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A Phase I Study of Everolimus and Docetaxel in Patients With Castration-Resistant Prostate Cancer

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Abstract

Activation of the phosphoinositide 3-kinase signaling cascade, often through loss of the phosphatase and tensin homologue deleted on chromosome 10 (PTEN) tumor suppressor, is frequent in castration-resistant prostate cancer (CRPC). We assessed the safety and efficacy of combining the mammalian target of rapamycin (mTOR) inhibitor everolimus with docetaxel in a phase I clinical trial of men with metastatic CRPC, and evaluated the ability of fluorine–18-fluorodeoxyglucose (FDG) positron emission tomography (PET) to predict response to treatment. The observed clinical activity of tolerable dose levels of everolimus with docetaxel was low. FDG-PET might serve as a biomarker for target inhibition by mTOR inhibitors in metastatic CRPC.

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This trial is registered at clinicaltrials.gov (NCT00459186).

Disclosure

MET, WKO, TMB have attended advisory boards and educational meetings sponsored by Sanofi Aventis.

Background—The PTEN tumor suppressor is frequently lost in CRPC, with activation of Akt-mTOR signaling, driving growth. We conducted a phase I trial of the mTOR inhibitor, everolimus, and docetaxel in CRPC.

Patients and Methods—Eligible patients had progressive, metastatic, chemotherapy-naive CRPC. Patients received everolimus 10 mg daily for 2 weeks and underwent a restaging FDG-PET/computed tomography scan. Patient cohorts were subsequently treated at 3 dose levels of everolimus with docetaxel: 5 mg to 60 mg/m^2, 10 mg to 60 mg/m^2, and 10 mg to 70 mg/m^2. The primary end point was the safety and tolerability of combination therapy.

Results—Accrual was 4 patients at level 1, 3 patients at level 2, and 8 patients at level 3. Common toxicities were hematologic and fatigue. Serum concentrations of everolimus when administered with docetaxel were 1.5 to 14.8 ng/mL in patients receiving 5 mg everolimus and 4.5 to 55.4 ng/mL in patients receiving 10 mg everolimus. Four patients had partial metabolic response (PMR) using FDG-PET, 12 had stable metabolic disease, and 2 had progressive metabolic disease after a 2-week treatment with everolimus alone. Five of 12 evaluable patients experienced a prostate-specific antigen (PSA) reduction ≥ 50% during treatment with everolimus together with docetaxel. All 4 patients with a PMR according to PET imaging experienced a PSA reduction in response to everolimus with docetaxel, and 3 of 4 had PSA declines ≥ 50%.

Conclusion—Everolimus 10 mg daily and docetaxel 60 mg/m^2 was safe in CRPC patients and these were the recommended doses in combination. FDG-PET response might serve as a biomarker for target inhibition by mTOR inhibitors.

Keywords

mTOR; PI3K; Positron emission tomography; Prostatic adenocarcinoma; PTEN

Introduction

Recent advances in treatment of metastatic castration-resistant prostate cancer (CRPC) have resulted in only modest improvements in overall survival. There remains a pressing need for novel therapies that exploit molecular growth pathways. The phosphatase and tensin homologue deleted on chromosome 10 (PTEN)/phosphoinositide 3-kinase (PI3K)/Akt signaling pathway is critical for the growth, proliferation, and survival of cancer cells and provides potentially relevant targets in CRPC.[1] PTEN is a lipid and protein phosphatase and tumor suppressor that antagonizes proliferative and survival signaling through PI3K. PTEN loss correlates with higher Gleason score and advanced stage, and PTEN inactivation occurs with increased frequency in metastases.[2,3] PTEN deletion is frequent in CRPC, in which biallelic loss correlates with worse disease-specific mortality.[4] Loss of PTEN lipid phosphatase activity results in activation of downstream effectors of PI3K signaling, including the serine-threonine kinases Akt and mammalian target of rapamycin (mTOR).[5,6] mTOR plays a central role in cell cycle regulation, protein translation, and energy homeostasis.[7]

The potential clinical utility of inhibiting mTOR in CRPC is supported by published reports of in vitro inhibition of prostate cancer growth by rapamycin and its derivatives.[8–11] Rapamycin, a macrolide antibiotic and a clinical immunosuppressant, exerts an

antiproliferative effect by inhibition of mTOR.[12,13] Everolimus (Afinitor, Novartis) is an orally bioavailable ester of rapamycin approved for treatment of metastatic renal cell carcinoma, pancreatic neuroendocrine tumors, and giant-cell astrocytomas associated with tuberous sclerosis.[14–16] Everolimus induces apoptosis of epithelial cells and completely reverses the neoplastic phenotype of mice expressing human Akt1 in the prostate.[17]

We predicted that everolimus alone would be unlikely to demonstrate clinical benefit in patients with metastatic CRPC. This prediction was supported by the low level of clinical activity observed in clinical trials of single-agent mTOR inhibitors in men with metastatic CRPC.[18–21] Everolimus failed to elicit prostate-specific antigen (PSA) or radiographic responses in men with chemorefractory metastatic CRPC in a phase II study.[18] A second phase II study evaluated the efficacy of everolimus in chemotherapy-naive subjects with metastatic CRPC with a primary end point of progression-free survival (PFS) at 12 weeks.[21] Of 37 recruited subjects, 13 (35%; 95% confidence interval [CI], 20–53) met the primary end point, but only 2 (5%) experienced PSA decline ≥ 50%.[21] PTEN loss was associated with response and longer PFS.[21] Additionally, a phase II trial of the mTORC1 inhibitor temsirolimus administered at 25 mg weekly in subjects with chemorefractory CRPC was stopped prematurely because of lack of efficacy/feasibility.[20] Preclinical data also supported the combined use of everolimus with docetaxel.[22] Androgen ablation combined with docetaxel induced PI3K/mTOR signaling in CRPC cell lines, providing impetus to assess whether concurrent inhibition of the PI3K/mTOR pathway can overcome docetaxel resistance.[23,24]

Our study evaluated the safety and tolerability of inhibition of mTOR with everolimus and tubulin depolymerization with docetaxel in metastatic CRPC. We explored the association between responses to baseline phosphorylation of the mTOR substrate ribosomal protein S6 and loss of PTEN expression in tumor samples. We also used fluorine–18-fluorodeoxyglucose (FDG) positron emission tomography (PET) imaging as a functional assessment of the pharmacodynamic effect of everolimus. Although FDG-PET has not been validated to assess treatment response in CRPC, mTOR signaling is linked to glucose uptake and regulation of glycolysis through Hif1α, and mTOR inhibition can impair tumor glycolysis.[25] We hypothesized that increased mTOR pathway activity would be reflected in increased glucose uptake and glycolysis in tumor cells, which would be impaired by suppression of mTOR activity with everolimus.[26]

Patients and Methods

Eligibility

Eligible patients were ≥ 18 years of age, with histologically proven prostatic adenocarcinoma. Patients had radiographic evidence of metastatic disease with subsequent progression of disease (biochemical or radiographic) with castrate levels of testosterone (testosterone < 50 ng/dL) during androgen deprivation therapy. Other eligibility criteria included a life expectancy of > 12 weeks, Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, ≥ 3 weeks since major surgery, ≥ 4 weeks since radiotherapy, ≥ 8 weeks since radiopharmaceutical treatment, and adequate bone marrow, liver, and renal function. Patients were ineligible if they had ongoing steroid use, Grade ≥ 2 peripheral

neuropathy, uncontrolled brain metastases, had received previous taxane therapy, chemotherapy for CRPC, mTOR inhibitors, or had uncontrolled medical conditions. The study was approved by the Scientific Review Committees and Institutional Review Boards at Dana-Farber Harvard Cancer Center and Oregon Health and Science University and informed written consent was obtained from patients. The trial was registered in clinicaltrials.gov (NCT00459186).

Drug Formulation and Administration

Everolimus was formulated as tablets of 5 mg, blister-packed under aluminum foil in units of 10 tablets and stored at temperatures < 30°C. The dose was 5 mg or 10 mg daily, as dictated by the dose level schema. Patients were instructed to take the drug fasting or after no more than a light, fat-free meal, in the morning. Patients kept treatment diaries to assess compliance, and unused tablets were returned and counted.

Docetaxel was administered intravenously every 21 days. The dose was dictated by the dose level schema. Participants received dexamethasone 8 mg orally (p.o.) 12 hours before, 1 hour before, and 12 hours after docetaxel. Prednisone was given at 5 mg p.o. twice per day starting on day 1 of cycle 1 of docetaxel/everolimus treatment.

Treatment and Dose Escalation

All patients had a baseline FDG-PET/computed tomography (CT) scan. Patients with positive FDG-PET scans were treated with everolimus (10 mg daily) only for 2 weeks. A follow-up FDG-PET/ CT scan was performed between days 10 and 14. On day 15, patients entered the phase I portion of the trial designed to evaluate the combination of everolimus and docetaxel with prednisone. Per the study protocol, patients with negative baseline FDG-PET scans would not receive treatment with everolimus alone and would proceed directly to treatment with everolimus with docetaxel. However, all enrolled patients had positive baseline FDG-PET scans. Standard cohorts of patients were treated at 3 escalating dose levels of everolimus with docetaxel: 5 mg/60 mg/m^2, 10 mg/60 mg/m^2, and 10 mg/70 mg/m^2, respectively, every 21 days with evaluation of dose-limiting toxicity (DLT) and pharmacokinetic levels.

Dose-Limiting Toxicity and Maximum Tolerated Dose

The DLT and maximum tolerated dose (MTD) were defined using the National Cancer Institute Common Toxicity Criteria version 3.0. DLT was defined as any of the following that were ascertained to be at least possibly drug-related: neutropenia Grade 4 for > 7 days; febrile neutropenia of Grade 3 or 4 (any duration); thrombocytopenia Grade 4 for > 7 days; or Grade 3 or 4 non-hematologic toxicity (excluding Grade 3 nausea, Grade 3/4 vomiting, or diarrhea in patients who had not received antiemetic or antidiarrhea agents, and Grade 3 alkaline phosphatase elevation). The MTD was defined as the dose below that at which > 33% (2 of up to 6 patients) of the patient population suffered a DLT due to the drug(s).

Patient Evaluation

Before enrollment and within 2 weeks before the initial pharmacokinetic measurement, each patient had a complete history and physical examination, electrocardiogram, chest x-ray,

urinalysis, and ECOG performance status assessment. Physical examination was performed at baseline and every 3 weeks. Laboratory investigations included complete blood count, electrolyte levels, and liver function tests. These were done weekly for the first cycle, and subsequently on day 1 of each 21-day cycle. Cholesterol and triglycerides were measured on day 1 of the first 3 cycles of combined treatment. PSA was measured at baseline and on day 1 of combined treatment and every 3 weeks. PSA progression was defined as a 25% increase over the baseline PSA (drawn on cycle 1 day 1 of the combination therapy portion of the trial), and an increase in the absolute value PSA level by at least 5 ng/mL, confirmed by a second increase in PSA level. Measurable lesions were documented using computed tomography and evaluable osseous lesions were documented using bone scan during screening and every 3 cycles thereafter. Lesions were measured according to Response Evaluation Criteria in Solid Tumors version 1.0 (RECIST). Progression on bone scan was defined as ≥ 1 new lesion.[27]

Statistical Methods

Design—The phase I component was designed using a standard dose escalation strategy using a 3 + 3 design. Using this dose escalation scheme, the probability of escalation was 91 % if the true DLT rate was 10%, 17% if the true DLT rate was 50%, and < 1% if the true DLT rate was ≥ 80%. The study was designed with a phase II component. However, this component was not executed because of the low level of clinical activity anticipated for the combination at the MTD, compared with single-agent docetaxel administered at the approved dose in this patient population.

Analysis—Data were summarized with respect to baseline characteristics, efficacy, safety, and pharmacokinetic measurements. Adverse events (AEs) were summarized by presenting, for each treatment group, the number or percentage of patients having any AE, having an AE in each body system, and having an AE for each individual. Ninety percent exact binomial confidence intervals were used to provide preliminary estimates of efficacy.

PET Imaging Correlative Analysis

Fluorine–18-fluorodeoxyglucose-PET/CT imaging was performed after the patients had fasted for at least 4 hours. Patients had blood glucose measurement and were given 10 to 20 mCi FDG intravenously. Scanning from the skull base to the proximal thighs was performed beginning 1 hour later, after emptying of the bladder. Helical CT imaging was performed over the same anatomic range of the PET scanning and CT-based attenuation correction was performed. Similar acquisition times relative to FDG injection times were used for the baseline and the day 10 to 14 scan.

A positive FDG-PET scan for the purposes of this study consisted of a visualized area of abnormal increased FDG uptake that matched the anatomic location of an abnormality seen on bone scan or CT imaging. Quantitative analysis was also performed using maximum standardized uptake value (SUVmax). Target lesions were identified on baseline FDG-PET images based on hypermetabolic uptake. Percent change in the summed SUVmax of target lesions was calculated at days 10 to 14, compared with baseline. Metabolic response was assessed for percent change in SUVmax according to the criteria of the European

Organization for Research and Treatment of Cancer[28]: partial metabolic response (PMR) ≥ −25%; stable metabolic disease ± 25%; and progressive metabolic disease ≥ 25%.[28] In addition, bone lesions were distinguished from soft tissue lesions. Descriptive statistics were used to characterize the percentage of patients with FDG-avid metastatic sites at baseline (measured using FDG-PET/CT scans) and the proportion of patients categorized by each metabolic response category after 10 to 14 days of everolimus therapy.

Pharmacokinetic Studies

Nonfasting blood samples were collected for everolimus blood levels on day 8 of cycle 1 and days 1 and 8 of cycles 2 and 3 during the everolimus with docetaxel treatment. In a phase I pharmacokinetic and pharmacodynamic study of everolimus in patients with advanced solid tumors, everolimus achieved peak serum concentrations within 1 hour of administration in most subjects, with a range of 1 to 6 hours, and maximum concentration was dose-proportional.[29] When possible, samples were drawn 3 to 5 hours after ingestion of everolimus. Analysis for everolimus was performed using Schimadzu Prominence high performance liquid chromatography pumps and autosampler on an AB SCIEX 4000 triple quad mass spectrometer using positive electrospray ionization. Samples were extracted using liquid–liquid extraction using 125 μL of whole blood sample. After evaporation of the extracts to dryness, extracts were reconstituted with 1 mL of 50/50 0.1% formic acid in water: 2 mM ammonium acetate in methanol before injection on a Waters Sunfire C18 3.5-μm 3.0 × 500-mm column operated at 50°C.

Biopsies: Prostate, Lymph Node, and Bone Biopsy

The first 11 participants enrolled in the study underwent tumor biopsies. Biopsies could be obtained from the prostate using ultrasound guidance if the patient had not received previous prostate radiation; from bone lesions using CT guidance; from bone marrow obtained from the iliac crest if a bone scan demonstrated cancer involvement; or from lymph nodes/other soft tissue using CT guidance. Eight bone biopsies, 1 lymph node biopsy, and 2 prostate biopsies were obtained before treatment. One patient underwent an optional follow-up bone biopsy after 2 weeks of everolimus treatment. The patients underwent standard preprocedural tests as defined in the study protocol to ensure safety. CT-guided bone biopsies or lymph node biopsies were performed on a spiral CT scanner with fluoroscopy capability (Siemens, Erlangen, Germany).

Histology and Immunohistochemistry

Tumor samples obtained from all biopsy specimens were assessed for PTEN and phosphorylation of the phosphorylated ribosomal protein S6 (phospho-S6). Hematoxylin and eosin and immunohistochemical staining were performed on 5-μm sections of formalin-fixed and paraffin-embedded samples. The following primary antibodies were used: rabbit anti-PTEN at 1:200 dilution (Zymed Laboratories, Inc, South San Francisco, CA) and rabbit anti–phospho-S6 ribosomal protein (Ser 235/236) (Cell Signaling Technology, Inc, Danvers, MA) at 1:100 dilution. Tissue sections were deparaffinized, rehydrated, and subjected to microwaving in 10 mmol/L citrate buffer (pH 6.0) in a 750-W microwave oven for 15 minutes. The primary antibody was applied at room temperature in an automated tissue stainer (i6000, Biogenex, San Ramon, CA). Standardized 3,3-diaminobenzidine

development times allowed accurate comparison of all samples. Antibody isotype-specific immunoglobulin G served as a negative control. Scoring of metastatic cells was performed by assigning scores of 0 to 4 according to the percentage of positive tumor cells (1 = negative; 2 = 1%-10%; 3 = 11%-50%; and 4 = 51%-100% positive cells) and the intensity of staining (0 = negative; 1 = weak; 2 = moderate; 3 = strong). The 2 scores were multiplied to give an overall score of 0 to 12, where values < 6 were considered low, 6 intermediate, and > 6 strong staining.

Results

Nineteen patients were registered. Eighteen patients were treated; 1 patient was removed from the study before beginning treatment. All patients had received previous treatment with first-generation antiandrogen therapy. Most patients had received additional previous therapies for CRPC (Table 1). Three patients did not receive combination therapy: 2 experienced persistently increased transaminase levels, and 1 had hyperglycemia after treatment with everolimus alone. Of the remaining 15 patients, accrual was 4 patients at level 1, 3 patients at level 2, and 8 patients at level 3 (Figure 1). One patient at dose level 1 and 2 at dose level 3 were not evaluable for DLTs because they did not receive uninterrupted treatment with everolimus and docetaxel for at least 1 full cycle.

The most common toxicities were hematologic, fatigue, fever, diarrhea, nausea, back pain, and bone pain. The most frequent Grade 3 or 4 toxicities were hematologic and febrile neutropenia (Table 2). Among the 15 patients who received everolimus with docetaxel, 13 experienced Grade 3 or 4 AEs. Eleven patients experienced Grade 3 or 4 AEs (4 Grade 3, 7 Grade 4) that were considered at least possibly related to treatment. One of 6 patients experienced a DLT (febrile neutropenia) at dose level 3. Two additional patients started treatment with everolimus and docetaxel at dose level 3, but were not evaluable for DLTs because they did not receive everolimus and docetaxel for the full 21 days of cycle 1. Both of these patients would have met criteria for a DLT had they received treatment for the full 21 days of cycle 1, the former because of persistent Grade 4 neutropenia, the latter because of Grade 3 febrile neutropenia. For this reason, the investigators determined that the MTD was dose level 2.

In patients treated at dose level 1 (5 mg everolimus daily), serum everolimus concentrations were 1.5 to 8.6 ng/mL on day 8, 5.0 to 7.9 ng/mL on day 22, 5.9 to 7.5 ng/mL on day 29, 4.1 to 14.8 ng/mL on day 43, and 7.1 to 10.6 ng/mL on day 50. In patients treated at dose levels 2 and 3 (10 mg everolimus daily), serum everolimus concentrations were 9.8 to 32.0 ng/mL on day 8, 7.4 to 44.2 ng/mL on day 22, 7.8 to 55.4 ng/mL on day 29, 6.1 to 21.8 ng/mL on day 43, and 6.8 to 47.6 ng/mL on day 50. Some patients treated at dose levels 2 and 3 received reduced doses of everolimus at different times during their treatment.

Although patients with negative baseline FDG-PET scans were eligible for this study, every enrolled patient had FDG-PET– positive metastatic disease. All patients had FDG-PET/CT scans positive for metastatic disease to bone, and 13 patients also had quantifiable FDG-avid soft tissue lesions. FDG-PET/CT analysis was repeated after 10 to 14 days of treatment with everolimus 10 mg daily to assess for metabolic response to everolimus alone. Representative

images before and after everolimus treatment are shown for patient 005 (Figure 2A). Based on FDG-PET/CT scan evaluations, 4 patients (22%; 90% CI, 8%-44%) experienced a PMR to treatment with everolimus alone, with a decline of > 25% in summed SUVmax (Figure 2B and C). Two of these patients experienced responses in bone lesions and did not have measurable soft tissue disease according to FDG-PET. One patient had a PMR principally attributable to a decline in soft tissue FDG uptake, and 1 patient had a PMR in bone and soft tissue lesions. Twelve patients (67%; 90% CI, 45%-84%) had stable metabolic disease: 10 in bone and soft tissue, and 2 in bone without detectable soft tissue disease. Two patients (11%; 90% CI, 2%-31%) had disease progression (1 in bone, and 1 in bone and soft tissue).

Patients were assessed for PSA response to everolimus and docetaxel. Twelve patients received at least 2 cycles of combination therapy and were evaluable for PSA response. Of these 12 patients, 5 (42%; 90% CI, 18%-68%) experienced ≥ 50% decline from baseline PSA, and 4 of these (33%) were sustained for at least 2 consecutive measurements (Figure 3A and B).[27] All 4 patients who had a PMR according to FDG-PET scans after 2 weeks of everolimus alone subsequently experienced a PSA decline with everolimus and docetaxel treatment, with 3 having ≥ 50% PSA reduction (Figures 2C and 3A and B). Six patients remained in the study and experienced PSA declines from baseline at 12 weeks (Figure 3C). Of the 4 patients who achieved a PMR according to FDG-PET scans after everolimus alone, 1 experienced a partial response (PR) according to RECIST after everolimus with docetaxel treatment, 2 had stable disease (SD), and 1 had progressive disease (PD). Overall, 1 of 12 patients (8%; 90% CI, < 1% to 34%) experienced a radiographic PR, 5 (42%; 90% CI, 18%-68%) had SD, and 6 (50%; 90% CI, 25%-75%) had PD. The median length of treatment was 77 days (range, 13–407 days).

The first 11 patients who were enrolled underwent tumor biopsies to evaluate activation of PI3K/mTOR signaling. These samples were immunostained with antibodies against PTEN and phospho-S6 ribosomal protein, the latter as a surrogate for mTORC1 activity. Only 5 of 11 biopsies contained tumor evaluable for PTEN and phospho-S6. Three of 5 biopsies showed low or no PTEN expression, 1 showed intermediate expression, and 1 was strongly positive for PTEN. Adjacent sections of these samples were stained for phospho-S6. Phospho-S6 staining tended to inversely correlate with PTEN expression (Table 3). In addition, phospho-S6 expression was not detected after everolimus treatment in the only patient to undergo biopsies before and after treatment (Figure 4).

Discussion

This phase I study of everolimus and docetaxel with prednisone in men with CRPC showed the combination to have satisfactory safety and tolerability profile at 10 mg daily everolimus with docetaxel 60 mg/m^2 every 3 weeks, the second dose level evaluated. Grade 3 and 4 toxicities included primarily neutropenia, with or without signs of infection.

Serum everolimus concentrations were variable, particularly in patients treated at dose levels 2 and 3 of the study combination. The observed everolimus serum concentrations when administered in combination with docetaxel were lower than the maximum concentrations of everolimus after 5 mg and 10 mg daily dosing previously reported in a phase I study of

everolimus in solid tumors that demonstrated a pharmacodynamic effect of everolimus.[29,30] Although it is difficult to draw direct comparisons between the pharmacokinetic results in these different studies, it is possible that serum concentrations of everolimus when given in combination with docetaxel were insufficient to adequately inhibit mTOR. This might have contributed to the low response rates to combination therapy.

The overall PSA response (≥ 50% PSA decline) was 5 of 12 (42%). Only 1 of 12 evaluable patients (8%) experienced a radiographic response according to RECIST. That patient was treated at dose level 3, which exceeded the MTD for combination therapy. In phase III testing, the PSA response rate of docetaxel (75 mg/m^2 every 3 weeks) in CRPC was 45% (of 291 evaluable subjects), and 12% of 141 had responses in measurable disease. Although this phase I study was not designed to assess tumor response as a primary end point, we concluded that the combination of everolimus and docetaxel at the MTD was unlikely to have response rates significantly greater than responses reported for single-agent docetaxel in CRPC.[31] We therefore decided not to proceed with phase II testing of this combination in CRPC.

We examined whether response to treatment with everolimus alone according to FDG-PET would correlate with subsequent treatment response to combination everolimus and docetaxel. The predictive value of FDG-PET for tumor response to targeted therapies has varied in previous studies. In a study involving patients with gastrointestinal stromal tumors, FDG-PET metabolic response preceded anatomic response on CT scans and was associated with subsequent clinical responses to imatinib mesylate (Gleevec, Novartis).[32,33] However, a study of patients treated with rapamycin reported that early FDG-PET response was not predictive of clinical response to mTOR inhibitor therapy.[34] In the current study, only 4 of 18 (22%) patients demonstrated a PMR according to FDG-PET after single-agent everolimus treatment, raising the possibility that effective mTOR inhibition was achieved in only this minority of patients. Patients with a PMR to everolimus alone according to FDG-PET were more likely to have a subsequent PSA response to combination therapy than those without a metabolic response to single-agent everolimus treatment (3 of 4; 75% vs. 2 of 8; 25%), but our data are too limited to draw definitive conclusions about the predictive clinical value of FDG-PET response after mTOR target inhibition.

In this study we also attempted to assess whether tumor PTEN and phospho-S6 expression correlates with radiographic or clinical response to combination everolimus with docetaxel. The purpose of these biopsies was to assess the pharmacodynamic response of patients' tumors to everolimus treatment. The feasibility of this endeavor was tested in the phase I portion of this study. The planned phase II study was intended to provide an initial assessment of PTEN and phospho-S6 tumor expression as potential biomarkers for treatment response to mTOR inhibition. In the completed phase I portion of the study, only 5 of 11 biopsy samples were evaluable for PTEN and phospho-S6, and only 1 patient consented to follow-up biopsy. These results underscore the challenges of obtaining serial biopsies of prostate cancer metastases for correlative studies, but these types of analyses remain critical to understanding response and resistance to targeted therapies.

The PI3K/mTOR pathway has emerged as an attractive therapeutic target in a number of cancers. How to best target this pathway in CRPC remains an open question, and new data have emerged since we initiated this study that shed light on potential mechanisms of resistance to mTOR inhibition by rapamycin analogues. mTOR is present in 2 separate multimolecular complexes, mTORC1 and mTORC2. Rapamycin and its analogues are believed to preferentially inhibit mTORC1, which can result in loss of feedback inhibition on the PI3K pathway and subsequent Akt activation.[13,35,36] Moreover, this Akt activation during mTOR inhibition is tightly associated with development of cell resistance to mTOR inhibitors, and as a consequence can counteract the anticancer efficacy of these agents.[35] Importantly, however, Akt activation by mTOR inhibitors might lead to a critical dependence on the PI3K/Akt/mTOR signaling pathway, sensitizing tumors to either PI3K- or Akt-directed inhibition, or multitarget inhibition of PI3K (or Akt), and mTOR as an effective treatment strategy.[35,36] Adding further complexity, in some cancers, prolonged rapamycin treatment appears to block mTORC2 assembly and inhibit Akt, and in other cancers mTORC2 remains resistant to rapamycin treatment.[37] The activation of components of the mitogen activated protein kinase (MAPK) signaling cascade after PI3K/mTOR inhibition in certain cancer cells provides yet another mechanism of potential resistance to PI3K/mTOR-targeted therapies.[38] In mice with CRPC driven by the combined effects of PTEN loss and surgical castration, treatment with the dual PI3K/mTOR inhibitor NVP-BEZ-235 failed to abrogate tumor progression, but concurrent treatment with the mitogen activated protein kinase kinase inhibitor AZD6224 suppressed tumor growth.[39] There is also preclinical evidence that PI3K pathway inhibition in PTEN-deficient prostate cancer might activate signaling through the androgen receptor (AR), and that AR inhibition can activate Akt.[40,41] Although combining everolimus with bicalutamide showed limited activity in patients with metastatic CRPC, combining PI3K/mTOR inhibitors with agents that target the MAPK pathway and more potent AR inhibitors might prove efficacious in CRPC.[42]

Conclusion

In this study, the feasibility of combining everolimus with docetaxel for the treatment of CRPC was demonstrated. Everolimus 10 mg/d in combination with docetaxel 60 mg/m^2 was reasonably well tolerated. However, we concluded that the response rate to combination therapy would be unlikely to exceed that of docetaxel alone administered at 75 mg/m^2. Despite the small number of patients, results of this study support additional investigation of the validity of FDG-PET as a biomarker for pharmacodynamic response to mTOR pathway inhibition in CRPC. Inhibitors that target key components of the PI3K/Akt/mTOR pathway remain important investigational agents being developed for prostate cancer treatment.

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Clinical Practice Points

- PTEN and activation of the PI3K/mTOR signaling cascade occurs frequently in CRPC. Preclinical studies support the therapeutic potential for PI3K/mTOR pathway inhibition in the treatment of prostate cancer.
- We conducted a phase I clinical trial of the mTOR inhibitor everolimus with docetaxel in men with metastatic CRPC and found that clinical activity was low at the MTD. FDG-PET might serve as a biomarker for target inhibition by mTOR inhibitors in metastatic CRPC.
- Taken together with recent findings concerning the interplay between multiple oncogenic drivers of CRPC, including the PI3K/mTOR, MAPK, and AR signaling pathways, results of this study support further exploration of combined inhibition of these pathways in subjects with metastatic CRPC.

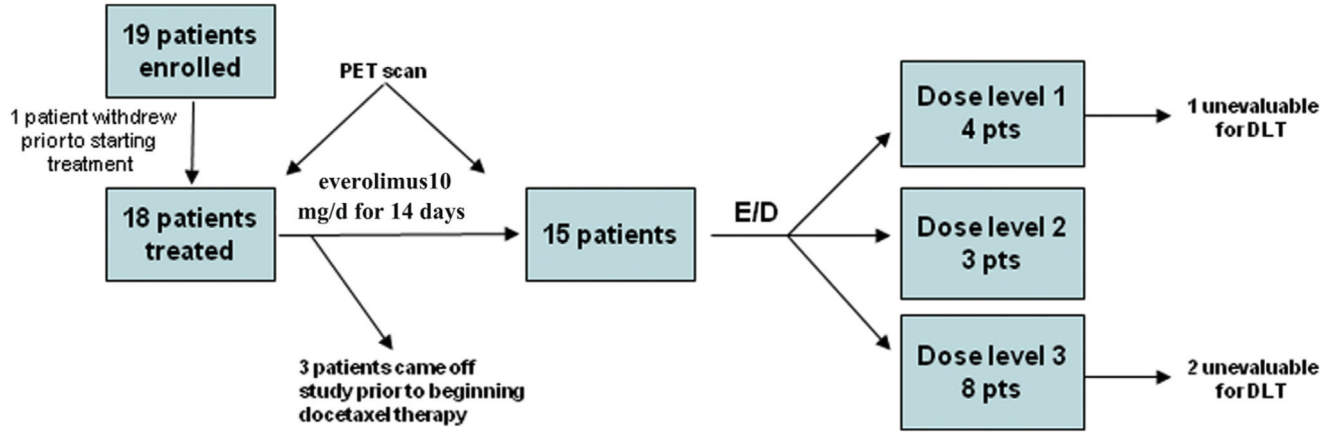

Figure 1.

Treatment Schema and Patient Flow for the Study

Abbreviations: DLT = Dose-Limiting Toxicity; E/D = Everolimus With Docetaxel Treatment; PET = Positron Emission Tomography; pts = Patients.

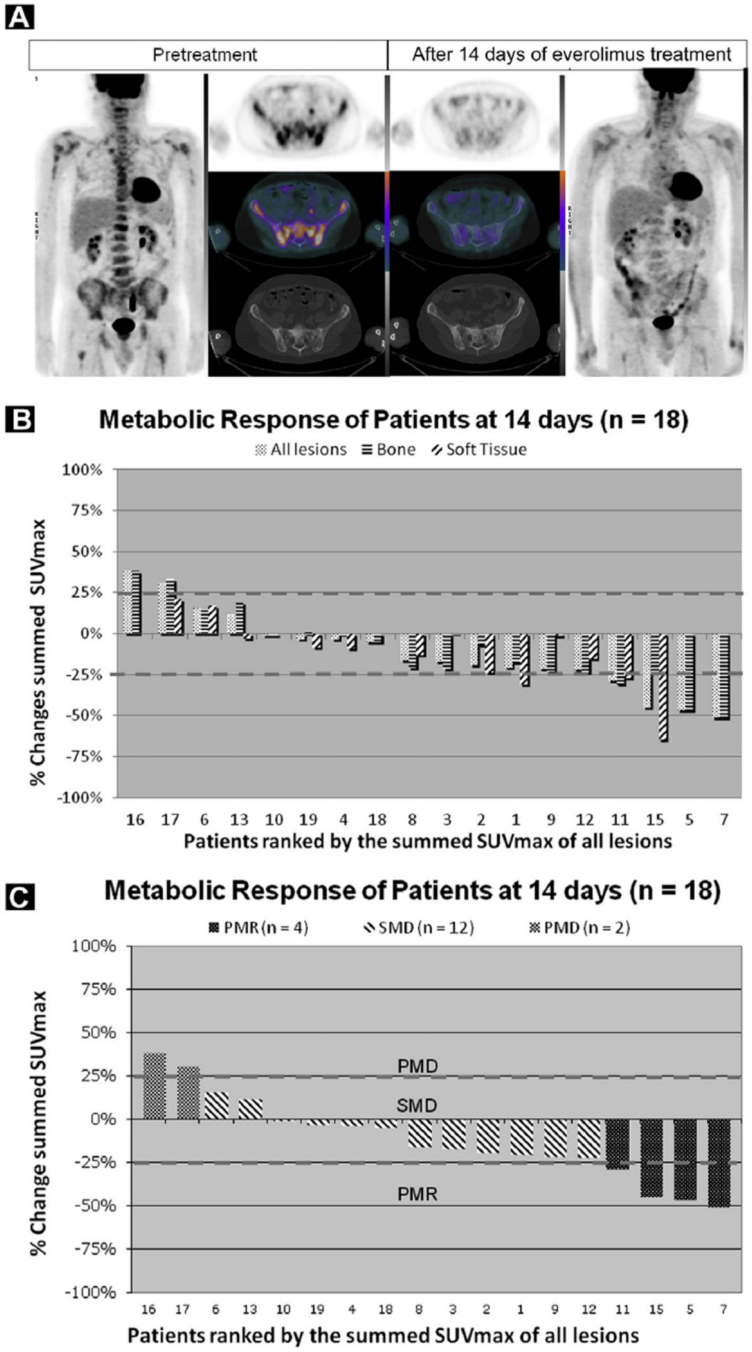

Figure 2.

Metabolic Response to Everolimus Treatment and Prostate-Specific Antigen (PSA) Response to Everolimus With Docetaxel (E/D) Treatment. Patients Underwent Fluorine-18 Fluorodeoxyglucose Positron Emission Tomography Imaging Before Treatment and Again After 10 to 14 Days of Treatment With Everolimus Alone to Assess for Metabolic Response. (A) Representative Images Before and After Everolimus Treatment Are Shown for Patient 005. (B) The Percent Change in Summed Maximum Standardized Uptake Value (SUVmax) Was Calculated for Bone, Soft Tissue, and Total Lesions. (C) After Everolimus

Treatment, 4 Patients Had a Partial Metabolic Response (PMR), 12 Had Stable Metabolic Disease (SMD), and 2 Had Progressive Metabolic Disease (PMD)

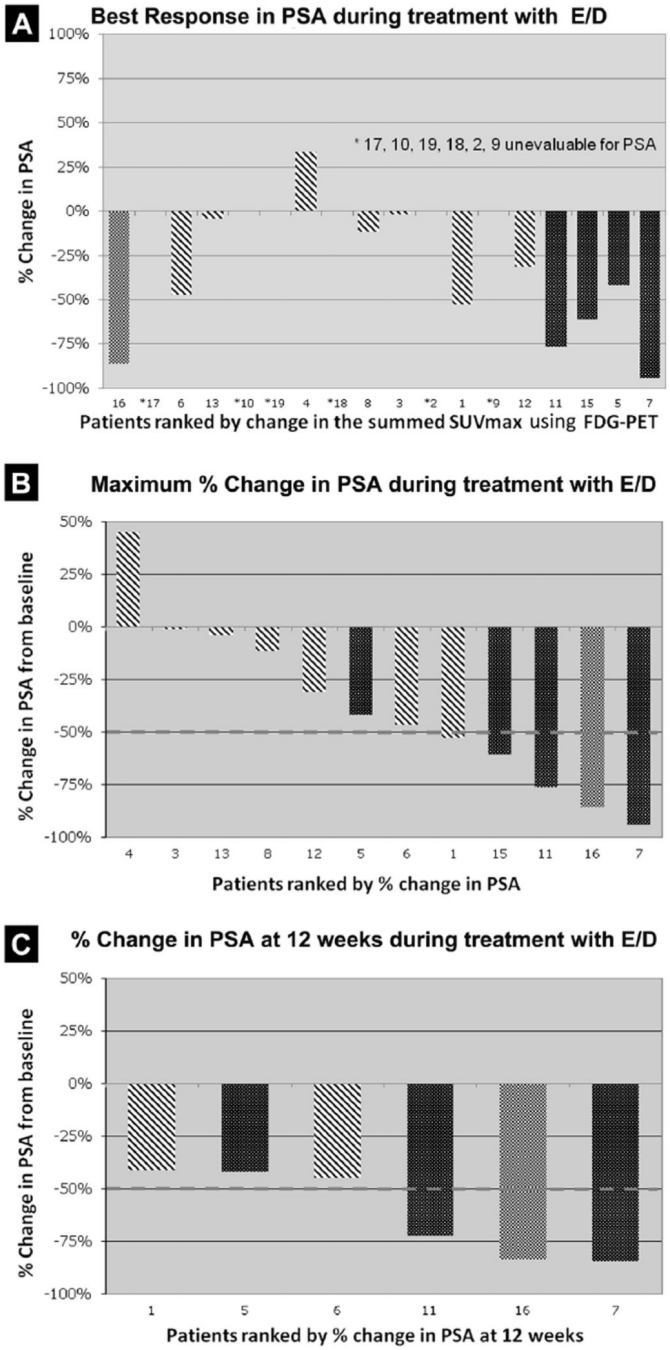

Figure 3.

(A) The Percent Change in Prostate-Specific Antigen (PSA) After Treatment With Everolimus and Docetaxel (E/D) Is Shown. Color Coding Matches That for Figure 2C to Permit Comparison With Metabolic Response to Treatment With Everolimus. Best PSA Response for All Patients Is Shown Ranked According to the Change in Summed Maximum Standardized Uptake Value (SUVmax) Using Fluorine-18 Fluorodeoxyglucose Positron Emission Tomography (FDG-PET) Corresponding to Figure 2C. (B) A Waterfall Plot of Maximal Change in PSA for All Patients Treated With E/D for at Least 2 Cycles. Patients 2,

9, 10, 17, 18, and 19 Were Not Evaluable for Change in PSA. (C) The Percent Change in PSA at 12 Weeks Is Shown for the 6 Patients Who Continued Treatment

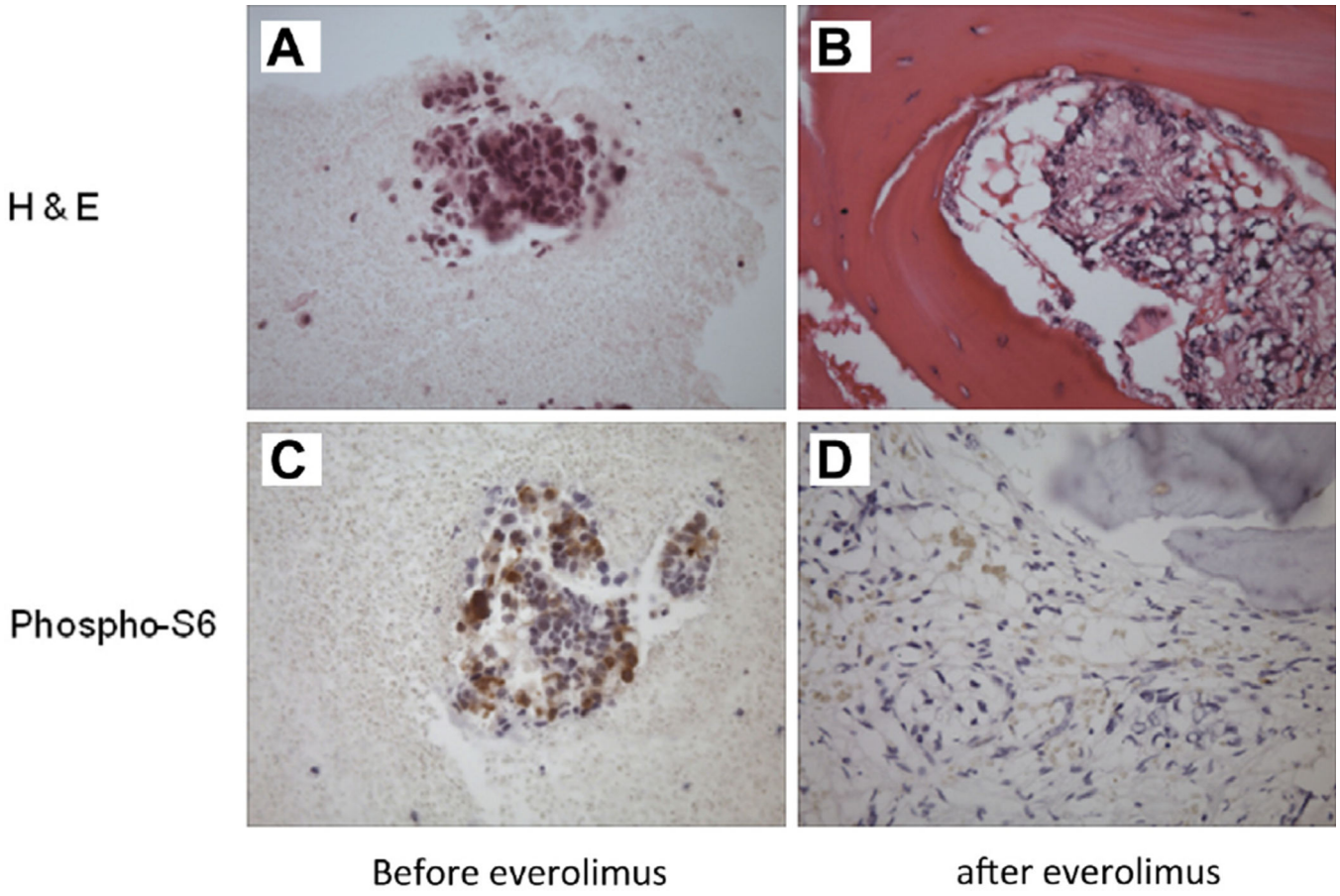

Figure 4.

Histologic and Immunohistochemical Response to Everolimus Treatment. Image-Guided Bone Biopsy Was Performed on Patient 3 Before and After Everolimus Treatment. Tissue Sections Were Stained With (A and B) Hematoxylin and Eosin (H & E) or (C and D) Antibody Against Serine-Phosphorylated Ribosomal Protein S6 (Phospho-S6)

Table 1

Baseline Patient Characteristics (n = 18)

Baseline Variable	Value	n (%) or Median Value
Age, Years	50–79	62
ECOG Performance Status	0	8 (44)
	1	10 (56)
PSA at Enrollment, ng/mL	7.3-3602.3	288.1
Caucasian Race	NA	18 (100)
Gleason Score	5	1 (5)
	6	2 (11)
	7	7 (39)
	8	3 (17)
	9	5 (28)
Measurable Disease	No	3 (17)
	Yes	15 (83)
Previous Radiation	No	4 (22)
	Yes	14 (78)
Previous Prostatectomy	No	13 (72)
	Yes	5 (28)
Previous Steroids	No	2 (11)
	Yes	16 (88)
Previous Ketoconazole	No	2 (11)
	Yes	16 (88)
Previous Estrogens[a]	No	12 (67)
	Yes	6 (33)
Previous 5α-Reductase Inhibitor	No	14 (78)
	Yes	4 (22)

Additional previous therapies include: sunitinib (2), trilostane (1), PC-SPES (1), celecoxib (1), sipuleucel-T (1), and enzalutamide (1).

Abbreviations: ECOG = Eastern Cooperative Oncology Group; PSA = prostate-specific antigen.

[a] Including diethylstilbestrol.

Table 2

Adverse Events Among 15 Patients Who Received Combination Therapy With E/D

Adverse Event	Grade 1/2	Grade 3	Grade 4
Allergic Rhinitis	2	0	0
Anemia	6	1	0
Leukopenia	3	4	2
Lymphopenia	0	1	0
Neutropenia	0	2	6
Thrombocytopenia	2	1	0
Hypertension	0	1	0
Fatigue	8	1	0
Fever Without Neutropenia	3	0	0
Rigors/Chills	2	0	0
Sweating	1	0	0
Weight Loss	1	0	0
Alopecia	3	0	0
Nail Changes	1	0	0
Rash/Desquamation	3	0	0
Hand-Foot Reaction	1	0	0
Skin Breakdown/Decubitus Ulcer	1	0	0
Ulceration	1	0	0
Skin-Other	1	0	0
Anorexia	3	0	0
Constipation	1	0	0
Diarrhea	8	1	0
Gastritis	1	0	0
Mucositis According to Examination of Oral Cavity	5	0	0
Mucositis (Symptom) Oral Cavity	1	0	0
Nausea	6	0	0
Taste Disturbance	2	0	0
Vomiting	1	0	0
Gastrointestinal-Other	1	0	0
Nose, Hemorrhage	4	0	0
Cholecystitis	0	1	0
Febrile Neutropenia	0	3	2
Infection With Grade 3/4 Neutrophils, Dental-Tooth	1	0	0
Infection With Grade 3/4 Neutrophils, Lung	0	1	0
Infection With Grade 0/2 Neutrophils, Dental-Tooth	1	0	0
Infection With Grade 0/2 Neutrophils, Lung	0	1	0
Infection With Grade 0/2 Neutrophils, Upper Airway	2	0	0

Adverse Event	Grade 1/2	Grade 3	Grade 4
Edema, Limb	3	0	0
Edema, Trunk/Genital	1	0	0
Hypoalbuminemia	1	0	0
Alkaline Phosphatase	3	1	0
Aspartate Aminotransferase	4	0	0
Hypercholesterolemia	5	0	0
Hyperglycemia	2	2	0
Hypokalemia	0	1	0
Hypernatremia	1	0	0
Hyponatremia	1	0	0
Hypertriglyceridemia	1	0	0
Dizziness	1	0	0
Extrapyramidal Movement	1	0	0
Depression	2	0	0
Neuropathy-Sensory	3	0	0
Abdomen, Pain	2	1	0
Back, Pain	6	0	0
Bone, Pain	7	0	0
Chest Wall, Pain	1	0	0
Dental/Teeth/Periodontal, Pain	1	0	0
Extremity-Limb, Pain	3	0	0
Joint, Pain	1	0	0
Muscle, Pain	1	0	0
Pelvis, Pain	2	0	0
Throat/Pharynx/Larynx, Pain	1	0	0
Pain-Other	1	0	0
Cough	4	0	0
Dyspnea	4	0	0
Pneumonitis/Pulmonary Infiltrates	2	0	0
Incontinence, Urinary	1	0	0
Obstruction-Urethral	0	1	0
Urinary Frequency/Urgency	2	0	0
Vascular Access/Thrombosis/ Embolism	0	1	0
Worst Degree	2	5	8

All adverse events, regardless of attribution, among 15 patients who received combination therapy are shown. The identity and total number of adverse events are presented. The total number of patients experiencing adverse events is presented.

Table 3

Immunohistochemistry Analysis of Tumor Biopsies for PTEN and Phospho-S6

Patient No.	PTEN Score			Phospho-S6 Score		
	Q	I	I x Q	Q	I	I x Q
1	3	2	**6**	3	2	**6**
2	NA	NA	NA	NA	NA	NA
3	1	0	**0**	2	3	**6**
3 After Therapy	1	0	**0**	1	0	**0**
4	2	1	**2**	4	3	**12**
5	NA	NA	NA	NA	NA	NA
6	4	2	**8**	3	1	**3**
7	NA	NA	NA	NA	NA	NA
8	NA	NA	NA	NA	NA	NA
9	3	1	**3**	4	2	**8**
10	NA	NA	NA	NA	NA	NA
11	NA	NA	NA	4	3	**12**

Immunohistochemistry analysis of tumor biopsies for PTEN and phospho-S6. Image-guided biopsies of tumors in the prostate (patients 1 and 9), bone (patients 2–7, 10, and 11), or lymph node (patient 8) were performed before treatment. Immunohistochemistry (IHC) for PTEN and phospho-S6 ribosomal protein were carried out on adjacent sections. A semiquantitative scoring taking into account quantity (Q; 1 = negative, 2 = 1%-10%, 3 = 11%-50%, 4 = > 50% positive tumor cells) and intensity (I; 0 = negative, 1 = weak, 2 = moderate, 3 = strong) of the staining was used to obtain a final score (I*Q; < 6 = low, 6 = intermediate, > 6 = high; I*Q score in bold typeface). Patient 3 underwent a repeat tumor biopsy and IHC analysis after everolimus treatment.

Abbreviations: phospho-S6 = phosphorylated ribosomal protein S6; PTEN = phosphatase and tensin homologue deleted on chromosome 10.