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# Phase II trial of temsirolimus in patients with metastatic breast cancer

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## Abstract

Preclinical models suggested that activating mutations of the *PIK3CA* gene are associated with sensitivity to inhibitors of the mammalian target of rapamycin (mTOR). In breast cancers, *PIK3CA* mutations are associated with estrogen receptor (ER) positivity. We therefore performed an open-label single arm phase II study of the rapamycin analog, temsirolimus, at a dose of 25 mg weekly, in women with pretreated breast cancers that were positive for ER, PR, or HER2. Archived formalin-fixed paraffin embedded tumor was collected for immunohistochemical evaluation of components of the PI3K/Akt/mTOR pathway and *PIK3CA* mutation analysis. Thirty-one patients were enrolled. There were no major objective responses; however, three patients had stable disease for over 24 weeks. Twenty-three tumor samples were available for mutational analysis. There were five tumors with *PIK3CA* mutations; no association was found between prolonged stable disease and *PIK3CA* mutation or any immunohistochemical marker. There was a trend toward improved progression free survival (PFS) for patients with positive nuclear staining for phospho-Akt308. One patient remains on study four and a half years after starting therapy; her tumor did not have a *PIK3CA* mutation. We conclude that single agent temsirolimus has minimal activity in a population of women with heavily pretreated breast cancer. We found no evidence that either absence of immunohistochemical staining for PTEN or mutations in the hotspot domains of *PIK3CA* in the primary tumor were associated with clinical benefit.

### Keywords

Rapamycin; Temsirolimus; Breast cancer; *PIK3CA*; Treatment; PTEN

## Introduction

The PI3K/AKT/mTOR signaling pathway mediates key cellular processes, including cell growth, proliferation, and survival. Aberrations in this pathway are common in human cancers, including breast cancer, and a variety of drugs targeting components of this pathway are under development as anticancer agents. Temsirolimus is an inhibitor of the mammalian target of rapamycin (mTOR) that is FDA approved for the treatment of kidney cancer. In preclinical studies, temsirolimus inhibited the proliferation of breast cancer cell lines that were estrogen dependent, overexpressed human epidermal growth factor receptor 2 (HER2), or were phosphatase and tensin homolog (PTEN)-deficient, and inhibited growth of PTEN-deficient cells in a nude mouse xenograft model [1].

In 2005, Kang et al. [2] reported that activating mutations in the *PIK3CA* gene (which encodes the p110$\alpha$ catalytic subunit of PI3K) are oncogenic, and that the cancers developing after the introduction of such mutations are sensitive to rapamycin (sirolimus). Twenty to 40% of primary breast cancers harbor mutations in *PIK3CA*. The majority are clustered at two hotspot regions in exon 9 (helical domain; E542K and E545K) and exon 20 (kinase domain; H1047R and H1047L). Initial reports suggested that these mutations are associated with estrogen receptor (ER) positivity and a good prognosis [3, 4] and that, in breast cancers, PTEN loss and *PIK3CA* mutation are usually mutually exclusive [5]. Preclinical

studies also suggested that *PIK3CA* mutations are associated with sensitivity to PI3K/AKT/mTOR inhibitors [6].

No marker for sensitivity to rapamycin or other agents targeting the PI3K/AKT/mTOR pathway has been confirmed in the clinical setting. In a multicenter European phase II study published in 2005, 108 breast cancer patients who had received one or two prior chemotherapy regimens for metastatic disease were randomly assigned to therapy with temsirolimus at 75 or 250 mg/m$^2$ weekly. An overall objective response rate of 9.2% with a clinical benefit rate of 13.8% [complete response (CR) plus partial response (PR) plus stable disease (SD) for at least 24 weeks] was reported. The median time to tumor progression was 12 weeks, and there was no difference in efficacy between the two arms [7]. An assessment of molecular characteristics was performed on a subset of 28 tumors from patients enrolled in the study. Three of the four tumors staining negative for PTEN and two of the three HER2 positive tumors were noted to have had an objective response to therapy [8]. A randomized phase II study of two different doses (10 mg daily vs. 70 mg weekly) of the oral mTOR inhibitor, everolimus, in patients with minimally pretreated (0–1 chemotherapy regimens) metastatic breast cancer reported a response rate of 12% to daily therapy and 0% to weekly therapy. One patient remained on study for 23 months. PTEN expression and pAKT were examined on archival tissue specimens; no response between these markers and efficacy was found [9]. *PIK3CA* mutations were not examined.

We hypothesized that breast cancers with a *PIK3CA* mutation would be more sensitive to therapy with an mTOR inhibitor than tumors not containing a *PIK3CA* mutation. To test this hypothesis, we designed a phase II trial of temsirolimus limited to patients whose primary breast cancer expressed the ER or the progesterone receptor (PR) or was HER2 positive. We hoped to enrich for *PIK3CA* mutations. Tumor blocks were requested for determination of *PIK3CA* mutations and for immunohistochemical staining for components of the PI3K/AKT/mTOR pathway, including PTEN.

## Patient population and methods

### Patients

Eligible patients were required to be at least 18 years of age and have measurable metastatic or recurrent breast cancer. The primary tumor or a metastatic lesion had to be positive for either ER or PR (≥1% by immunohistochemical staining) or HER2 [3+ by immunohistochemical staining or positive by fluorescent in situ hybridization (FISH)]. There were no limitations on the number of prior therapy regimens allowed, but prior exposure to mTOR inhibitors was prohibited. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 and, after the first 6 months of the trial, albumin of at least 3.3 mg/dl were required (albumin was initially required to be ≥3.0 mg/dl). Other laboratory requirements were a baseline absolute neutrophil count (ANC) of at least 1,500/μl, platelets of at least 100,000/μl, bilirubin less than institutional upper limits of normal, cholesterol ≤350 mg/dl, and triglycerides ≤400 mg/dl. Concomitant long-term steroid use was prohibited, as were strong Cytochrome P450 3A4 (CYP3A4) inducers. The protocol was approved by the institutional review board of both participating institutions, and all subjects signed an approved written informed consent document prior to enrollment.

### Study conduct

The protocol was conducted at two sites, the University of Chicago and Washington University. Temsirolimus was supplied as commercially labeled kits by the pharmaceutical management branch of the National Cancer Institute and was administered at a dose of 25 mg intravenously every week after premedication with diphenhydramine. Doses were held

for a platelet count of <100,000/μl or an ANC of <1,500/μl, and reduced in 5 mg increments for subsequent cycles. Patients who missed more than two successive doses for reasons of toxicity were removed from study. Hyperglycemia and lipid elevations did not necessarily mandate dose reduction, but could be treated with glucose-and lipid-lowering medications. A complete blood count was performed weekly; serum chemistries were performed every 2 weeks, and cholesterol and triglycerides were checked every 4 weeks. Four weeks were considered one cycle. Tumor measurements were repeated every 8 weeks. Overall survival was not an endpoint, and patients were not followed for survival. Response evaluation criteria in solid tumors (RECIST) criteria version 1.0 was used for response evaluation.

## Correlative studies

A formalin-fixed paraffin embedded (FFPE) tumor block or unstained slides were obtained from the primary tumor. Immunohistochemical staining was performed at the University of Chicago Core Facility for: Cyclin D1, PTEN, AKT, pAKT (Thr308), pAKT (Ser473), SGK-1, pSGK-1 (Ser422), mTOR, p-mTOR, and PDK1. The histological slides from 25 cases were deparaffinized by two xylene rinses followed by two rinses with 100% ethanol. Antigen retrieval was performed by heating the slides in a water bath filled with 7.5 mM sodium citrate (pH 6). After rinsing in 2× Tris-buffered saline (TBS) at pH 8, slides were incubated for 30 min in 3% hydrogen peroxide in methanol to block endogenous peroxidase activity. Slides were then incubated with 0.3% bovine serum albumin in 1× TBS for 30 min at room temperature to reduce non-specific background staining and then subjected to three washes in TBS containing 0.01% Triton. Slides were incubated for 1 h at room temperature with each of the primary antibodies listed in the table below. After TBS rinses slides were incubated for 30 min with goat anti-rabbit or anti-mouse IgG conjugated to a horseradish peroxidase-labeled polymer (Envision+System, DAKO, Carpinteria, CA), or with high sensitivity Immunodetector HRP/DAB avidin–biotin (ABC) kit (BioSB, Santa Barbara, CA) in case of PTEN antibody. This incubation was followed by TBS rinses, visualization with diaminobensidine chromogen (DAKO, Carpinteria, CA), and then counterstained with hematoxylin. For positive controls, we used various tissues according to manufacturer's recommendations, and negative controls were prepared by omitting the primary antibody step and substituting it with non-immune rabbit or mouse serum. A summary of primary antibodies and immunohistochemical methods follows:

All of the slides were jointly reviewed by two pathologists (Husain Sattar and Maria Tretiakova); cytoplasmic and nuclear staining was scored separately for normal, DCIS, and invasive carcinoma areas if present. Staining of >5% of the cells was considered positive. Staining intensity in positive cases was graded on a scale of 1–3, where a staining intensity of 3 was considered the strongest.

*PIK3CA* sequencing analysis was performed at Washington University. Tumor FFPE DNA samples were isolated using DNeasy Blood/Tissue Kit (Qiagen); instructions were modified to use prolonged overnight protease K digestion. After being matched with an adjacently cut hematoxylin and eosin (H&E) stained FFPE slide, the areas rich in tumor cells were scraped off from a non-HE stained FFPE slide. The tumor cellularity reached 70% and above for the purpose of somatic tumor DNA isolation and mutational analysis. The known regions of frequent mutations, exon 9 and exon 20, of *PIK3CA* were subjected to genomic polymerase chain reaction (PCR) and direct nucleotide sequence analysis. PCR was carried out and followed by either gel purification (Gel Extraction Kit, Qiagen) or enzymatic purification (ExoSAP-IT, Affymetrix-USB) of PCR products followed by sequencing cycling (BigDye Terminator v. 3.1, Applied Biosystems, ABI) of the PCR products using nested primers. The raw sequencing data were collected by a 3730 capillary DNA analyzer (ABI). The mutational analysis was completed by utilizing both the chromatogram viewer, Finch TV 1.4 (PerkinElmer-Geospiza) and the analytical software, Mutation Surveyor v.3.01

(Softgenetics). The PCR primers for exon 9 were 5-ctgtaaatcatctgtgaatccagag-3′, forward, and 5-ctgctttatttattccaatagg-3′, reverse. The PCR primers for exon 20 were 5-ctattcgacagcatgccaatc-3′, forward, and 5-tgtggaatccagagtgagctttc-3′, reverse. The related and nested sequencing primers for the products of exon 9 were 5-ccagaggggaaaaatatgac-3′ and 5-ccaataggtatggtaaaaacatgc-3′; and, for the products of exon 20 were 5-gccaatctcttcataaatcttttctc-3′ and 5-ccagagtgagctttcattttctca-3′. Taq LA DNA Polymerase (Sigma-Aldrich) was used for the PCR reaction. The annealing temperatures for the PCR program were 63°C for the first five cycles after initial denaturing and then 58°C for rest of 35 cycles in the PCR thermal cycler (iCycler, Bio-Rad).

### Statistical design

The primary endpoint of the trial was clinical benefit rate, defined as complete response (CR) plus partial response (PR) plus stable disease for at least 24 weeks (i.e., must not have progressed at the 24 week evaluation). A clinical benefit rate of 28% would indicate that further study was warranted. A Simon's two-stage design was used. Thirty-one patients were to be enrolled in the first stage. If four or fewer patients with clinical benefit were observed, the trial would terminate. If five or more patients with clinical benefit were observed, an additional 27 patients would be enrolled, with the agent considered promising if at least 12 total patients had clinical benefit. In addition, if, after the first stage, there was any evidence that responses/clinical benefit were limited to patients with tumors that exhibited *PIK3CA* mutations or PTEN deficiency, the trial would be re-opened only to subjects whose tumors had those characteristics. Response status was to be correlated with immunohistochemical and mutation results using Fisher's exact test; given the multiplicity of markers tested, these analyses were exploratory.

## Results

Thirty-one patients were enrolled between June 20, 2006, and August 9, 2007. Patient characteristics are shown in Table 1. Patients had received a median of four prior chemotherapy regimens. A median of two cycles were administered, with a range of 1–57. No major objective responses were observed. The median time to progression was 7.9 weeks (95% CI 6–15 weeks). Three patients (9.7%) had stable disease for more than 24 weeks. Two patients with stable disease for more than 24 weeks had ER positive disease; all three had HER2 negative disease. Seven other patients had stable disease for at least 16 weeks. One patient achieving stable disease remains on treatment at four and a half years after starting drug. She had received two prior chemotherapy regimens. Her tumor was ER positive, PR positive, HER2 negative, *PIK3CA* wild-type, with no PTEN nuclear or cytoplasmic staining, and 2+ phosphoAkt308 nuclear staining.

The adverse event profile was compatible with that reported in other trials of therapy with single agent temsirolimus. The most common adverse events of any grade considered at least possibly related to temsirolimus included fatigue ($n = 20$), anemia ($n = 16$), thrombocytopenia ($n = 16$), transaminitis ($n = 15$), and hypercholesterolemia ($n = 14$). Adverse events considered at least possibly related to temsirolimus and occurring in at least six participants are shown in Table 2.

Twenty-three and 24 patients, respectively, had tissue available for mutation and IHC analysis; in six patients not all the immunohistochemical stains could be completed because there was not sufficient tissue. Five patients had tumors with *PIK3CA* mutations, including two of the four patients with invasive lobular carcinomas. *PIK3CA* mutation status was not associated with progression free survival (PFS); the median PFS was 6.6 weeks for patients whose tumor had a mutation and 7.9 weeks for those whose tumor did not. Of the three patients with prolonged SD, one had a *PIK3CA* mutation. The tumor of the patient still

receiving treatment at 4½ years did not have a *PIK3CA* mutation although it was negative for PTEN expression. Neither HER2 status nor PTEN staining were associated with PFS. Nuclear pAkt308 expression was associated with prolonged PFS ($P = 0.03$). Characteristics of the tumors with *PIK3CA* mutations are shown in Table 3; relationships of the immunohistochemical markers to PFS are shown in Table 4.

## Discussion

We found no evidence that *PIK3CA* mutation status is associated with response to temsirolimus. Our findings may be falsely negative, either because our sample size of tumors with a mutation was too small and the overall activity of single agent temsirolimus in breast cancer too slight, because *PIK3CA* status in the primary tumor does not reflect mutation status in metastatic disease, or because heavily pretreated tumors become resistant despite a mutation. However, it is possible that *PIK3CA* status is, in fact, not necessarily associated with response to mTOR inhibitors, and that some other overall marker of PI3K pathway activation, whether primary or secondary activation, will be predictive.

Was our sample size simply too small? This problem is exacerbated since there may be different biologic implications of exon 9 (helical domain) and exon 20 (kinase domain) mutations. Barbareschi et al. [10] published that exon 9 mutations occurred more frequently in lobular carcinomas and were associated with a significantly worse prognosis, whereas exon 20 mutations were associated with a good prognosis. Baselga et al. [11] performed a randomized phase II study of neoadjuvant letrozole with or without everolimus, and published that tumors with an exon 9 mutation showed a poor response to letrozole alone, but a good response to letrozole plus everolimus. Tumors with an exon 20 mutation did not appear to benefit more from everolimus than patients with wild-type *PIK3CA*. However, while our numbers are small, they do not appear to suggest any increased sensitivity to mTOR inhibitor therapy for tumors with an exon 9 (helical domain) mutation, and the one patient with a very prolonged benefit from temsirolimus had no *PIK3CA* mutation.

Were our patients too heavily pretreated, or our dose not optimal? We allowed heavily pretreated patients on our study because we hypothesized that if *PIK3CA* alterations were indeed a driving mutation, efficacy of agents abrogating the signaling should be preserved even in the setting of prior therapy. This concept is supported by a recent publication that analyzed results of patients with diverse tumor types treated on phase I trials (hence presumably heavily pretreated) with agents targeting the PI3K/AKT/mTOR pathway. Six of 17 (35%) patients with documented *PIK3CA* mutations had a partial response versus 15 of 241 (6%) patients with no documented mutation (either no mutation found or no tissue available) [12]. However, data recently published suggest that single agent temsirolimus is markedly less active in women with endometrial carcinoma who have received prior chemotherapy [13]. We selected a dose of 25 $mg/m^2$ as it was the approved dose for renal carcinoma, and a dose shown to have efficacy in the treatment of endometrial carcinoma. Some other trials have used higher doses, and neither the optimal biologic dose nor the optimal schedule for mTOR inhibitor therapies is known.

Might there be discordance between *PIK3CA* status in the primary tumor and at metastatic sites? Dupont Jensen et al. [14] reported that in a study of tumors from 100 breast cancer patients with paired primary and asynchronous metastatic samples, 33 changed *PIK3CA* genotype: 21 from wild type to mutant, and 11 from mutant to wild type. Similarly, Gonzalez-Angulo et al. reported that 18% of matched primary tumors and metastases were discordant for *PIK3CA* mutation status (8% gain and 10% loss), and 26% were discordant for PTEN status. However, biopsy of a metastatic site might not result in better predictive power. Metastases themselves might be discordant; in the report of Gonzalez-Angulo et al.

[15] there were two cases in which more than one metastasis was biopsied and in both of these the two metastatic sites were discordant for PTEN status, although concordant for *PIK3CA* status.

Is the preclinical data on *PIK3CA* mutation and response to mTOR inhibition consistent? Resistance to endocrine therapy would be expected with PI3K pathway activation, but a recent analysis of samples from four clinical trials showed that *PIK3CA* mutations are only weakly negatively associated with response to neoadjuvant endocrine therapy [16]. Earlier preclinical data showed that cell lines with PTEN deficiency were inhibited by agents targeting mTOR [17–19]. Recent data on molecular determinants of response to mTOR inhibitors and other inhibitors of the PI3K/Akt/mTOR pathway have been mixed. Stemke-Hale et al. [20] examined 12 ER-positive breast cancer cell lines and found that loss of PTEN expression predicted for greater response to PI3K inhibitors than *PIK3CA* mutations. Dan et al. [21] examined a panel of 39 human cancer cell lines derived from various anatomical sites, and found that the efficacy of 25 PI3K pathway inhibitors did not correlate with either gain-of-function mutations of *PIK3CA* or PTEN loss in, but rather with phospho-Akt and *KRAS/BRAF* mutations. Di Nicolantonio et al. [22] reported that *PIK3CA* mutations predicted for sensitivity to everolimus in glioblastoma, breast, ovarian, prostate, endometrial and colorectal cancer cells except when *KRAS* mutations occurred concomitantly. The PI3K/mTOR dual inhibitor NVP-BEZ235 has been reported to selectively induce cell death in breast cancer cells harboring *PIK3CA* mutations, but not in cells lacking PTEN function [23, 24]. This is in agreement with data published by Weiglet et al. [25] that breast cancer cell lines with *PIK3CA* mutations are sensitive to mTOR inhibitors, but cells with PTEN loss of function are not, and with data from O'Brien et al. [26] that suggest breast cancer cell lines that amplify HER2 or have *PIK3CA* mutations, but not those with PTEN deficiency, are sensitive to PI3K pathway inhibitors. They also identified some sensitive cell lines with neither HER2 amplification nor *PIK3CA* mutations. Noh et al. found that phosphorylated Akt was associated with rapamycin sensitivity independently of PTEN status, suggesting that general pathway activation rather than a specific alteration might be important. In this context, our finding of a trend toward improved progression survival for women whose tumors had increased nuclear staining for phosphoAkt308 is interesting, but will require confirmatory studies. We did not see a correlation with immunodetection of phospho-Akt473. Marty et al. [27] suggested that stathmin protein might represent a marker for PTEN-dependent PI3K pathway activation, and this might provide a more robust readout than staining for phospho proteins, as performance of phospho-antibodies on surgical FFPE specimens may not be reliable.

Are there biologic reasons that resistance develops quickly to single agent mTOR inhibitors in most tumors, despite pathway activation? It has been described that rapamycin and its analogs, such as temsirolimus, suppress mammalian target of rapamycin complex 1 (mTORC1) but do not acutely suppress mammalian target of rapamycin complex 2 (mTORC2). Feedback activation of PI3K and AKT is believed to possibly limit the efficacy of these compounds [28], and newer agents that inhibit mTORC1 as well as mTORC2 and other components of the pathway are currently under development. Another approach is to try to interrupt the feedback loop upregulating pAKT after mTOR inhibition. This upregulation may be partially moderated by the insulin-like growth factor receptor (IGF-1R), and we are currently conducting a trial of the combination of temsirolimus and an anti-IGF-1R monoclonal antibody in women with metastatic breast cancer.

Current data suggest that mTOR inhibitors will need to be used in combination for the treatment of breast cancer. Preclinical data show that mTOR pathway activation increases resistance to chemotherapy, trastuzumab, and hormonal therapies [19, 29, 30], and recent trials have shown that combining an mTOR inhibitor with trastuzumab or hormonal therapy

may be able to reverse this resistance [31–34]. Possibly, at least for hormonal therapy, this may be particularly relevant to acquired resistance. A large randomized phase III trial of letrozole alone versus letrozole plus oral temsirolimus was terminated because of lack of benefit with the combination [35], whereas several trials performed in the setting of acquired hormone resistance, including a randomized trial of everolimus plus exemestane versus everolimus alone (BOLERO-2) have preliminarily reported very promising results. However, all studies report that the addition of mTOR inhibitor therapy to other agents adds significant toxicity, and a marker predicting which patients will benefit from such a combination would be of great value.

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## Abbreviations

| | |
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| **PIK3CA** | Gene encoding phosphoinositide-3-kinase, catalytic subunit |
| **mTOR** | Mammalian target of rapamycin |
| **mTORC1** | Mammalian target of rapamycin complex 1 |
| **mTORC2** | Mammalian target of rapamycin complex 2 |
| **ER** | Estrogen receptor |
| **PR** | Progesterone receptor |
| **HER2** | Human epidermal growth factor receptor 2 |
| **ECOG** | Eastern Cooperative Oncology Group |
| **ANC** | Absolute neutrophil count |
| **CYP3A4** | Cytochrome P450 3A4 |
| **RECIST** | Response evaluation criteria in solid tumors |
| **FFPE** | Formalin-fixed paraffin embedded |
| **PTEN** | Phosphatase and tensin homolog |
| **SGK** | Serum and glucocorticoid-induced protein kinase |
| **PDK** | Pyruvate dehydrogenase kinase |
| **TBS** | Tris-buffered saline |
| **H&E** | Hematoxylin and eosin |
| **PCR** | Polymerase chain reaction |
| **CR** | Complete response |
| **PR** | Partial response |
| **IHC** | Immunohistochemistry |
| **PFS** | Progression free survival |
| **IGF-1R** | Insulin-like growth factor receptor |
| **FISH** | Fluorescent in situ hybridization |
| **Ig** | Immunoglobulin |

**NA** Not available

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| Antibody | Source | Catalog# | Type | Dilution | Pretreatment |
|---|---|---|---|---|---|
| Akt | Cell signaling | 4685 | Rabbit | 1:50 | WB-citrate * |
| Akt-phospho (308) | Abcam | ab38449 | Rabbit | 1:100 | WB-citrate |
| Akt-phospho (473) | Cell signaling | 3787 | Rabbit | 1:50 | WB-citrate |
| Cyclin D1 | DAKO | N1619 | Mouse | 1:25 | WB-citrate |
| mTOR | Abcam | ab32028 | Rabbit | 1:50 | WB-citrate |
| mTOR-phospho | Abcam | ab51044 | Rabbit | 1:100 | WB-citrate |
| PDK1 | Abcam | ab31406 | Rabbit | 1:100 | WB-citrate |
| PTEN | Cascade | ABM-2052 | Mouse | 1:100 | WB-BioSci |
| SGK1-C terminal | Enzo | NA | Rabbit | 1:150 | WB-citrate |
| SGK-phospho | Upstate | 36-002 | Rabbit | 1:150 | WB-citrate |

* WB-water bath

**Table 1**

Patient characteristics

| | ***n*** |
|---|---|
| Total Enrolled | 31 |
| No. prior chemotherapy regimens | |
| Median (range) | 4 (1–9) |
| ECOG performance status | |
| 0 | 15 |
| 1 | 16 |
| Histologic subtype | |
| Ductal | 21 |
| Lobular | 5 |
| Not specified/other | 5 |
| Receptor status | |
| ER or PR+ and HER2− * | 21 |
| ER or PR+ and HER2+ | 4 |
| ER and PR− and HER2+ | 6 |

* Two patients had unknown HER2 status

## Table 2

Adverse events at least possibly related to temsirolimus and occurring in at least six patients

| Grade | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| ANC | 2 | 6 | 2 | – |
| Lymphocytes | 4 | 5 | 7 | – |
| Plt | 10 | 3 | 2 | 1 |
| Hb | 13 | 2 | 1 | – |
| ALT | 10 | 1 | 1 | – |
| AST | 10 | 4 | 1 | – |
| ↑Glucose | 4 | 2 | – | 2 |
| ↑Triglycerides | 7 | 2 | 1 | – |
| ↑Cholesterol | 11 | 3 | – | – |
| Nausea | 8 | 4 | – | – |
| Mucositis | 6 | 5 | – | – |
| Anorexia | 5 | 4 | 1 | – |
| Fatigue | 9 | 9 | 1 | 1 |
| Rash | 8 | 3 | – | – |

**Table 3**

Tumor characteristics and relationship to PFS

| | No of patients | Median PFS | *P* Value |
|---|---|---|---|
| ER | | | |
| − | 10 | 7.9 | |
| + | 21 | 7.7 | 0.71 |
| PR | | | |
| − | 12 | 7.9 | |
| + | 19 | 7.7 | 0.79 |
| HER2 | | | |
| − | 21 | 8 | |
| + | 10 | 7 | 0.36 |
| Dermal lymphatic invasion | | | |
| − | 9 | 7 | |
| + | 5 | 16.3 | 0.045 |
| *PIK3CA* mutation | | | |
| No | 19 | 7.9 | |
| Yes | 5 | 6.6 | 0.61 |
| pSGK1 cytoplasm | | | |
| − | 1 | NA | |
| 1+ | 9 | 7.1 | |
| 2+ | 3 | 7.9 | |
| 3+ | 7 | 15.1 | 0.73 |
| pSGK1 nuclear | | | |
| − | 2 | 7.7 | |
| 1+ | 3 | 24 | |
| 2+ | 5 | 5.3 | |
| 3+ | 10 | 7.1 | 0.56 |
| PDK1 cytoplasm | | | |
| − | 1 | NA | |
| 1+ | 3 | 5.1 | |
| 2+ | 8 | 16.3 | |
| 3+ | 8 | 7.9 | 0.68 |
| SGK1 cytoplasm | | | |
| − | 3 | 7 | |
| 1+ | 12 | 15.1 | |
| 2+ | 4 | 3.7 | 0.31 |
| SGK1 nuclear | | | |
| − | 1 | NA | |
| 1+ | 10 | 7.9 | |
| 2+ | 7 | 16.3 | |
| 3+ | 1 | NA | 0.73 |

| | No of patients | Median PFS | *P* Value |
|---|---|---|---|
| mTOR cytoplasm | | | |
| − | 2 | 5.1 | |
| 1+ | 6 | 5 | |
| 2+ | 8 | 8 | |
| 3+ | 4 | 5.3 | 0.99 |
| pMTOR cytoplasm | | | |
| − | 12 | 7.1 | |
| 1+ | 7 | 16.3 | |
| 2+ | 1 | NA | 0.07 |
| − | 12 | 7.1 | |
| Any + | 8 | 16.3 | 0.08 |
| pMTOR nuclear | | | |
| − | 9 | 16.3 | |
| 1+ | 5 | 7.9 | |
| 2+ | 5 | 5.1 | |
| 3+ | 1 | NA | 0.29 |
| PTEN cytoplasm | | | |
| − | 9 | 8 | |
| 1+ | 3 | 5.3 | |
| 2+ | 5 | 23 | |
| 3+ | 3 | 4 | 0.43 |
| − | 9 | 8 | |
| Any + | 11 | 7 | 0.38 |
| Cyclin D1 cytoplasm | | | |
| − | 7 | 7.7 | |
| 1+ | 8 | 8 | |
| 2+ | 5 | 16.3 | 0.83 |
| Cyclin D1 nuclear | | | |
| − | 5 | 7 | |
| 1+ | 3 | 7.7 | |
| 2+ | 9 | 23 | |
| 3+ | 3 | 8 | 0.61 |
| AKT, pan cytoplasm | | | |
| − | 8 | 7 | |
| 1+ | 7 | 15.1 | |
| 2+ | 3 | 23 | |
| 3+ | 2 | 3.7 | 0.56 |
| pAKT 308 cytoplasm | | | |
| − | 11 | 8 | |
| 1+ | 6 | 7.9 | |
| 2+ | 3 | 5 | 0.74 |
| pAKT 308 nuclear | | | |

| | No of patients | Median PFS | *P* Value |
|---|---|---|---|
| − | 4 | 5.1 | |
| 1+ | 8 | 7.1 | |
| 2+ | 6 | 23 | |
| 3+ | 2 | 15.1 | 0.08 |
| − | 4 | 5.1 | |
| Any + | 16 | 15.1 | 0.02 |
| pAKT 473 cytoplasm | | | |
| − | 16 | 7.9 | |
| 1+ | 2 | 3.7 | |
| 2+ | 1 | NA | 0.95 |
| pAKT 473 nuclear | | | |
| − | 15 | 7.9 | |
| 1+ | 5 | 23 | 0.89 |

* *P* values calculated from log-rank test

## Table 4

Characteristics of tumors with *PIK3CA* mutations

| Pts with *PIK3CA* mutation | Domain mutated | Her2/Neu | ER/PR | Weeks of TEM received | IHC results (0–3) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | PTEN cyto | pmTOR cyto/nuc | pAKT 308 cyto/nuc | pAKT 473 cyto/nuc | PDK1 cyto |
| #9 | H domain | – | +/+ | 6 | NA | NA | NA | NA | NA |
| #22 | H domain | – | +/− | 5 | 1 | 0/0 | 0/0 | NA | 0 |
| #29 | H domain | – | +/− | 34 | 2 | 0/1 | 1/1 | 0/0 | 2 |
| #11 | H & K domain | – | +/+ | 2 | 2 | 1/0 | 2/0 | 0/0 | 1 |
| #21 | K domain | – | +/− | 16 | 3 | 1/0 | 1/3 | 0/0 | 3 |