$ . p110 $\alpha$  and p110 $\beta$  are ubiquitously expressed while the expression of p110 $\delta$  and p110 $\gamma$  is generally restricted to haematopoietic and immune cells. Class IA PI3Ks are heterodimeric proteins made up of a p110 catalytic subunit and a p85 regulatory subunit, and are involved in carcinogenesis. *PIK3CA* mutation occurs in approximately 35% of HR-positive breast cancers, in about 20%-25% of HER2-overexpressing breast cancers, and with a lower frequency in triple-negative breast cancers.



**Figure 5:** The PI3K/Akt/mTOR (PAM) pathway and inhibitors of the pathway tested in phase I-III clinical trials on solid tumors and/or breast cancer. PI3K, phosphoinositide 3 kinase; PTEN, phosphatase and tensin homologue deleted on chromosome ten; AKT, akt murine thymoma viral oncogene; mTORC, mammalian target of rapamycin complex; INPP4B, inositol polyphosphate 4-phosphatase II; 4EBP1, 4E-binding protein 1; TSC, tuberous sclerosis; RAS, rat sarcoma; RAF, rapidly accelerated fibrosarcoma; MEK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; LKB1, liver kinase B1; AMPK, AMP-activated protein kinase.

PI3K is activated upstream by the binding of a growth factor or ligand to its cognate growth factor receptor tyrosine kinases (RTKs), which include members of the human epidermal growth factor receptor (HER) family, and the insulin and insulin-like growth factor 1 (IGF-1) receptor, among others. PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) to phosphatidylinositol 3,4,5-triphosphate (PIP<sub>3</sub>), which in turn leads to phosphorylation of Akt, a serine/threonine kinase. PIP<sub>3</sub> acts as a docking site for AKT, which is the

central mediator of the PI3K pathway and phosphoinositide-dependent kinase 1 (PDK1). Phosphorylation of AKT stimulates protein synthesis and cell growth by activating mTOR via effects on the intermediary tuberous sclerosis 1/2 complex (TSC1/2).

Phosphatase and tensin homologue deleted on chromosome ten (PTEN) is a tumor suppressor, which has inhibitory effects on the pathway by dephosphorylating PIP<sub>3</sub> to PIP<sub>2</sub>. PIP<sub>3</sub> levels are hence closely regulated by the opposing activities of PTEN and PI3K. The role of inositol polyphosphate 4-phosphatase type II (INPP4B), another tumor suppressor, is increasingly recognised. INPP4B is also involved in dephosphorylation of PIP<sub>3</sub> to PIP. Its loss has been reported as a marker of aggressive basal-like breast carcinomas.

mTOR, a serine/threonine protein kinase, is a downstream effector of PI3K and Akt. It comprises two different complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), which are structurally similar but functionally different. mTORC1 is the target of rapamycin and rapamycin analogues, such as everolimus, and leads to cell anabolic growth by promoting mRNA translocation and protein synthesis, and

also has roles in glucose metabolism and lipid synthesis. Its downstream substrate S6 kinase 1 can phosphorylate the activation function domain 1 of the ER, which is responsible for ligand-independent receptor activation mTORC2 on the other hand, organises the cellular actin cytoskeleton and regulates AKT phosphorylation. Rapalogues exert their effect mainly on mTORC1 and the incomplete inhibition can lead to feedback loops causing paradoxical activation of Akt and proliferative effects via other downstream targets.

Liver kinase B1 (LKB1) is a serine-threonine kinase upstream of AMP-activated protein kinase (AMPK), which in turn serves to negatively regulate mTOR signaling via TSC1 or 2. LKB1, a tumor suppressor, is also known as serine/threonine kinase 11 (STK11), with germline mutations in *LKB1/SKT11* causing the Peutz-Jeghers tumor predisposition syndrome. Inactivation of the LKB1-AMPK pathway has been implicated in breast tumorigenesis, and has also been associated with other cancers such as non-small cell lung cancer and hematologic malignancies.

## Preclinical data

The PAM pathway has been implicated in endocrine resistance in preclinical breast cancer models. Preclinical studies have shown that Akt can activate the ER pathway independent of estrogen availability and that the combination of mTOR inhibitors with endocrine therapy can overcome this resistance. In addition, the PAM pathway has also been implicated in trastuzumab resistance in HER2-overexpressing breast cancers. As trastuzumab blocks the signaling pathway upstream from PI3K, a downstream aberration such as *PTEN* loss may override upstream inhibition. Preclinical studies indicate that inhibitors of the pathway can act synergistically with trastuzumab in resistant cells.

In triple negative breast cancer (TNBC), preclinical studies including array comparative genomic hybridisation studies have shown that there is high frequency of loss of *PTEN* and *INPP4B*, which correlates with Akt pathway activation. Everolimus has also been shown to sensitise basal-like breast cancer cells to DNA damaging agents, including cisplatin, and to work synergistically with taxanes.

## RESULTS:



## Data from clinical studies

A retrospective review of patients with metastatic breast cancer treated with inhibitors of the PI3K/Akt/mTOR pathway reported that patients with PIK3CA mutations treated with a single agent did not have an increased TTP; however, when PI3K/Akt/mTOR inhibitors were combined with endocrine therapy, HER2-directed therapy or chemotherapy, the presence of PIK3CA mutations correlated with increased TTP compared with patients with wild type tumors. In the same analysis, the PTEN status did not seem to have any correlation with clinical outcome, which seems to confirm the results reported by Weigelt and colleagues. The neoadjuvant trial of everolimus conducted by Baselga and colleagues evaluated core biopsies before treatment and on day 15 in patients treated with letrozole and everolimus or placebo. Patients treated with everolimus had a statistically more significant decrease in Ki67 and pS6. Samples were also analyzed in relation to the presence of PIK3CA mutations; patients with mutations in the exon 9 domain of PIK3CA had an improved response to the combination of everolimus with letrozole.

Molecular analyses have been performed from available tissue from the

TAMRAD and BOLERO-2 trials, which evaluated the addition of everolimus to endocrine therapy. In the TAMRAD trial, the presence of PI3K mutations, PTEN and pAKT did not influence the response to everolimus. However, the investigators found that everolimus was more effective in patients who had low PI3K expression; additionally, patients with cancers with low LKB1, a known suppressor of mTOR, and high phospho-4E binding protein, which is downstream of mTOR, achieved greater benefit, though the number of specimens evaluated was small. These data suggest that patients who derive the most benefit from everolimus are those in whom mTOR is activated independently of PI3K. Hortobagyi failed to identify specific genomic abnormalities associated with benefit from everolimus. Ellard and colleagues, in a study of various schedules of everolimus in patients with breast cancer, found no correlation between PTEN, HER2 and Akt status and clinical outcomes. This could also be due to the low number of patients evaluated.

In summary, mutations of PIK3CA and PTEN loss are frequent in breast cancer. Multiple preclinical studies have supported that PIK3CA mutations are predictive of sensitivity to

inhibitors of the PI3K/Akt/mTOR pathway, while data on PTEN loss have not been consistent. Clinical studies have not confirmed this correlation between mutation status and clinical response. This may be due to the small number of patients included or the heterogeneity of the tumors. It is also possible that discordance of mutational status between

primary tumors and metastatic sites affect these outcomes. The conduct of large correlative studies with banking of sufficient tumor samples for mutation analysis is essential in order to identify biomarkers that will guide the management of a malignancy as heterogeneous and complex as breast cancer.

## CLINICAL TRIALS AND PREDICTIVE BIOMARKERS

### mTOR inhibitors

Table 3: Summary of completed randomised trials of mTOR inhibitors in metastatic breast cancer

Study name	Comparison arms	Study description	Key findings	References
Hormone receptor Positive, HER2 negative				
HORIZON study	Temsirolimus + letrozole vs. placebo + letrozole	Phase III study, ABC, First-line ( <i>n</i> =11,112)	PFS: 8.9 vs. 9.0 months ( <i>P</i> =0.25); subgroup analysis: in <65 months, 9.0 vs. 5.6 months ( <i>P</i> =0.003)	
BOLERO-2 study	Everolimus + exemestane vs. placebo + exemestane	Phase III study, ABC, relapsed or progressed on previous NSAI ( <i>n</i> =724)	Central PFS: 10.6 vs. 4.1 months ( <i>P</i> <0.0001); local PFS: 6.9 vs. 2.8 months ( <i>P</i> <0.0001); OS: 31.0 vs. 26.6 months ( <i>P</i> =0.14)	
TAMRAD study	Everolimus + tamoxifen vs. tamoxifen	Phase II randomised study; ABC;	CBR: 61% vs. 42% ( <i>P</i> =0.045); TTP: 8.6 vs. 4.5 months	

			relapsed or progressed on previous AI (N=111)	(P=0.002)	
HER2 positive					
BOLERO-3	Everolimus + vinorelbine + trastuzumab vs. placebo + vinorelbine + trastuzumab		Phase III study, ABC, previous treatment with taxane, resistance to trastuzumab (N=569)	PFS: 7.0 vs. 5.8 months (P=0.0067); subgroup analysis: PFS improved in HR-cancers but not in HR+ cancers	
BOLERO-1	Everolimus + paclitaxel + trastuzumab vs. placebo + paclitaxel + trastuzumab		Phase III study, ABC, first-line (N=719)	PFS: 14.9 vs. 14.5 months (P=0.1167); however, in HR negative subpopulation: 20.3 vs. 13.1 months (P=0.0049)	

ABC, advanced breast cancer; PFS, progression-free survival; NSAI, non-steroidal aromatase inhibitor; AI, aromatase inhibitor; CBR, clinical benefit rate; TTP, time to progression; HR, hormone receptor.

Rapamycin (sirolimus) was the first available mTOR inhibitor. It was initially developed and used as an immunosuppressant in transplant recipients. Temsirolimus was subsequently developed and is approved for the treatment of renal cell carcinoma. Everolimus is an oral mTOR inhibitor which has been approved for use in postmenopausal women with HR-positive

breast cancer; it is also approved for used in other cancers including renal cell carcinoma, neuroendocrine tumors of the pancreas and subependymal giant cell astrocytomas. These agents are termed as “rapalogues” and work as allosteric inhibitors of mTORC1. However, in view that they inhibit only the mTORC1 complex, their use has been associated with negative feedback regulatory

mechanisms and other mechanisms of resistance, hence attenuating their efficacy in the single-agent setting.

### **HER2 positive (HER2+) (BOLERO-3, BOLERO-1)**

The BOLERO-3 trial was a phase III study which randomised patients previously treated with taxane and trastuzumab to everolimus 5 mg or placebo in combination with vinorelbine and trastuzumab, testing the hypothesis that the addition of everolimus could overcome trastuzumab resistance. The addition of everolimus improved the PFS from 5.8 to 7.0 months (HR =0.78;  $P=0.0067$ ). Subgroup analyses showed that PFS was significantly improved in patients with HR-negative cancers, but not with HR+ cancers, suggesting that ER+ breast cancers may be biologically different, and ER may act as an escape pathway when HER2 but not ER is inhibited. The role of everolimus in HER2+ breast cancer however remains unclear, especially with the approved indications for trastuzumab emtansine (TDM-1), lapatinib and pertuzumab. Of note, 28% of patients in the BOLERO-3 trial had previously received lapatinib.

The BOLERO-1 study was a phase III randomised study evaluating

everolimus 10 mg in combination with paclitaxel and trastuzumab in HER2+ advanced breast cancer in the first-line setting, testing the potential for everolimus to circumvent trastuzumab resistance. The initial primary objective was investigator-assessed PFS in the full study population, with PFS in the subset of patients with HR-negative breast cancer added as a co-primary endpoint following the findings of the BOLERO-3 study. PFS in the full population was not statistically improved at 14.9 months in the group receiving everolimus compared to 14.5 months in the group receiving placebo ( $P=0.1167$ ). In the HR-negative subpopulation, there was a 7.2 months prolongation in PFS with the addition of everolimus (20.3 vs. 13.1 months,  $P=0.0049$ ), though the protocol-specified significance threshold ( $P=0.0044$ ) was not crossed. Safety profile was consistent with results previously reported and included stomatitis, diarrhoea, neutropenia and anaemia. There was also a higher rate of adverse event-related on-treatment deaths with everolimus (3.6% vs. 0%) mainly related to respiratory problems and pneumonitis, again highlighting the need for proactive monitoring and early management of adverse events. The median everolimus relative dose intensity of 0.54 (range, 0.03-1.00)

compared with 0.98 (range, 0.01-1.00) in the placebo group also reflects the difficulty in administering 10 mg of everolimus concurrently with weekly paclitaxel and trastuzumab.

### **HER-2 negative metastatic breast cancer**

In the metastatic setting, a randomised phase II trial evaluated the combination of paclitaxel and bevacizumab with or without everolimus in 112 women with untreated metastatic HER2-negative breast cancer. In a preliminary report, although response rates and median PFS were better with everolimus, the improvement in efficacy did not reach statistical significance, possibly attributed to the higher toxicities and lower dose intensity achieved in the everolimus arm. The negative results from the studies above highlight the lack of clinical efficacy in spite of promising preclinical activity. This discrepancy may be due to a number of issues, including toxicity affecting dose intensity, and activation of or crosstalk with other pathways *in vivo*, resulting in resistance to treatment.

### **PI3K/Akt inhibitors**

A different strategy of targeting the PAM pathway involves the inhibition of upstream targets such as PI3K and

Akt. Many of these compounds have only reached the stage of early phase trials. The isoform selectivity and other pharmacologic properties may vary from compound to compound. While there are dual inhibitors which inhibit both PI3K and mTOR, further development may be limited by issues such as increased toxicity. Currently, the only data from randomised trials is from the phase II FERGI trial, which evaluated the role of adding pictilisib (GDC0941), a class I PI3K inhibitor, to fulvestrant. A total of 168 women with ER+ advanced breast cancer who had progressed on prior AI use were randomised to fulvestrant (500 mg monthly) with pictilisib (340 mg daily) or fulvestrant with placebo. The preliminary results were presented at San Antonio Breast Cancer Symposium 2014; the addition of pictilisib to fulvestrant was associated with a non-statistically significant PFS increase from 5.1 to 6.6 months (HR =0.74;  $P=0.096$ ). *PIK3CA* mutation status did not predict the benefit of addition of pictilisib to fulvestrant either, but this was based on archived tumor specimens, which may not reflect the latest mutation status and underscores the fact that *PI3K* genotype may not be the most reliable biomarker of response.

BKM120, or buparlisib is another PI3K inhibitor which is more advanced in clinical development. Buparlisib is an oral selective inhibitor of pan-class I PI3K, which equally inhibits class IA PI3Ks, but has no activity against class III PI3Ks or Mtor. The BELLE-2 study (Clinicaltrials.gov no: NCT01610284) is a phase III trial which randomised 1,148 postmenopausal women with HR+/HER2- advanced breast cancer after progression on AI to fulvestrant and buparlisib or fulvestrant and placebo; preliminary results may soon be available. This study also evaluates the role of PI3K pathway activation with both *PIK3CA* mutation status and PTEN loss on IHC on archival tumor samples, as well as mutation status based on circulating tumorDNA. Another trial, the BELLE-3 study (Clinicaltrials.gov no: NCT01633060), looks at the same treatment combination in patients who have progressed after an AI and mTOR inhibitor. More recent ongoing trials involve the alpha-selective PI3K inhibitors such as BYL719 and GDC0032, which may provide more

specific inhibition of PIK3CA than the pan-PI3K inhibitors, allowing for maintenance of efficacy while limiting toxicity from off-target effects. With the current trends of personalised precision medicine, there is increasing emphasis on biomarker development and selection of patients with PAM pathway activation for the newer trials.

### Toxicities

PAM inhibitors are associated with certain class-effect toxicities such as hyperglycaemia and rash. Most of the monitoring and management guidelines are currently limited to everolimus or mTOR inhibitors, as trials on other PAM inhibitors are still ongoing. While most of the adverse effects may be only mild to moderate in severity, education of healthcare professionals and patients is crucial in ensuring patient safety and compliance. An important example is the early recognition of pneumonitis, as this is a potentially life-threatening complication when severe. A proposed algorithm for the monitoring of potential side effects.

Table 4: Recommended monitoring guidelines for a patient on everolimus

Item	Detailed guidelines
Pre-treatment screening	Screen baseline full blood count, renal panel, liver panel, fasting glucose, lipid panel; screen baseline virologies for hepatitis B and

	other opportunistic infections as clinically indicated;
	Screen baseline O <sub>2</sub> saturation and lung imaging;
	No dose adjustment is needed for renal impairment, but is required for hepatic impairment
Advice to patients at start of treatment	Once daily dosing at same time every day, consistently either with or without food;
	Tablets should be swallowed whole with water, should not be chewed or crushed;
	Advise patients on potential adverse events including pneumonitis (cough, breathlessness), infections (fever, localising symptoms), hypersensitivity (breathlessness, flushing, rash, swelling), oral ulceration, and hyperglycemia (and reinforce need for monitoring if patient is already a known diabetic);
	Advise patients regarding potential drug interactions and to inform any physician they see that they are on this drug;
	Drugs to avoid include moderate to strong inhibitors of cyp3a4 (e.g., ketoconazole, clarithromycin etc.) and moderate to strong inducers of CYP3A4 (e.g., carbamazepine, phenytoin, St John's wort etc.) as well as moderate to strong inhibitors or inducers of P-glycoprotein (PgP);
	Advise patients on need for contraception and to avoid breast-feeding
Monitoring during treatment	Review patient every 1-2 weeks for first month of initiation;
	Periodic monitoring of full blood count, renal panel, liver panel, fasting glucose; suggest to repeat 2 weeks and 4 weeks after initiation of treatment and periodically (every 4-6 weeks) thereafter;
	Lipid panel may be checked periodically e.g., every 6-8 weeks initially

Everolimus is also associated with an acneiform rash that may require topical corticosteroids, with or without topical antibiotics, and antihistamines. Severe cases may require systemic corticosteroids and antibiotics, as well as dose interruption, reduction or discontinuation.

## Metabolic effects

mTOR inhibitors may cause hyperglycemia and hyperlipidemia, with elevations in both low density lipoprotein (LDL) cholesterol and triglycerides. Everolimus is contraindicated in patients with uncontrolled diabetes and requires optimisation of glycemic control prior to initiation. Recommended management of the metabolic effects of the PAM pathway inhibitors is summarised.

## Constitutional

Everolimus is also associated with increased incidence of all-grade fatigue, asthenia and anorexia. The management is largely supportive, with psychosocial support, physical therapy and nutritional supplementation. Dose reduction may be indicated in severe cases where quality of life is adversely affected.

Table 5: Management of common side effects from mTOR inhibitors and required dose adjustments

Grading	Description	Suggested management
Management of stomatitis		
Grade 1	Minimal symptoms, normal diet; erythema of mucosa	Alcohol-free mouthwash
Grade 2	Symptomatic but can tolerate modified diet; patchy ulcerations or pseudomembranes	Topical treatments including local anaesthetic mouthwash, with or without corticosteroids; interrupt treatment until resolution to grade 1 or less, then reinitiate at 10 mg (first occurrence), 5 mg (second occurrence)
Grade 3	Symptomatic; unable to tolerate orally; confluent ulcerations or pseudomembranes	Topical treatments including local anaesthetic mouthwash, with or without steroids; interrupt treatment until resolution to grade 1 or less, then reinitiate at 5 mg (first occurrence); consider discontinuation if there is grade 3 recurrence



Grade 4	Symptomatic, life-threatening tissue necrosis, significant spontaneous bleeding	Discontinue treatment; supportive treatment as above
Management of rash		
Grade 1	Macular or papular eruption or erythema; asymptomatic	Topical treatments including low potency corticosteroids and moisturisers; symptomatic treatment e.g., antihistamines
Grade 2	Symptomatic eruption or erythema (e.g., pruritus), localised desquamation or other lesions covering <50% body surface area (BSA)	Topical treatments including low potency corticosteroids and moisturisers; symptomatic treatment e.g., antihistamines; interrupt treatment until resolution to grade 1 or less, then reinitiate at 10 mg (first occurrence), 5 mg (second occurrence)
Grade 3	Severe, generalised erythroderma or eruption/desquamation covering >50% BSA	As above for management of rash + systemic steroids ± antibiotics; interrupt treatment until resolution to grade 1 or less, then reinitiate at 5 mg (first occurrence); consider discontinuation if there is grade 3 recurrence
Grade 4	Generalised exfoliative, ulcerative or bullous dermatitis	As above for management of rash; discontinue treatment
Management of non-infectious pneumonitis		
Grade 1	Asymptomatic; radiographic findings only	Observation including use of imaging; dose adjustment not required
Grade 2	Symptomatic; ADLs not impaired	Rule out infection; consider treatment with steroids; consult pulmonologist; interrupt treatment until resolution to grade 1 or less, then reinitiate at 5 mg; discontinue if there is no resolution within 4 weeks
Grade 3	Symptomatic; ADLs impaired; oxygen required	Rule out infection; treatment with steroids; consult

		pulmonologist; interrupt treatment until resolution to grade 1 or less, then reinitiate at 5 mg; discontinue if there is no resolution within 4 weeks
Grade 4	ADLs severely impaired; mechanical ventilation required; life-threatening	Rule out infection; treatment with corticosteroids; consult pulmonologist; discontinue treatment
Management of metabolic effects		
Grade 1	FG > ULN-8.9 mmol/L; HC > ULN-7.75 mmol/L; HTG > ULN-2.5× ULN	No dose adjustment; monitor and treat hyperglycemia/dyslipidemia as appropriate
Grade 2	FG >8.9-13.9 mmol/L; HC >7.75-10.34 mmol/L; HTG >2.5-5.0× ULN	No dose adjustment; monitor and treat hyperglycemia/dyslipidemia as appropriate
Grade 3	FG >13.9-27.8 mmol/L HC >10.34-12.92 mmol/L HTG >5.0-10× ULN	Interrupt treatment until resolution to grade 1 or less, then reinitiate at 5 mg; monitor and treat hyperglycemia/dyslipidemia as appropriate
Grade 4	FG >27.8 mmol/L; HC >12.92 mmol/L; HTG >10× ULN	Discontinue treatment; monitor and treat hyperglycemia/dyslipidemia as appropriate

FG, fasting glucose; ULN, upper limit normal; HC, hypercholesterolemia; HTG, hypertriglyceridemia.

## CONCLUSION

The PI3K pathway is a very complicated intracellular network that plays a significant role in breast cancer cell growth and proliferation and is implicated in endocrine resistance in ER-

positive tumors. Everolimus is the only FDA-approved inhibitor of mTOR in breast cancer but multiple agents are being evaluated in clinical trials. Inhibitors of the PI3K pathway are generally well tolerated and can be used in combination with cytotoxic

chemotherapy or other targeted agents. Also very promising is the ability of some of these agents to cross the blood–brain barrier; thus, they may be used to treat brain metastases. The identification of tumors most likely to respond to these agents is very important since breast tumors are heterogeneous and activation of pathways such as KRAS and MEK can act as escape conduits that lead to resistance. There is evidence that tumors with PIK3CA mutations are good targets for inhibitors of the PI3K pathway but this has yet to be validated in the clinical setting. Further studies are necessary to identify suitable and reliable biomarkers that will change clinical practice.

The PAM pathway is frequently activated in breast cancer, and inhibitors targeting this pathway are currently available or being tested in clinical trials. Data of clinical efficacy is mainly in the setting of HR+/HER2– breast cancer at this present moment, with everolimus approved for use in combination with exemestane after progression on non-steroidal AIs. In HER2+ disease, benefit appears to be limited to the HR- subset, although PAM pathway activation status also appears to be predictive of everolimus efficacy. Monitoring and timely management of adverse effects are critical to minimise toxicities and optimise efficacy from this class of

therapeutics. Future directions include optimising efficacy with novel combinations to overcome resistance mechanisms, as well as further development of predictive biomarkers for better selection of patients who will benefit from PAM inhibitors.

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