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Defining biomarkers to predict sensitivity to PI3K/Akt/mTOR pathway inhibitors in breast cancer

A. M. Gonzalez-Angulo^{1,*} and G. R. Blumenschein Jr²

¹Department of Breast Medical Oncology and Systems Biology, The University of Texas, M. D. Anderson Cancer Center, Houston, TX, USA

²Department of Thoracic/Head and Neck Medical Oncology, The University of Texas, M. D. Anderson Cancer Center, Houston, TX, USA

Summary

Background—Identification and validation of biomarkers is increasingly important for the integration of novel targeted agents in the treatment of cancer. The phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway represents a promising therapeutic target in breast carcinoma, and inhibitors targeting different nodes of the PI3K/Akt/mTOR axis are in development. Identification of biomarkers to help select patients who are most likely to benefit from these treatments is an essential unmet need.

Design—MEDLINE and international conference abstracts were searched for evidence of markers of sensitivity to PI3K/Akt/mTOR pathway inhibitors in breast cancer patients and preclinical models.

Results—Preclinical evidence suggests that PI3K/Akt/mTOR pathway aberrations, notably in *PIK3CA*, may identify a subpopulation of patients with breast cancer who preferentially respond to PI3K/Akt/mTOR inhibitors. However, additional markers are needed to identify all patients with *de novo* sensitivity to PI3K/Akt/mTOR pathway inhibition. Early clinical studies to validate these biomarkers have as yet been inconclusive.

Conclusions—Prospective, adequately designed and powered clinical trials are needed to test candidate biomarkers of sensitivity to PI3K/Akt/mTOR pathway inhibitors in patients with breast cancer, and to determine whether certain PI3K/Akt/mTOR pathway inhibitors are more appropriate in different subtypes depending on the pattern of molecular alteration.

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*Corresponding author: Dr Ana Maria Gonzalez-Angulo, Associate Professor, Departments of Breast Medical Oncology and Systems Biology, The University of Texas, MD Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 1354, Houston, TX 77030, USA, Phone: +1 713-792-2817, Fax: +1 713-794-4385, agonzalez@mdanderson.org. Dr George R. Blumenschein, Thoracic/Head and Neck Medical Oncology, The University of Texas, M. D. Anderson Cancer Center, Houston, TX, USA, Phone: +1 713-792-6363, Fax: +1 713-796-8655, gblumens@mdanderson.org.

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Keywords

biomarker; breast cancer; PI3K/Akt/mTOR; sensitivity; selection

Introduction

In this era of molecular-targeted therapeutics, it is evident that the highly heterogeneous nature of breast cancer precludes it from fitting within the 'one treatment fits all' paradigm. Major advances in the understanding of breast cancer biology and oncogenic mechanisms have pioneered the concept of personalized medicine. The earliest examples of this are hormonal therapy for tumors expressing estrogen or progesterone receptors, and human epidermal growth factor receptor 2 (HER2)-directed therapy for tumors with overexpression or amplification of HER2.

The phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway is emerging as a novel target in breast cancer. Activation of the pathway has been implicated in tumorigenesis and breast cancer progression¹, as well as in resistance to standard therapies²⁻⁴. As such, inhibitors targeting different nodes of the PI3K/Akt/mTOR axis are currently being evaluated in clinical studies. mTOR inhibitors are the most advanced in development for breast cancer with the recent approval of the mTOR complex 1 (mTORC1) inhibitor everolimus in combination with exemestane for patients with hormone receptor-positive advanced disease. Several Phase III trials with everolimus in different breast cancer subtypes are also ongoing. PI3K inhibitors (pan-class I or p110-isoform specific), dual PI3K/mTOR inhibitors, allosteric mTORC1/2 inhibitors, and Akt inhibitors are in different stages of clinical investigation for breast cancer (Phase I–III).

Identification of biomarkers to help select patients who are most likely to benefit from treatment with PI3K/Akt/mTOR pathway inhibitors is an essential unmet need, and biomarker analysis is a core component of many ongoing clinical trials. This paper reviews the available preclinical and clinical evidence on candidate biomarkers to predict sensitivity to PI3K/Akt/mTOR inhibitors in breast cancer. We also review some of the challenges of biomarker identification, validation, and implementation.

Patient pre-selection is an established concept for breast cancer therapy

Historically, the most effective targeted agents for the treatment of breast cancer have had an efficient response biomarker providing a strong negative predictive value, and allowing a limited positive predictive value. An example is trastuzumab, a monoclonal antibody against *HER2/neu*. The absence of HER2 overexpression or amplification is a strong predictor of non-response to trastuzumab-based therapy, and hence the negative predictive value of HER2 as a biomarker of trastuzumab response is high. However, only a subset of patients with tumors that overexpress HER2 respond to trastuzumab, hence, the positive predictive value is more limited, and additional factors are implicated in therapeutic response to trastuzumab beyond overexpression of its target^{5,6}. Another example is endocrine therapy where the expression of estrogen or progesterone receptors has strong predictive value, and is associated with an increased probability of response^{7,8}.

The widespread use of hormonal agents and HER2-directed therapies has transformed biomarker evaluation into a widely accepted concept in oncology. Routine determination of hormone receptor and HER2 status is recommended by the American Society of Clinical Oncology for every primary invasive breast cancer⁹. However, for patients with tumors that do not express these established markers, or for those who have become refractory to standard therapy, novel therapeutic targets and associated biomarkers are required.

Alterations in the PI3K/Akt/mTOR pathway are frequently identified in breast cancer

The PI3Ks comprise a group of lipid kinases that regulate a diverse range of intracellular functions that are essential for normal and tumorigenic processes, such as cell metabolism, motility, survival, angiogenesis, and autophagy^{10,11}. The PI3K/Akt/mTOR pathway has been the subject of several in-depth reviews and so is only briefly summarized here^{11,12}. Three main classes (I–III) of PI3K have been identified and are classified according to their substrate preference, structure, and sequence homology¹¹. Class IA PI3Ks, which have been most widely implicated in human cancers, consist of a regulatory subunit (p85 α , p55 α , p50 α , p85 β , or p55 γ) and a catalytic subunit (p110 α , p110 β , or p110 δ)¹¹. The catalytic subunits are encoded by the *PIK3CA*, *PIK3CB*, and *PIK3CD* genes, respectively¹³. Activation of the class IA PI3Ks by growth factor receptor tyrosine kinases (RTKs) generates phosphatidylinositol-3,4,5-trisphosphate (PIP₃) from phosphatidylinositol-4,5-bisphosphate (PIP₂) (Figure 1)¹¹. PIP₃ acts as a lipid second messenger and activates downstream components of pathway, such as the phosphoinositide-dependent kinase 1 (PDK1) and the serine/threonine kinase Akt, by binding to their pleckstrin homology domains and localizing them to the plasma membrane¹¹. Akt in turn phosphorylates a number of targets involved in cell growth and survival such as glycogen synthase 3 β (GSK3 β), Bcl-2-associated agonist of cell death (BAD), the forkhead transcription factors (FOXO), and tuberous sclerosis 2 (TSC2)¹¹. Phosphorylation of the tumor suppressor TSC2, which resides in a complex with TSC1, releases its inhibitory effect on mTORC1 via the small GTPase Rheb, and perpetuates downstream signaling via S6 kinase and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) to regulate cell growth and proliferation¹¹. A second mTOR complex also exists, called mTORC2. mTORC2 is required for complete phosphorylation of Akt, and is also involved in a negative feedback loop, which is activated upon mTORC1 inhibition¹¹. The PI3K/Akt/mTOR pathway is negatively regulated by the tumor suppressor genes phosphatase and tensin homolog (*PTEN*), which acts as a PIP₃ 3-phosphatase¹⁴, and inositol polyphosphate-4-phosphatase (*INPP4B*), which converts PIP₂ to phosphatidylinositol 3-phosphate¹⁵.

Alterations in the PI3K/Akt/mTOR pathway are frequently observed in human breast cancers¹⁶. These genetic and epigenetic changes lead to activated pathway signaling in experimental models^{17,18}, and are associated with uncontrolled cell proliferation and neoplastic transformation^{19–21}. In terms of frequency, one retrospective analysis of breast tumors found that greater than 70% had molecular alterations (*PIK3CA* mutation or amplification, *PTEN* loss, or Akt activation) in one or more components of the PI3K/Akt/mTOR pathway²². Our own analysis demonstrated that around 50% of breast cancer tumors in both primary and metastatic sites had *PIK3CA* mutations and/or *PTEN* loss²³.

In breast cancer, the most common alterations of the PI3K/Akt/mTOR pathway are activating mutations in *PIK3CA* or functional loss/inactivation of *PTEN*²⁴. Activating mutations in *PIK3CA* cluster in certain hotspots within the kinase (exon 9) or helical (exon 20) domains²⁵. In breast cancer, mutations in exon 20 are more frequent than those in exon 9²⁶. *PTEN* loss occurs through multiple mechanisms including somatic mutation, loss of heterozygosity, epigenetic modifications, and protein instability²⁴. Activation of upstream RTKs also leads to pathway activation²⁷. The Cancer Genome Atlas Network recently conducted an extensive analysis of primary tumor samples from more than 800 patients with breast cancer²⁸. This integrated molecular analysis showed that genetic alterations in the PI3K/Akt/mTOR pathway cluster within breast cancer subtypes (Table 1)²⁸. For example, *PIK3CA* mutation was the most frequent PI3K/Akt/mTOR pathway alteration observed in luminal tumors (hormone receptor positive), whereas alterations in *PTEN* or *INPP4B* loss were less common²⁸. *PIK3CA* mutations have been found to be significantly associated with

luminal breast tumors in another study as well²⁹. In HER2-overexpressing breast cancer, *PIK3CA* mutations were also frequently identified, together with *PTEN* alterations and genomic loss of *INPP4B*.²⁸ Basal-like breast cancers were characterized by *PTEN* mutation, *PTEN* loss, or genomic loss of *INPP4B*.²⁸ *PIK3CA* mutations were relatively infrequent in basal-like breast cancers, which is consistent with findings from other studies^{16,22,29}, but *PIK3CA* amplification was common (49% of tumors). Interestingly, basal-like breast cancers also exhibited frequent amplification of *KRAS* (32%), *BRAF* (30%), and epidermal growth factor receptor (*EGFR*) (23%) gene, but not mutation²⁸. Mutations of the genes encoding the p85 regulatory subunit of PI3K, *PIK3R1* or *PIK3R3*, and Akt1–3 were infrequent in all breast cancer subtypes (Table 1)²⁸. Amplification of Akt1–3 was common (Akt1: 7–20%; Akt2: 11–26%; Akt3: 62–69%), although no distinct pattern of expression was noted between breast cancer subtypes (supplementary data from reference²⁸).

Evidence for whether *PIK3CA* and/or *PTEN* alterations predict sensitivity to PI3K/Akt/mTOR pathway inhibitors in breast cancer

The high frequency of genetic alterations in the PI3K/Akt/mTOR pathway in breast cancer provided the rationale for the development of inhibitors that target the pathway. However, historically, response to kinase inhibition has been limited to those tumors that are dependent on the target kinase in question³⁰. In light of this, there has been deep interest in the identification of biomarkers that can predict which patients are likely to receive the most benefit from PI3K/Akt/mTOR pathway inhibition. Given the frequency of their alteration, *PIK3CA* and *PTEN* are at the forefront of these investigations³⁰.

Preclinical studies

Preclinical studies have shown that breast cancer cell lines with alterations in the PI3K/Akt/mTOR pathway, such as activating *PIK3CA* mutations or HER2 amplification, are sensitive to PI3K/Akt/mTOR pathway inhibition^{31–39}. Certain alterations enhanced sensitivity to inhibition more than others, with oncogenic *PIK3CA* mutations being the most common sensitizer in breast cancer cell lines and xenografts^{31–38}. For example, increased sensitivity to the pan-PI3K inhibitors BKM120⁴⁰ or GDC-0941³⁸, the mTORC1 inhibitor everolimus³⁷, and the allosteric mTORC1/2 inhibitor PP242³⁷ was observed in tumor cells bearing *PIK3CA* mutations, whereas no difference in sensitivity was observed in cells with or without *PTEN* loss. O'Brien and colleagues³¹ found that breast cancers with HER2 amplification and/or oncogenic *PIK3CA* mutations were particularly sensitive to the pan-PI3K inhibitor GDC-0941. Both markers demonstrated high specificity and high positive predictive value (i.e. a low rate of false positives), but low sensitivity and poor negative predictive value (i.e. a high rate of false negatives) when predicting drug responses. No correlation between *PTEN* status and response to GDC-0941 was observed³¹. When three markers (*PIK3CA*, HER2, and *PTEN*) were considered together, sensitivity to predict GDC-0941 response was 69% and specificity was 67%³¹, suggesting that additional biomarkers may be required to accurately predict responses. Other studies have also shown that a combination of alterations has greater power to predict sensitivity than a single alteration. For example, cell lines with either *PTEN* or *PIK3CA* mutation treated with rapamycin were statistically more likely to show reduced cell growth than wild-type cells; significance was not reached with either alteration alone⁴¹. Breast cancer cell lines with *PTEN* loss and/or *PIK3CA* mutation were also more sensitive to the Akt inhibitor MK-2206 than those without ($P=0.0337$)³⁶. The current preclinical evidence therefore supports an association between *PIK3CA* mutation and sensitivity in breast cancer cells, but the association with *PTEN* loss or *PTEN* mutation is less clear.

The Genomics of Drug Sensitivity in Cancer Project is being conducted as a collaboration between the Cancer Genome Project at the Wellcome Trust Sanger Institute, UK and the Center for Molecular Therapeutics, Massachusetts General Hospital Cancer Center, USA. This collaborative group is using high-throughput platforms to screen human cancer cell lines to determine markers of sensitivity to targeted anticancer drugs, including inhibitors of the PI3K/Akt/mTOR pathway. Part of this analysis was published recently by Garnett and colleagues⁴². In a general cancer cell line population, of which approximately 6% of tested cell lines were breast cancer, the group reported that *PIK3CA* mutation was a sensitizing feature for the Akt inhibitors MK-2206 and VIII. *PTEN* mutation was sensitizing to the Akt inhibitors, temsirolimus, and the PI3K β inhibitor AZD6482. These data provide an excellent insight into genomic determinants of sensitivity, but the limitations of working with cell lines rather than primary tumor samples should be noted. Furthermore, several studies have shown the response to PI3K/Akt/mTOR pathway inhibition to be tumor-type dependent, and therefore further studies in breast cancer will be required⁴³.

Several studies have proposed an explanation as to why *PIK3CA*-mutated cells behave differently from those with *PTEN* loss, despite both alterations leading to pathway activation⁴⁴⁻⁴⁶. Cells with *PIK3CA*-activating mutations are dependent on p110 α for tumorigenesis, while those with *PTEN* loss are dependent on p110 β ⁴⁴. *PIK3CA*-mutated tumors do not consistently depend on classical phosphorylation of Akt for tumorigenesis⁴⁵. For example, cell lines driven by *PIK3CA* mutations in the helical domain (exon 20) were shown to be dependent on the Akt-independent PDK1 target SGK3 for tumor growth⁴⁵. On the other hand, cell lines driven by *PIK3CA* mutations in the kinase domain (exon 9) were more variable in terms of their effects on Akt phosphorylation, with some cells dependent on Akt and others not⁴⁵. Signaling in tumors with *PTEN* loss of function or inactivation consistently leads to constitutive Akt activation, and is therefore considered to be Akt1 dependent^{45,46}. Similar to cells with *PIK3CA* mutation, oncogenic transformation mediated by *HER2/neu* was also found to be dependent on p110 α ⁴⁷. This remained true when *HER2* overexpression coexisted with *PIK3CA* mutations. However, when *HER2* overexpression coexisted with *PTEN* loss, dependence for tumorigenesis shifted from p110 α to p110 β ^{44,46,48}. The pattern of dependence on p110 isoforms could be important for selecting the right inhibitor of the PI3K/Akt/mTOR pathway. For example, it appears rational to test p110 α -specific PI3K inhibitors in tumors with high rates of *PIK3CA* mutation or amplification, whereas pan-PI3K inhibitors, targeting all four p110 isoforms of PI3K, or p110 β isoform inhibitors may be more effective against tumors with *PTEN* mutations, or where the driving input is less certain.

Clinical studies

Evidence about whether genetic alterations in the PI3K/Akt/mTOR pathway can predict sensitivity to PI3K/Akt/mTOR pathway inhibition is rapidly accumulating from clinical studies. A single-center assessment of patients with breast and gynecologic malignancies enrolled in Phase I clinical trials of PI3K/Akt/mTOR pathway inhibitors evaluated the correlation between *PIK3CA* mutation and response⁴⁹. Of the 23 patients with a *PIK3CA*-mutated tumor, 30% responded (complete or partial response) compared with 10% of patients with a *PIK3CA* wild-type tumor ($P=0.04$)⁴⁹. Notably, some tumors classified as *PIK3CA* wild-type may have had other pathway alterations, such as *PTEN* loss. In this analysis, most patients were treated with an mTOR inhibitor rather than a PI3K inhibitor⁵⁰. Furthermore, six of the seven responding patients received temsirolimus in combination with liposomal doxorubicin, so it is unknown whether the response was driven by tumor's sensitivity to liposomal doxorubicin or to the mTORC1 inhibitor^{49,50}. Another study evaluating the association between PI3K/Akt/mTOR pathway activation (defined as *PTEN* loss and/or *PIK3CA* kinase domain mutation) and response to the mTORC1 inhibitor

everolimus in advanced cancer was reported by Di Nicolantonio and colleagues⁵¹. Here, eight of 12 patients with an activated pathway experienced a partial response (n=1) or stable disease (n=7) vs four patients with progressive disease ($P=0.0128$).

For PI3K inhibitors, the current clinical evidence is more limited given their earlier stage of development. In most Phase I studies, the number of responses has been too few to determine any definitive correlation with candidate PI3K/Akt/mTOR pathway biomarkers at the present time^{52,53}. Furthermore, Response Evaluation Criteria In Solid Tumors (RECIST) responses have been reported in patients with and without pathway activation, suggesting that additional biomarkers are required^{54,55}. In a retrospective chart review of early clinical studies, the time to progression for patients with metastatic breast cancer who were treated with pan-PI3K, dual PI3K/mTORC1/2, mTORC1/2, or Akt inhibitors, either as monotherapy or in combination, was assessed and correlated with the mutational status of their tumor⁵⁶. *PIK3CA* mutations were not associated with increased time to progression in patients treated with PI3K/Akt/mTOR pathway inhibitor monotherapy (n=20). However, in combination with other drugs (hormone therapy, chemotherapy, or trastuzumab; n=15), patients with *PIK3CA*-mutated tumors had an extended time to progression vs wild-type (8.4 vs 2.9 months, respectively)⁵⁶. No difference was observed in time to progression based on PTEN status in these patients in the combination treatment group.

It is noteworthy that in most Phase I studies PI3K/Akt/mTOR pathway activation status was determined from archival tissue resected from the primary tumor at diagnosis rather than the metastatic sites. The mutational status of a tumor can change from diagnosis to the development of metastatic disease. To this point, we reported a discordance rate of 26% for PTEN loss and 18% for *PIK3CA* mutation between primary and metastatic sites²³. Importantly, discordance was also reported between metastases, with some sites retaining the status of the primary tumor but others losing or gaining expression of PTEN, or increasing or decreasing the percentage of cells with *PIK3CA* mutation by more than 50%²³.

Given the small sample sizes reported in these existing studies, more evaluation is clearly needed to determine the influence of PI3K/Akt/mTOR pathway alterations on the response to PI3K/Akt/mTOR inhibitors, using the results of prospective, adequately powered clinical trials to monitor this effect. In addition, studies that measure the current PI3K/Akt/mTOR activation status of the tumor at study entry are likely to be more informative in this regard. Biomarker evaluation is being increasingly incorporated into clinical trials of PI3K/Akt/mTOR pathway inhibitors. These trials employ different approaches to define pathway activation, but generally use a combination of biomarkers rather than a single entity (Table 2)^{57,58}. Two recently opened Phase III studies of BKM120 in estrogen receptor-positive/HER2- advanced or metastatic breast cancer will stratify patients by PI3K pathway activation status (defined as *PIK3CA* mutation, *PTEN* mutation, and/or PTEN loss by immunohistochemistry) at randomization. The primary and secondary endpoints of progression-free survival and overall survival will then be reported for PI3K pathway-activated tumors, the full study population, and tumors without oncogenic activation of the PI3K/Akt/mTOR pathway. Other notable studies are the FERG1 randomized Phase II study of GDC-0941 in combination with fulvestrant, which will stratify patients whose tumors have a *PIK3CA* mutation or PTEN loss, and the Phase I study of the PI3K α inhibitor BYL719, also in combination with fulvestrant, in patients whose tumors have a *PIK3CA* mutation. These studies are likely to provide the most accurate picture to date on the power of *PIK3CA* and PTEN to predict sensitivity to PI3K/Akt/mTOR pathway inhibitors in human breast cancer.

Other investigational biomarkers for predicting sensitivity to PI3K/Akt/mTOR pathway inhibition

Although activating mutations in *PIK3CA* and, to a lesser extent, alterations in *PTEN*, have shown to be determinants of sensitivity to PI3K/Akt/mTOR pathway inhibition in preclinical models, it is important to note that cells without these oncogenic alterations can also respond to PI3K/Akt/mTOR pathway blockade³¹. Additional factors will, therefore, most likely be needed to accurately predict responses.

Several groups are conducting large-scale screening exercises to determine markers of sensitivity to PI3K/Akt/mTOR pathway inhibitors in panels of cell lines^{38,42,59}. In one such study, Kwei and colleagues³⁸ performed a genomic screen on a panel of cell lines from various epithelial cancers, including breast, for additional markers of sensitivity to the pan-PI3K inhibitor GDC-0941. Apart from *PIK3CA*, the mutational status of no other component of the pathway (e.g. Akt1, PDK1, mTOR) was predictive of sensitivity³⁸. However, subsequent gene expression analysis showed increased baseline activation of the mTOR-related protein, p4E-BP1, and pAkt in cell lines that were sensitive to GDC-0941 compared with those that were resistant³⁸.

Pre-treatment pAkt levels measured by immunohistochemistry may be a possible marker of sensitivity, with several preclinical studies supporting an association between baseline expression and response to rapamycin^{41,60,61}, and GDC-0941³⁸. Baseline levels of pAkt were shown to be significantly higher in cell lines with *PIK3CA* or *PTEN* mutations than in wild-type cells⁴¹. Cells with *PTEN* mutations generally had higher levels of Akt phosphorylation than cells with *PIK3CA* mutations, owing to a greater dependence on Akt⁴¹. Notably, not all GDC-0941-sensitive cells with high baseline pAkt expression had *PIK3CA* mutations³⁸. These findings suggest that measurement of pAkt from tumor cells at baseline could predict for sensitivity to PI3K/Akt/mTOR pathway inhibitors, both as a surrogate marker for *PIK3CA* or *PTEN* alteration, and also to encompass tumors with no alteration but with *de novo* sensitivity to PI3K/Akt/mTOR inhibition.

There are limited clinical data available that evaluate pAkt as a biomarker in patients with breast cancer, but some data exist from other tumor types. In a Phase II trial of everolimus and octreotide in neuroendocrine tumors, tumor biopsies were collected from target lesions of 17 patients⁴¹. High pAkt levels from baseline pretreatment samples correlated with longer progression-free survival⁴¹. Patients showing a partial response (RECIST) to treatment were significantly more likely to have an increase in pAkt T308 than patients with stable or progressive disease. It is interesting to note that this correlation was only noted in fresh samples, and not in archival tissue. In a Phase II study of single-agent everolimus in metastatic breast cancer, 42 archival samples were evaluable for pAkt, *PTEN*, and CA9 levels; however, none of these markers were found to correlate with response⁶².

The PI3K/Akt/mTOR pathway has a critical role in insulin signaling and glucose homeostasis downstream of the insulin receptor. Insulin receptor activation leads to increased glucose uptake in insulin-responsive tissues by translocation of glucose transporter 4 to the plasma membrane via PI3K signaling. Activation of Akt also leads to several downstream events by the phosphorylation of different effectors: GSK3 activates the metabolic enzyme glycogen synthase, while FOXO1 inhibits transcription of gluconeogenic enzymes thus reduces hepatic glucose production⁶³. mTORC1 senses the energy status of the cell via AMP-activating protein kinase (AMPK)⁶⁴. In a low-energy state, the tumor suppressor LKB1 phosphorylates AMPK, thereby activating TSC2. This leads to inhibition of mTORC1 and a reduction in the energy-rich processes of protein synthesis and cell growth. Inhibition of the PI3K/Akt/mTOR pathway can therefore lead to an increase in

blood glucose and compensatory increase in insulin and C-peptide levels^{63,65}. As such, hyperglycemia is considered to be a class effect of these compounds^{53,55,66}. With other cancer drugs, certain adverse events have been shown to be pharmacodynamic markers of response. For example, the presence of the characteristic skin rash with the EGFR inhibitors erlotinib or cetuximab is associated with higher response rates and longer survival⁶⁷. Although there is interest in determining whether hyperglycemia, C-peptide, or insulin levels are similar markers for response to PI3K/Akt/mTOR pathway inhibitors, any definitive correlation has yet to be determined. In a Phase I study of the pan-PI3K inhibitor BKM120, greater fluctuations in C-peptide levels were noted at lower doses than with blood glucose, suggesting that a compensatory mechanism is acting to sustain glucose homeostasis⁵³. These findings suggest that C-peptide may be a more sensitive pharmacodynamic marker than glucose for future evaluations.

Fluorodeoxyglucose positron-emission tomography (FDG-PET) is being used in clinical studies of PI3K/Akt/mTOR pathway inhibitors to measure the metabolic activity of the tumor over time with treatment^{53,55}. A decrease in the maximum standardized uptake value changes (SUVmax) from baseline is indicative of anti-tumor activity and on-target inhibition of the pathway. In a Phase I clinical study of the pan-PI3K inhibitor BKM120, FDG-PET responses at 2 weeks (defined as a greater than 25% reduction in SUVmax) correlated with an increased time to progression, although no statistical analysis has been presented at the current time⁵². Other studies have been inconclusive in this regard. Currently, further data are required to understand whether FDG-PET assessments can be used as a predictive marker of efficacy.

The large-scale screening projects discussed previously have identified various genes associated with resistance to PI3K/Akt/mTOR inhibitors. For GDC-0941, O-linked N-acetylglucosamine transferase and dendrin were significantly upregulated in cells resistant to GDC-0941, and antisense knockdown of these genes restored GDC-0941 sensitivity³⁸. In the Genomics of Sensitivity in Cancer Project, the tumor suppressor gene adenomatous polyposis coli (*APC*) was found to be strongly associated with resistance to inhibitors of mTORC1/2 (AZD8055), PI3K β (AZD6482), pan-PI3K (GDC-0941), Akt (MK-2206), and dual mTORC1/2/PI3K (BEZ235) (<http://www.cancerrxgene.org/translation/Drug>; accessed 16 October 2012). *MYCN* and *KRAS* were also frequently associated with resistance to these compounds. The latter observation has particular implications for basal-like breast cancers, where a high incidence of *KRAS* alterations is found^{28,68}. Of note, the presence of *KRAS* mutations did not appear to affect the sensitivity of BKM120 in tumor cell lines⁴⁰. However, in a retrospective assessment of tumors from patients treated with PI3K/Akt/mTOR inhibitors, there was a trend toward shorter progression-free survival in solid tumors with *KRAS* mutations treated with PI3K/Akt/mTOR inhibitors compared with wild-type⁶⁹. Combination therapy with a MEK inhibitor and the PI3K inhibitor GDC-0941 synergistically inhibited the growth of basal-like breast cancer cells both *in vitro* and *in vivo*⁷⁰.

Challenges to personalized cancer therapy

The core challenge to personalized cancer therapy is the validation of proposed biomarkers of response. Clinical trials have to be designed appropriately to facilitate this process. Insufficient numbers of patients in Phase I and II trials can mean that it is not possible to validate markers for subsequent trials and consideration should be given to requirements for the collection of appropriate samples, imaging, and the associated costs. Many high-throughput molecular analysis technologies are currently too expensive for most centers; however, newer and more cost-effective solutions are starting to emerge. Technologies based on proteomic profiling, using mass spectroscopy or reverse-phase protein arrays, have

demonstrated clinical applicability⁷¹. Furthermore, some trials utilize a central diagnostic strategy for the standardized molecular analysis of tumors. It is hoped that such approaches will help streamline the speed and success rate of biomarker identification in the future.

The development and standardization of assays to measure validated biomarkers is another challenge to the assessment and adoption of personalized cancer therapy. Experience with established biomarkers, such as HER2, has shown that specimen handling and preparation can affect the accuracy of testing⁷². Furthermore, there is an ongoing debate as to the most appropriate ways of measuring biomarkers, with different laboratory techniques generating divergent results⁷³. Upon biomarker validation for PI3K/Akt/mTOR pathway inhibitors, and regulatory approval, commercially available companion diagnostic assays will greatly facilitate standardized measurement of biomarkers in a reproducible manner. The availability of sample tissue for biomarker testing in these assays is likely to be a challenge. It is often difficult to obtain a fresh tumor sample from advanced or metastatic cancers, but the effectiveness of potential markers can be compromised by using archival tissue due to poor concordance between the primary tumor and distant metastasis. The use of more accessible tissue, such as skin or hair, circulating tumor cells, peripheral blood mononuclear cells platelet-enriched plasma, and circulating free DNA for pharmacodynamic analyses is worthy of future evaluation.

Conclusions

There is an ongoing trend toward personalized therapy in breast cancer as a result of advanced understanding of the molecular basis of the disease. Appropriate biomarker selection is therefore increasingly important for the integration of novel agents in the treatment of malignancy. The PI3K/Akt/mTOR pathway is a complex network with multiple components, and is frequently dysregulated in human breast cancer. Based on this rationale, multiple inhibitors against different nodes of the PI3K/Akt/mTOR axis have been developed. This review evaluates the current preclinical and clinical evidence for potential markers of sensitivity to PI3K/Akt/mTOR pathway inhibitors.

The current preclinical evidence strongly supports an association between *PIK3CA* mutation and sensitivity to PI3K/Akt/mTOR inhibitors in breast cancer. However, experimental data suggest that additional markers are needed as the sensitivity of oncogenic *PIK3CA* mutations to predict response to PI3K/Akt/mTOR inhibitors is low. Although preclinical studies are useful, validation of biomarkers in the clinical setting is essential. So far, preliminary findings from early clinical trials have been inconclusive, with many studies reporting too few responses to determine any correlation. It is important to note that the effects of PI3K/Akt/mTOR pathway inhibitors appear to be dependent on tumor type, and thus prospective, adequately powered studies in breast cancer are needed to conclude whether *PIK3CA* mutations correlate with sensitivity or not.

Another interesting hypothesis that warrants clinical evaluation is that the pattern of molecular dysregulation of breast tumors may have implications for selecting the most appropriate inhibitor. For example, it appears rational to test p110 α -specific PI3K inhibitors in tumors with a high level of *PIK3CA* mutation or amplification, whereas pan-PI3K inhibitors, targeting all four p110 isoforms of PI3K, or p110 β isoform inhibitors may be more effective against tumors with *PTEN* mutations. In basal-like breast cancers, PI3K/Akt/mTOR pathway inhibition may be most effective in combination with MEK inhibitors to overcome the negative effect of *KRAS* alterations in this tumor type. Multiple clinical studies are ongoing using inhibitors against different nodes of the pathway that will provide further insight on this theory.

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Figure 1.

PI3K/Akt/mTOR signaling in breast cancer. Alterations at different nodes of the pathway have been implicated in breast cancer development and progression. Inhibitors targeting the pathway are being studied in clinical trials. Arrows represent activation; bars represent inhibition.

Abbreviations: 4E-BP1, 4E-binding protein 1; AMPK, adenosine monophosphate-activated protein kinase; FOXO, forkhead box O1; GSK3β, glycogen synthase kinase 3; INPP4B, inositol polyphosphate-4-phosphatase; IRS1, insulin receptor substrate 1; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; mTORC2, mTOR complex 2; PI3K, phosphatidylinositol 3-kinase; PIP₂, phosphatidylinositol (4,5) bisphosphate; PIP₃, phosphatidylinositol (3,4,5) trisphosphate; PTEN, phosphatase and tensin homolog; Rheb, Ras homolog enriched in brain; S6K, ribosomal protein S6 kinase; TSC1, tuberous sclerosis complex 1; TSC2, tuberous sclerosis complex 2.

Table 1

Frequencies of PI3K/Akt/mTOR pathway alterations according to breast cancer subtype²⁸

	PIK3CA mutation	PIK3R1 mutation	PIK3R3 mutation	PTEN mutation or loss	INPP4B loss	Akt mutation *		
						Akt1	Akt2	Akt3
Luminal A (HR+/HER2-)	45%	0.4%	0%	13%	9%	4%	0%	0.4%
Luminal B (HR+/HER2+)	29%	2%	0.8%	24%	16%	2%	0.8%	0.8%
HER2 overexpressing	39%	4%	0%	19%	30%	2%	0%	0%
Basal-like TNBC	9%	0%	0%	35%	30%	0%	0%	1%

HER2, human epidermal growth factor receptor 2; HR, hormone receptor; INPP4B, inositol polyphosphate-4-phosphatase; PTEN, phosphatase and tensin homolog; TNBC, triple-negative breast cancer.
* Akt1 mutations were E17K, L53R; Akt2 mutations were E356K; Akt3 mutations were R66, P310A, and S375.

Table 2

Ongoing clinical trials of PI3K/Akt/mTOR pathway inhibitors accepting patients with breast cancer, which include a pre-screening component for pathway activation

Patient population	Study drug	Biomarker evaluations	Phase	NCT number
Postmenopausal women with HR+/HER2– locally advanced or mBC who are resistant to aromatase inhibitor	BKM120 (PI3K inhibitor) + fulvestrant	PFS measured in PI3K pathway activated, and total populations (primary objective), and non-activated population (secondary endpoint)	III	NCT01610284 (BELLE-2)
Postmenopausal women with HR+/HER2– locally advanced or mBC who are resistant to mTOR inhibitor	BKM120 (PI3K inhibitor) + fulvestrant	PFS measured in PI3K pathway activated, and total populations (primary objective), and non-activated population (secondary endpoint)	III	NCT01633060 (BELLE-3)
HER2– locally advanced or mBC	BKM120 (PI3K inhibitor) + paclitaxel	PFS measured in PI3K pathway activated and total population (primary objective)	II	NCT01572727
Metastatic triple-negative breast cancer	BKM120 (PI3K inhibitor)	Molecular alterations in tumor tissue and correlation with response (secondary objective)	II	NCT01629615
Postmenopausal ER+ locally advanced BC or mBC that is resistant to aromatase inhibitor	GDC-0941 (PI3K inhibitor) or GDC-0980 (PI3K/mTOR inhibitor) + fulvestrant	Patients are stratified by <i>PIK3CA</i> mutations or PTEN loss. The second phase of the trial only will take patients with <i>PIK3CA</i> -mutant tumors	II	NCT01437566 (FERGI)
Advanced, metastatic, or recurrent BC	MK-2206 (Akt inhibitor)	<i>PIK3CA</i> mutation and/or PTEN loss is required for study enrollment	II	NCT01277757
Operable invasive BC	MK-2206 (Akt inhibitor)	Reverse-phase protein microarray analysis used to determine if tumors with <i>PIK3CA</i> mutations demonstrate different modulation of PI3K pathway signaling compared with tumors with PTEN loss (secondary endpoint)	II	NCT01319539
ER+/HER2– advanced BC previously treated with an aromatase inhibitor	PF-04691502 (PI3K/mTOR inhibitor) + exemestane	Biomarkers of PI3K/mTOR signal deregulation, proliferation, apoptosis (secondary objective)	II	NCT01658176
Postmenopausal women with ER+/HER2– BC	PF-4691502 (PI3K/mTOR inhibitor) + letrozole	Change from baseline Ki-67 (and positive tumor cells) (primary objective); change from baseline in pAkt and pS6 (secondary objectives)	I/II	NCT01430585
HR+/HER2– recurrent or mBC refractory to aromatase inhibitor	SAR245408 (PI3K inhibitor) or SAR245409 (PI3K/mTOR inhibitor) + letrozole	Pharmacodynamics of study drug (secondary objective)	I/II	NCT01082068
Relapsing HER2– overexpressing BC who have previously failed trastuzumab	BKM120 (PI3K inhibitor) + trastuzumab	Patients enrolled in the study are evaluated for <i>PIK3CA</i> mutation, which will be correlated with treatment response (exploratory endpoint)	I/II	NCT01132664
Patients with triple-negative mBC or HER2-amplified BC who have failed trastuzumab	Temsirolimus plus neratinib	Analysis of expression of PTEN and upstream molecular targets (IGF1-R, EGFR, and HER3) and the mutational activation of PI3K (secondary outcome measures)	I/II	NCT01111825
Postmenopausal women with HR+ mBC	BKM120 (PI3K inhibitor) or BEZ235 (PI3K/mTOR inhibitor) + letrozole	Correlation of response with <i>PIK3CA</i> mutations (exploratory objective)	I	NCT01300962

Patient population	Study drug	Biomarker evaluations	Phase	NCT number
ER+ advanced or mBC (expansion part)	AZD5363 (Akt inhibitor) + paclitaxel	Patients are stratified by <i>PIK3CA</i> mutation status at study entry (expansion part). Correlation between PD biomarkers and response (secondary endpoint)	I	NCT01625286
Postmenopausal, estrogen receptor-positive Stage IV BC	BKM120 (PI3K inhibitor) + fulvestrant	Patients are evaluated for <i>PIK3CA</i> mutation, which will be correlated with treatment response (tertiary endpoint)	I	NCT01339442
mBC for whom treatment with capecitabine is a reasonable choice	BKM120 (PI3K inhibitor) or BEZ235 (PI3K/mTOR inhibitor) + capecitabine	Primary tissue is evaluated for predictive biomarkers of response (PI3K activating mutations, pAkt, mTOR) (exploratory objective)	I	NCT01300962
Postmenopausal, estrogen receptor-positive, locally advanced or mBC whose tumors have a <i>PIK3CA</i> alteration	BYL719 (PI3K α inhibitor) + fulvestrant	<i>PIK3CA</i> mutation is required for study enrollment	I	NCT01219699
Estrogen receptor-positive/HER2- negative mBC, with histologic grade 2 or 3 and Ki67 15%	Ridaforolimus $\pm$ dalotuzumab, or dalotuzumab alone	Patients are assessed for mean change from baseline in Growth Factor Signature* score (primary endpoint)	I	NCT01220570
In the dose-expansion phase, patients with mBC that have received a maximum of 3 lines of therapy	MK-2206 (Akt inhibitor) + paclitaxel	<i>PIK3CA</i> and PTEN status will be measured in the primary tumor and in metastases, and correlated with clinical response (secondary endpoint)	I	NCT01263145
In the dose-expansion phase, patients with HER2+ advanced BC	MK-2206 (Akt inhibitor) + lapatinib	Tumor tissue oncogenic status will be assessed and correlated with clinical response (exploratory endpoint)	I	NCT01245205
ER+/HER2- BC	BKM120 (PI3K inhibitor) or BEZ235 (PI3K/mTOR inhibitor)	Tumor status for <i>PIK3CA</i> mutation, <i>PTEN</i> mutation, Akt, pAkt, mTOR, p110, S6K, 4E-BP1, hormone receptors, and IGF1R before and after treatment (primary objective). Correlation between tumor markers and response (secondary objective)	0	NCT01513356

Source: Clinicaltrials.gov (accessed October 2012).

BC, breast cancer; EGFR, epidermal growth factor receptor; ER, estrogen receptor; HER, human epidermal growth factor receptor; HR, hormone receptor; IGF1-R, insulin-like growth factor 1; mBC, metastatic breast cancer; mTOR, mammalian target of rapamycin; PD, pharmacodynamics; PTEN, phosphatase and tensin homolog.

* Growth Factor Signature score represents a gene signature that is responsive to alterations in the PI3K/Akt/mTOR pathway⁵⁸.