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Phase 2 clinical trial of TORC1 inhibition with everolimus in men with metastatic castration-resistant prostate cancer

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Abstract

Background: Activation of the PI3K-Akt-mTOR signaling pathway is common in advanced castration resistant prostate cancer (CRPC), typically through PTEN loss. Preclinical studies suggest that Akt-driven CaP cells are genetically susceptible to mammalian target of rapamycin (mTOR, or TORC1) inhibition. Everolimus is a Food and Drug Administration-approved inhibitor of TORC1.

Materials and Methods: We performed a phase II study of everolimus in patients with mCRPC, who were refractory to standard of care hormonal and chemotherapeutic agents. Patients received everolimus 10 mg daily until unacceptable adverse events or disease progression. The primary efficacy outcome was confirmed 50% or greater prostate-specific antigen (PSA) response, using a 2 stage design with futility rules. Paired biopsies were utilized to assess for treatment effect on downstream TORC1 targets as well as tumor cell proliferation and apoptosis.

Results: Out of 35 men enrolled with heavily pretreated mCRPC, 32 were evaluable for clinical efficacy. No PSA responses were observed, the median progression-free survival time was 3.6 months (95% confidence interval = 2.9−4.8) and the median overall survival time was 10.4 months (95% confidence interval = 5.8−15.8). Several patients had declines in serum PSA upon cessation of everolimus. Thus, the study was closed due to clinical futility. The most common toxicities were mucositis, fatigue, anorexia, hypertriglyceridemia, and thrombocytopenia and were largely low grade. Pathologic evaluation of paired metastatic biopsies demonstrated consistent inhibition of pS6, a downstream mTOR pharmacodynamics biomarker, but the tumor proliferation marker Ki-67 increased with therapy.

Conclusions: Everolimus demonstrated predictable toxicity in advanced and heavily pretreated patients with mCRPC. No clinical or clear pathologic effects despite downstream TORC1 target inhibition, suggesting that single agent everolimus has no clinical utility in men with mCRPC.

Keywords: TORC1 inhibitor; Everolimus; mCRPC; Progression

1. Introduction

Metastatic prostate cancer (CaP) frequently harbors activation of the phosphatidyl-3-inositol kinase (PI3K) survival pathway due to PTEN loss or activating mutations in PI3K isoforms [1]. PTEN is a dual phosphatase that functions as a negative regulator of the PI3K/Akt/TORC1 pathway [2,3], leading to downstream signaling through mammalian target of rapamycin (mTOR)/TORC1 signaling, activation of p70 S6K and 4E-BP1, and enhanced protein translation [4], ribosomal biogenesis, cell growth and size, and apoptosis resistance. Consequently, loss of PTEN function results in dysregulation of multiple cellular processes critical to cancer progression.

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Rapamycin is a naturally occurring inhibitor of mTOR, from which derivatives such as everolimus have demonstrated clinical utility in kidney and breast cancer [5,6]. Preclinical studies using a transgenic mouse model with restricted expression of a myristoylated Akt resulted in constitutive Akt activation and prostate intraepithelial neoplasia. With mTOR inhibition, prostate epithelium reversed to that of normal mouse ventral prostate after 14 days with a marked decrease in the phosphorylation of downstream targets of mTOR activity (S6K), proliferation index (PI; MIB-1) and an increase in apoptosis [7]. Therefore, we hypothesized that inhibition of mTOR in men with mCRPC would induce similar phenotypic changes and clinical benefits.

In order to test this hypothesis, we designed a phase II study of continuous oral daily everolimus (Afinitor, Novartis Pharmaceuticals) in patients with mCRPC. At the time this study was conducted, no prior trials of mTOR inhibition in mCRPC had been conducted. To evaluate the pathologic and molecular biomarkers in metastatic tumor specimens impacted by mTOR inhibition, we performed image-guided bone biopsies of pelvic metastases before and while on treatment.

2. Methods

2.1. Patients

Patients eligible for this study included adults (≥18 years) with histologically confirmed prostate adenocarcinoma with clinical/radiographic evidence of disease progression despite castrate levels of testosterone (<50 ng/dl), by either 2 consecutive prostate-specific antigen (PSA) level increases of ≥50% above nadir achieved on Androgen Deprivation Therapy (ADT), or radiographic progression defined by RECIST 1.0. Patients were required to have good performance status (ECOG 0−1) and acceptable bone marrow, kidney and liver function, and a total cholesterol <300 mg/dl and triglycerides <350 mg/dl. Prior chemotherapy, radiation, surgery, immunotherapy, or other investigational products were permitted provided that they stopped 4 weeks prior to study entry. In addition, patients must have had at least one site amenable for biopsy (bone or lymph node). Additional eligibility details are provided on clinicaltrials.gov NCT00629525. All patients provided written informed consent. The study was approved by the institutional review board of Duke University Medical Center and was conducted in accordance with the Declaration of Helsinki.

2.2. Study design

The study was a single arm, single center, open label phase II trial conducted at the Duke University Medical Center. Patients underwent screening and pretreatment studies, including baseline laboratory assessments, imaging and tumor biopsy, after which they were started on everolimus 10 mg by mouth daily. Patients were evaluated for adverse events using the National Cancer Institute Common Toxicity Criteria version 3.0 every 2 weeks for the first cycle (4 weeks) and then every 4 weeks. Tumor volume was measured at baseline by computed tomography or magnetic resonance imaging and responses were assessed after every 8 weeks using RECIST 1.0 [8].

2.3. Biopsy procedures

Image-guided biopsies of pelvic bone or lymph node metastases were performed at baseline and day 28 to assess for tumor proliferation and pharmacodynamic biomarkers. An 18 gauge achieve biopsy needle was used for core biopsies, including a total of 3 to 6 cores per procedure. One core was placed in formalin (error) fixative and the rest were flash frozen at −80°C and stored until batched analysis.

2.4. Statistical analysis plan

The primary objective of this study was to estimate the ≥50% PSA response rate of mCRPC patients treated with everolimus, requiring confirmation of PSA decline ≥2 weeks later. We used the Simon 2-stage minimax design [9], assuming a minimum promising response rate of 15% (alternative hypothesis) and maximum unfavorable response rate of 5% (null hypothesis), a one-sided type I of 0.10 and power of 90%. Under these assumptions, the design calls for enrolling 39 patients in stage 1; if <2 of 39 patients respond the trial will be terminated due to futility. However, if ≥2 responses are observed, 21 additional patients would be enrolled, for a total of 60 patients. Treatment would be considered promising if ≥6 patients respond. The 95% confidence interval for the proportion of responses will be estimated within 6.5 percentage points.

The Kaplan-Meier method was used to estimate overall survival (OS) and progression-free survival (PFS) distributions. PFS was defined as the date from registration to date of radiographic progression by RECIST 1.0, clinical deterioration, or death, whichever occurred first. OS was defined as the date from registration to date of death or last follow-up.

2.5. Pathologic and pharmacodynamic evaluations

Apoptotic index was assessed on all tissue biopsies from all patients using the TdT nick-end labeling technique. Negative controls were generated by omitting dUTP. From each slide, sections without necrosis were evaluated and data expressed as % apoptosis (apoptotic nuclei/normal nuclei × 100) in cancer sections. Apoptotic index was calculated using representative sections from 3 non-necrotic tumor regions and scored as apoptotic positive cells/total tumor cells per hpf × 100.

The Ki-67 PI was assessed on tissue biopsies from all patients. Following a 20 minutes blocking step with 1.5%

horse serum, sections were treated with anti−Ki-67 at 1:100 dilution and secondary biotinylated anti-Immunoglobulin G (IGG) avidin-peroxidase complex. Treatment with secondary antibody alone served as a normal control. Western blot and immunohistochemical analysis using phospho-specific S6 Kinase was performed as previously described [10]. Phospho-S6 Kinase and Ki-67 was scored using an H-score system of frequency of expression (0% −100%) multiplied by intensity (0−3), for a total possible score of 0 to 300, and was described at baseline, on treatment, and for differences between paired biopsies in individual men.

3. Results

3.1. Demographics and baseline characteristics

We accrued 35 men to this study over 39 months. Baseline demographics are described in Table 1. This was an advanced mCRPC population at the time (2006−2009), prior to the availability of abiraterone, enzalutamide, radium-223, and cabazitaxel. Our patients had a median age of 71 years and 17% were African-American. Overall, patients were heavily pretreated and all had progressed on androgen deprivation therapy; 89% of patients had received prior treatment with taxane chemotherapy and 20% had received >2 courses of chemotherapy. On imaging, 71% of patients had osseous-only metastases while 11% had measurable lymph node metastases and 17% had visceral metastases with or without bone/nodal metastases. Baseline median PSA level was 200 ng/ml.

Table 1
Baseline characteristics of the patients.

Baseline characteristics	$n = 35$
Age, median (range), years	71 (52−91)
Race and Ethnicity	
White	29 (83%)
Non-Hispanic Black	6 (17%)
Gleason score, median	7 (6−9)
% Grade Group 4−5	40%
Karnofsky performance status ≥90	63%
Primary treatment	
Radical prostatectomy	11
Radiation	10
Salvage radiation	7
None (Systemic only)	7
Time from diagnosis to entry, years	8 (1−14)
Duration of prior hormonal therapy, years	5 (1−14)
Number of secondary hormonal therapies	
0	6 (17%)
1−2	25 (71%)
3 or more	4 (11%)
Number of prior chemotherapy regimens	
0	4 (11%)
1−2	24 (69%)
>2	7 (20%)
Prior palliative radiation (% yes)	12 (34%)
Pattern of spread	
Bone metastases only	71%
Visceral metastases ± bone/node metastases	17%
Node only metastases	11%
Presence of significant pain (%)	25%
Laboratory Studies	
PSA, median (range), ng/dl	200 (4.5−3193.3)
LDH median (range), IU/l	565 (5.89−2004)
Alkaline phosphatase median (range), U/l	140 (3.4−2137)

3.2. Efficacy

Of the 35 patients enrolled, 32 had post-treatment evaluable PSA levels for assessment of PSA decline. For the primary objective of ≥50% confirmed PSA decline, no responses were observed (0/32) and accrual was terminated early since the likelihood of 2 or more PSA responses after 39 patients was 0% to 10%. As shown in the PSA waterfall plot, best PSA decline from baseline suggests that the vast majority of patients had a rapid PSA rise with a median PSA doubling time from baseline of 1.47 months (Fig. 1a), with only 2 patients having any transient PSA declines.

In many cases PSA levels declined once everolimus was discontinued, suggesting that PSA expression may have been influenced by mTOR inhibition and demonstrating an mTOR inhibitor withdrawal response (Table 2). Lactate dehydrogenase (LDH) is highly prognostic in men with mCRPC [11,12], is predictive of the benefits of mTOR inhibition in renal cell carcinoma [13], and was downregulated in our preclinical model of prostate cancer with everolimus treatment (10). We hypothesized that serum LDH levels, as a reflection in part of tumor mTOR activity and LDH production, would decrease with everolimus exposure. Instead, we observed an increase in median LDH, from 565 to 864 U/l (Table 2). These LDH levels appeared to stabilize after an initial rise at month 1 even at the end of study (time of disease progression). Interestingly, we observed a decrease in LDH levels in a subset of patients followed off study without further treatment again suggesting an mTOR inhibitor withdrawal response.

The median PFS time was 3.61 months (95% confidence interval 2.92−4.82 months) while the median OS was 10.41 months (95% confidence interval 5.75−15.77 months) including 25 recorded deaths at the time of study completion (Fig. 1b). The median follow-up time among the 10 surviving patients at the time of study completion is 17.9 months.

3.3. Adverse events

Overall everolimus demonstrated predictable toxicity in this heavily pretreated patient population as described in Tables 3a and 3b. The most common toxicities observed were mucositis and fatigue. Adverse events tended to develop in cycles 1 to 2 (Table 3a). Adverse events occurring in 10% to 50% of patients included anemia, anorexia, constipation, diarrhea, dry skin, fever, headache, hypercholesterolemia, hypoalbuminemia, hypertriglyceridemia,

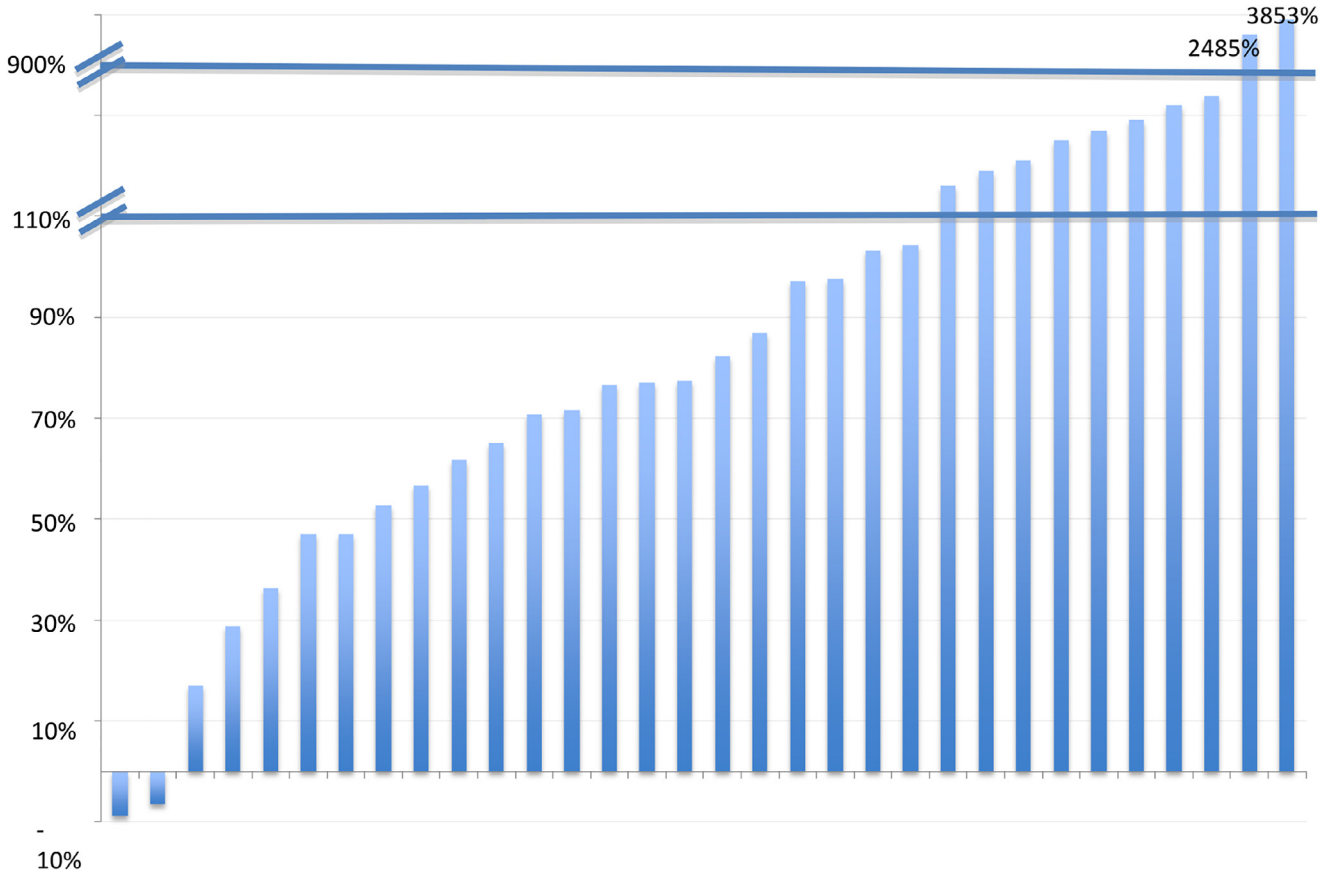


Fig. 1a. Waterfall plot of best overall PSA decline over time with everolimus.

elevated aspartate aminotransferase (AST)/ALT, nausea, neutropenia, rash, dyspnea, taste alterations, thrombocytopenia, vomiting, and weight loss. Almost all events were limited to grades 1 to 2. However, several grade III and IV toxicities occurred (Table 3b), including fatigue (5/35, 14%), mucositis (4/35, 11%), and anemia (3/35, 9%). Individual grade IV toxicities included anemia, hypertriglyceridemia, hypophosphatemia, increased troponin T, and acute myocardial infarction.

Table 2
Summary of PSA and LDH changes over time.

n = 32	Median	Range
LDH	U/l	U/l
Baseline	565	5.89–2004
Cycle 2 Day 1	864	164–4639
End of Study	859	209–4639
1-month follow-up	555 *(n = 8)*	167–2169
PSA	ng/ml	ng/ml
Baseline	197	4.5–3193.3
Cycle 2 Day 1	290	4.7–6591.8
End of Study	413.9	4.1–4237.5
1-month follow-up	363 *(n = 15)*	15.2–2157.0
Baseline PSA doubling time (months) on treatment	1.47	−1.95 to 8.85

3.4. Tumor biopsies and pathologic pharmacodynamic analyses

In this study, mandatory pretreatment metastatic tumor biopsies in 35 men; of these, 13 had viable tumor evaluable for biomarker analyses. Post-treatment on study day 28 biopsies were also mandatory and 33 patients had a day 28 biopsy. Of these day 28 biopsies, 12 had viable tumor tissue evaluable for biomarker analyses. A total of 9 patients had evaluable baseline and day 28 biopsy tumor tissue for pharmacodynamics studies. We assessed whether everolimus treatment led to a reduction in the activity of phospho-S6 kinase, a known downstream phosphorylation target of mTOR, using immunohistochemistry studies of paired metastatic biopsies before and following treatment with everolimus. Immunohistochemistry (IHC) for pS6 kinase in 9 pairs of metastatic samples for which paraffin embedded tissue was available demonstrated consistent pS6 kinase staining in all biopsies analyzed prior to treatment. We observed consistent decreases in staining intensity following 1 month of treatment with everolimus in 100% (9 out of 9) cases, and reduction of 50% or greater in pS6 kinase frequency/intensity with therapy was observed in 6 of 9 cases (Fig. 2A and B). While pS6 Kinase was not completely suppressed in most of the patients, the consistent decrease in phosphorylation of S6 Kinase suggests that everolimus

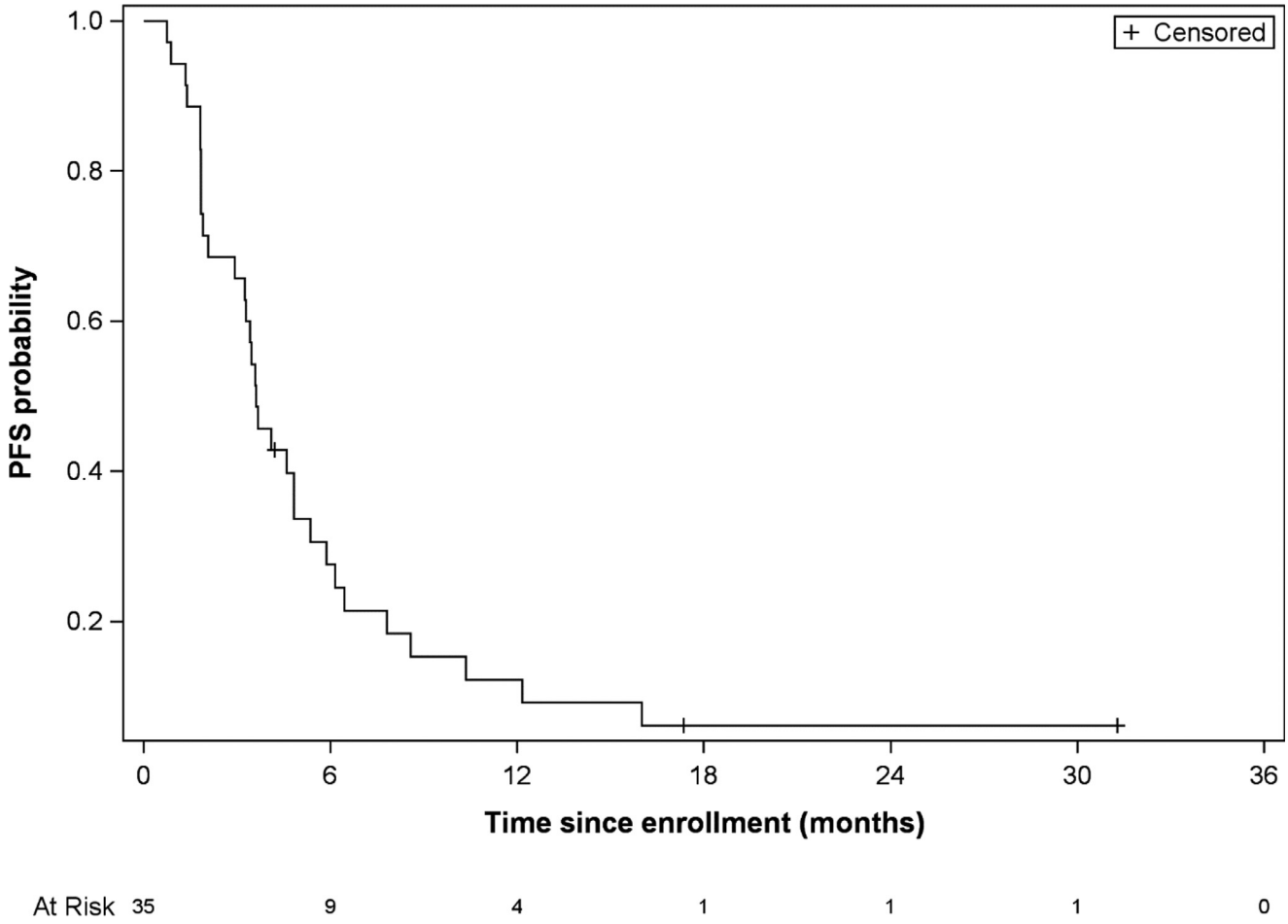


Fig. 1b. Kaplan-Meier plots of progression-free survival in months.

did achieve at least partial mTOR pharmacodynamic inhibition.

Of the 35 bone biopsies taken, 12 patients had evaluable tumor tissue with Ki-67 staining from their pretreatment biopsies, and 6 had evaluable tumor from post-treatment bone biopsies, 5 of which were pairs that contained evaluable pre- and post-treatment metastatic tumor tissue (Fig. 2C). Median Ki-67 staining was higher in the post-treatment samples than pretreatment samples (median Ki-67 42.5 vs. 17 cells/hpf). TUNEL staining was performed to evaluate apoptosis, which was evaluable in 8 pretreatment samples, 5 post-treatment samples; 3 were pairs with no clear treatment effect (data not shown).

4. Discussion

Based on the common activation of the PI3 kinase/Akt pathway in men with mCRPC, we anticipated that single agent mTOR (TORC1) inhibition would provide clinical benefits to these men, due to efficacy observed in preclinical models of prostate cancer with Akt activation. Despite the success in preclinical models and consistent downstream S6 activity inhibition in paired metastatic samples, we observed no clinical activity, with no PSA declines or objective responses, and primary resistance to therapy in all patients with rapid progression. In this phase 2 trial conducted prior to the availability of multiple Food and Drug Administration-approved agents for mCRPC, we also observed PSA withdrawal responses to everolimus discontinuation, suggesting that everolimus may result in reciprocal activation of AR signaling in certain tumors [14,15]. Based on the nearly 50% prevalence of PTEN loss in this disease state and nearly 100% prevalence of PI3K/mTOR pathway activation, we did not employ a biomarker selection strategy for the men with mCRPC in this trial. Our study provides strong evidence that PTEN loss is clearly not an actionable target for treatment with single agent mTOR inhibition in men with mCRPC. In addition, our data mirror that observed with single agent pan-PI3K inhibition in men with mCRPC [16], suggesting that combination approaches will be needed to overcome redundant signaling and reciprocal feedback systems poised to promote rapid resistance.

Our experience with everolimus therapy in this heavily pretreated group of men with mCRPC is similar to those studies that have examined other mTOR inhibitors such as rapamycin and temsirolimus, and everolimus itself with or without bicalutamide [17−21], where limited activity has

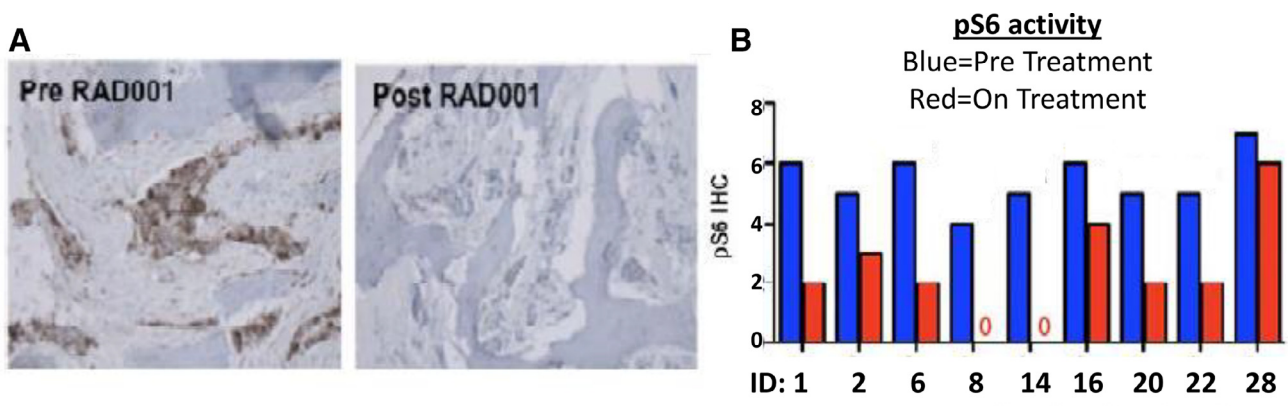
Table 3a
Adverse events of special interest expected for everolimus, including timing and maximal grade observed.

Adverse event	*n*	Median cycle of onset	Median maximum grade
Anemia	14	2	2
Anorexia	17	1	1
Constipation	6	1	1
Dehydration	3	3	3
Diarrhea	11	1	1
Dizziness	3	2	1
Dry skin	7	2	1
Fatigue	8	1	2
Fever	5	1	1
Headache	4	1	1
Hypercholesterolemia	11	1	1
Hyperglycemia	3	3	1
Hypertriglyceridemia	17	1	1
Hypoalbuminemia	6	2	1
Increased ALT	6	1	1
Increased AST	15	1	1
Leukopenia	8	1	2
Mucositis	21	1	2
Nausea	12	1	1
Neutropenia	12	1	2
Pneumonitis	3	4	3
Rash	13	1	2
Shortness of breath	7	2	1
Taste alterations	9	2	1
Thrombocytopenia	17	1	1
Truncal rash	8	1	1
Vomiting	10	1	1
Weight loss	11	2	1

Table 3b
Summary of all adverse events observed by grade ($n = 35$).

Adverse event	Grade 3/4	All grades
Acute myocardial infarction	0/1	1
Acute renal failure	1/0	1
Anemia	2/1	14
Anorexia	2/0	17
Dehydration	2/0	3
Fatigue	5/0	17
Hypertriglyceridemia	0/1	17
Hypophosphatemia	0/1	2
Increased PT/INR	1/0	2
Increased troponin T	0/1	1
Leukopenia	1/0	8
Lymphopenia	1/0	3
Mucositis	4/0	19
Nausea	2/0	12
Neutropenia	1/0	12
Pneumonitis	2/0	3
Rash	3/0	5
Sepsis	1/0	1
Truncal Rash	2/0	8
UTI	1/0	1
Vomiting	1/0	10
Weakness	1/0	2

been observed in unselected men, and a lower clinical benefit has been observed than predicted by the prevalence of PI3K/mTOR pathway activation. In a preprostatectomy study of rapamycin, pharmacodynamic inhibition of phospho-S6 was established in tissue, without a significant impact on tumor cell proliferation or apoptosis, similar to our findings in metastatic biopsies. Targeting upstream



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Ki-67	N	Mean (SD)	Median (range)
Pre-Treatment	12	50 (64)	17 (3-210)
Post-Treatment	6	52 (51)	43 (2-120)
Post-Pre Difference	5	21 (72)	12 (-55-140)

Fig. 2. (A) Pharmacodynamic inhibition of S6 kinase in paired metastatic biopsies on study. A) IHC examples of p-S6 kinase staining in pre- and post-treatment samples. RAD001 = everolimus. (B) Plot of pre- and post-treatment pS6 kinase H-Score. (C) Summary of pharmacodynamic findings in bone metastatic biopsies taken before or during everolimus treatment.

oncogenic targets in the PI3K pathway has also proven to be challenging. Little activity was observed with buparlisib, a pan-PI3K inhibitor, alone or in combination with enzalutamide [16], suggesting a generalizable limitation of single agent therapy for this complex disease with a range of genomic alterations in additional pathways, including RAS/MAPK, TP53, RB1, AR, WNT/beta catenin, DNA repair, and epigenetic pathways [22,23].

It is likely that the preclinical models that supported single agent mTOR inhibition in prostate cancer were overly simple and artificially dependent on a single pathway [7]. It will thus be critical to recapitulate human CRPC heterogeneity in a range of preclinical models to better evaluate novel targeted drug combinations to overcome these resistance mechanisms. Thus, while many studies support the common activation of the mTOR pathway in human mCRPC [4−7], these same studies show that men with CRPC do not harbor these alterations in isolation, and addressing this genomic and phenotypic heterogeneity will be critical. Earlier preclinical studies for example used a genetically manipulated and well-defined tissue model that resulted in premalignant prostate intraepithelial neoplasia, while our study population included a wide range of patients with heavily pretreated metastatic disease and a range of demographic, clinical, pathologic and host features. Autopsy series have indicated that prostate cancer in the late stage setting is extremely heterogeneous [24], and thus it is not surprising we were not able to recapitulate the pathologic effects in our patient population.

The technical obstacle of obtaining tumor biopsies was substantial, particularly from patients with bone only metastases. We have previously published on the predictors of obtaining viable tumor tissue from metastatic core biopsies in men with mCRPC from this trial, including long term zoledronic acid use [25]. As such, we were only able to obtain viable tissue for analysis in a subset of patients. Nonetheless, pS6K analysis demonstrated clear pharmacodynamic inhibition with everolimus in metastatic biopsies. Perhaps the most concerning observation from this study was the clear lack of efficacy in patients and even the possible suggestion of an acceleration of disease proliferation. The near universal PSA and LDH progression seen as a best response (Figs. 1a and 1b) suggest that everolimus exposure results in a tumor stress or reciprocal AR pathway activation, supported by an increase in tumor Ki-67 as a marker of proliferation. This may be due to TORC1/2 inhibition resulting in upstream Akt activation driving greater cell proliferation and glycolysis [18]. In addition, inhibition of mTOR may lead to reciprocal feedback activation of AR signaling through inhibition of PHLPP [14,15,26]. Additional resistance pathways also include activated PI3K beta [27], Ras/MAPK signaling [28], AR-variant signaling, mitochondrial metabolism [29], surface receptor activation [30], Wnt/beta-catenin signaling [31,32] and immune evasion [33], and TP53/RB1 and epigenetic mediated lineage plasticity [34]. Activity has been observed with dual abiraterone and Akt inhibition with ipatasertib in men with mCRPC harboring PTEN loss or PI3K pathway activating mutations [35], which has led to an ongoing phase 3 trial in this setting (NCT03072238). However, given the relatively rapid resistance observed even with dual AR and Akt inhibition in men with PTEN null mCRPC, it is likely that additional agents will need to be employed as part of a multitargeted approach.

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