A Randomized Phase II Study of Androgen Deprivation Therapy with or without Palbociclib in RB-positive Metastatic Hormone-Sensitive Prostate Cancer

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ABSTRACT

Purpose: Palbociclib, a cyclin-dependent kinase (CDK) 4/6 inhibitor, blocks proliferation in a RB and cyclin D–dependent manner in preclinical prostate cancer models. We hypothesized that cotargeting androgen receptor and cell cycle with palbociclib would improve outcomes in patients with metastatic hormone-sensitive prostate cancer (mHSPC).

Patients and Methods: A total of 60 patients with RB-intact mHSPC were randomized (1:2) to Arm 1: androgen deprivation (AD) or Arm 2: AD + palbociclib. Primary endpoint was PSA response rate (RR) after 28 weeks of therapy. Secondary endpoints included safety, PSA, and clinical progression-free survival (PFS), as well as PSA and radiographic RR. Tumors underwent exome sequencing when available. Circulating tumor cells (CTC) were enumerated at various timepoints.

Results: A total of 72 patients with mHSPC underwent metastatic disease biopsy and 64 had adequate tissue for RB assessment. A total of 62 of 64 (97%) retained RB expression. A total of 60 patients initiated therapy (Arm 1: 20; Arm 2: 40). Neutropenia was the most common grade 3/4 adverse event in Arm 2. Eighty percent of patients (Arm 1: 16/20, Arm 2: 32/40; $P = 0.87$) met primary PSA endpoint ≤4 ng/mL at 28 weeks. PSA undetectable rate at 28 weeks was 50% and 43% in Arms 1 and 2, respectively ($P = 0.5$). Radiographic RR was 89% in both arms. Twelve-month biochemical PFS was 69% and 74% in Arms 1 and 2, respectively ($P = 0.72$). TP53 and PIK3 pathway mutations, 8q gains, and pretreatment CTCs were associated with reduced PSA PFS.

Conclusions: Palbociclib did not impact outcome in RB-intact mHSPC. Pretreatment CTC, TP53 and PIK3 pathway mutations, and 8q gain were associated with poor outcome.

Introduction

Metastatic prostate cancer is an incurable disease with an estimated 33,330 deaths in the United States in 2020 (1). Androgen deprivation therapy (ADT) with or without androgen receptor (AR) blockade was the standard of care for men with newly diagnosed metastatic hormone-sensitive prostate cancer (mHSPC) for many decades (2). While most men treated with ADT experience a clinical response, the majority will relapse and progress to castration resistance within 2–3 years (3, 4). The overall survival for men with mHSPC treated with ADT as the sole therapy is around 4 years (3–6). Data from phase III trials, CHAARTED, STAMPEDE, LATITUDE, ENZAMET, and TITAN have demonstrated significant survival benefits from early treatment intensification with docetaxel (six cycles), abiraterone/prednisone, enzalutamide, or apalutamide for patients with newly diagnosed mHSPC (3–8). These data illustrate that early intensive therapy for mHSPC results in significant clinical benefits. Despite these major advances, molecular methods to direct treatment intensification choice for mHSPC are lacking and targeting therapy to a specific tumor molecular profile is not yet validated.

Androgens drive prostate cancer proliferation by upregulation of cyclin D which complexes with cyclin-dependent kinase (CDK) 4/6 resulting in phosphorylation of RB, a cell-cycle brake, which in turn allows the cell to progress through the cell cycle. Loss of RB (*RB1*) by mutation, deletion, or silencing has been shown in multiple preclinical models to promote the development of castrate resistance and is associated with poor outcome (9, 10). RB expression is lost in 1%–20% of patients with localized prostate cancer (11) and up to 30%–40% of patients with heavily treated metastatic castrate-resistant prostate cancer (mCRPC; ref. 12). The frequency of RB loss in newly diagnosed M1 prostate cancer may be around 5% (13). In addition, CDK4/6 directly associates with AR increasing the AR-associated transcriptional activity (14, 15). These results suggest that therapeutic targeting of the CDK4/6 pathway may be important in previously untreated mHSPC which largely retain RB expression.

Palbociclib is a highly selective reversible inhibitor of CDK 4 and 6 which has been shown to significantly improve progression-free survival (PFS) when combined with letrazole in estrogen receptor (ER)-positive, HER2-negative advanced breast cancer (16). It is administered orally on a 3 week on, 1 week off treatment schedule. Preclinical data demonstrated that treatment of prostate cancer cell lines and primary tumors which retained RB expression with palbociclib resulted in reduced proliferation in *in vitro* and *in vivo* models (17). Trials exploring the use of CDK 4/6 inhibitors in CRPC are

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Clin Cancer Res 2021;27:3017–27

doi: 10.1158/1078-0432.CCR-21-0024

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Translational Relevance

Cotargeting of hormonal and cell-cycle pathways by the cyclin-dependent kinase (CDK) 4/6 inhibitor, palbociclib, has demonstrated clinical activity in patients with hormone receptor–positive breast cancer leading to its FDA approval. Metastatic hormone-sensitive prostate cancer (mHSPC) also retains dependence on hormonal signaling and RB protein–related cell-cycle checkpoints suggesting that cotargeting CDK4/6 and androgen signaling might offer benefit to patients with prostate cancer. In this randomized phase II clinical study, we assessed the utility of palbociclib in patients with mHSPC undergoing androgen deprivation therapy. The study did not identify a clinical benefit for palbociclib but did establish that metastatic tissue-based biomarker preselected trials are feasible in mHSPC.

ongoing (NCT02905318 and NCT02494921). However, we hypothesized that targeting this pathway in mHSPC with retained RB expression might be an ideal strategy because RB loss contributes to the development of castration resistance, is rare in mHSPC, and because CDK4/6 participates with AR to promote prostate cancer growth.

To test this hypothesis, we conducted a multi-institutional randomized phase II trial in which patients with newly diagnosed mHSPC whose tumors retained RB expression were randomly assigned to ADT [luteinizing hormone-releasing hormone (LHRH) analog plus bicalutamide] versus ADT plus palbociclib. Primary endpoint was PSA response (≤4 ng/mL) after 28 weeks of therapy, an intermediate endpoint for efficacy (18, 19). Secondary endpoints include safety/tolerability, biochemical and clinical PFS, PSA, and radiographic response rate (RR). Circulating tumor cells (CTC) were enumerated from patients at various timepoints and mutations were examined in metastatic (and selected matched primary) tumor samples.

Patients and Methods

Trial participants

Eligible patients had pathologic diagnosis of prostate cancer, hormone-sensitive metastatic (M1) disease as evidenced by soft tissue and/or bone metastases, and were untreated or had started ADT for mHSPC less than 2 weeks prior to registration. Patients had to have a baseline PSA ≥ 5 ng/mL within 60 days of registration or prior to ADT initiation for patients starting prior to study registration; an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2; and prior neoadjuvant/adjuvant hormonal therapy for nonmetastatic disease if 12 months had elapsed since completion. Patients with known brain metastases and other uncontrolled illness were disqualified from the study.

Trial design

This phase II randomized biomarker-preselected multicenter trial involved registration of patients with newly diagnosed mHSPC (**Fig. 1**). All eligible patients provided written informed consent and all studies were conducted in accordance with recognized ethical guidelines (e.g., Declaration of Helskinki, CIOMS, Belmont Report, U.S. Common Rule) and approved by Institutional Review Boards. All registered patients underwent a metastatic disease biopsy unless metastatic archival samples were available. Metastatic biopsy tissue was evaluated for RB expression by IHC (**Fig. 2**). Patients with RB-positive (RB^+) tumors were stratified by ADT start prior to registration and disease extent: limited (disease confined to spine, pelvic bones, and/or lymph nodes) versus extensive disease (ribs, long bones, and/or visceral organs) as described previously (20) and randomized 1:2 to ADT alone (LHRH agonist + bicalutamide 50 mg daily) or ADT (LHRH agonist + bicalutamide 50 mg daily) plus palbociclib (125 mg orally daily, days 1–21 of a 28-day cycle).

PSA and safety laboratory testing was monitored on a monthly basis. Radiologic assessments [including either CT or MRI abdomen/pelvis; either chest x-ray (CXR), CT, or MRI chest; and a bone scan] occurred after 28 weeks of therapy, then every 24 weeks for 2 years and then annually until study therapy discontinuation. Patients came off study for clinical, radiographic, or PSA progression; toxicity; or per patient request. The full protocol is available on request. This multisite study was conducted at seven institutions with coordination at the University of Michigan (Ann Arbor, MI).

Assessment of RB status

RB expression in tumor tissue was assessed by IHC for total RB expression in a central Clinical Laboratory Improvement Amendments–certified laboratory per previously published protocol (21). IHC was performed using a mouse anti-RB mAb (BD Biosciences, G3-245) using automated IHC staining on the Ventana Medical Systems Benchmark Ultra autostainer. Nuclear RB staining intensity in tumor cells was assessed in 100 tumor cells and assigned a value of negative (0), weak (1+), moderate (2+), or strong (3+). Samples were considered positive for RB expression and eligible for trial if >5% of cells had at least moderate staining or if >20% cells had at least weak RB staining (representative results; **Fig. 2**).

Trial endpoints

The primary endpoint was the rate of PSA ≤ 4 ng/mL after 28 weeks of treatment in patients treated with ADT versus those treated with ADT + palbociclib. This endpoint has been previously demonstrated to be a strong predictor of overall survival for patients with mHSPC treated with ADT (18, 19). Secondary endpoints included rate of undetectable PSA (<0.2 ng/mL) after 28 weeks, best PSA response [complete response (CR) = <0.2 ng/mL; partial response (PR) = >0.2 ng/mL and <4 ng/mL; stable disease (SD; not CR, PR, or progressive disease (PD)); PD = 25% increase over baseline or nadir, whichever is lower, and a confirmed increase in the absolute value of PSA by 2 ng/mL], biochemical and clinical PFS, radiographic response rate and safety, and tolerability of ADT plus palbociclib.

Correlative endpoints

Tumor biopsy specimens also underwent additional molecular and genomic analyses. Flash-frozen biopsies were processed for genomic DNA and total RNA isolation using Qiagen AllPrep Kit and then underwent targeted exon sequencing and capture transcriptome analysis at University of Michigan (Ann Arbor, MI), as detailed previously (22). The variant reporting protocol followed the guidelines set out for reporting germline variants by American College of Medical Genetics and the guidelines for reporting somatic variants as presented by CAP/AMP. Specifically, the current version of the ClinVar database was queried for all variants with a population frequency less than 1% and the ClinVar status for all germline alleles designated as pathogenic or likely pathogenic are reported as such. For truncating alleles with no ClinVar entry in genes with known pathogenic truncating alleles, the allele was reported as likely pathogenic if it is 5′ to the most 3′ known pathogenic allele of that gene. For splice site mutations, the splicing

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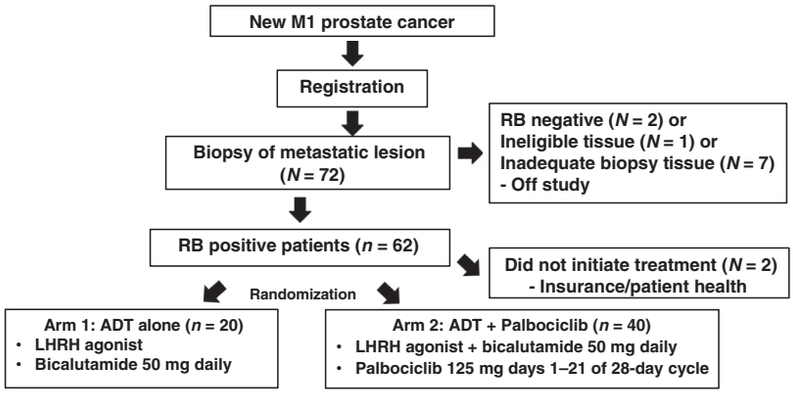

Figure 1.
Study schema.

pattern in the matched tumor RNA sequencing (RNA-seq) library was reviewed using IGV, to aid in assessing functional effects. For missense mutations, expression levels in the matched RNA-seq libraries were considered when assessing significance. For somatic mutations, recurrence in both COSMIC and the OncoSEQ databases were examined, along with entries in the online version of OncoKB, MyCancerGenome, the Jackson Laboratory clinical Knowledgebase, and the Atlas of Genetics and Cytogenetics in Oncology and Haematology. Literature searches for rare nonrecurrent alleles were additionally performed using PubMed. Genes with absolute copy number of 4–6 were reported as a gain. Genes with absolute copy number of 7 or greater were reported as amplified.

CTC numbers were also measured using Epic Biosystems platform at baseline, 12 weeks of therapy, 28 weeks of therapy, and after progression to determine whether CTC corresponded to treatment response. CTCs were stained as described previously (23–25). Total CTC/mL were measured using immunofluorescence and microscopy.

Statistical analyses

The primary endpoint was to compare the proportion of patients who had a PSA ≤ 4 ng/mL after 28 weeks of protocol treatment between patients randomized to combined ADT: LHRH agonist + bicalutamide and those randomized to combined ADT + palbociclib. Prerandomization, the patients were stratified by preregistration ADT treatment and extent of disease. For purposes of the primary endpoint, day 1 is the randomization date for patients with preregistration ADT treatment and the treatment start date for patients' naïve to ADT at registration. ADT alone (Arm 1) was expected to result in 70% of

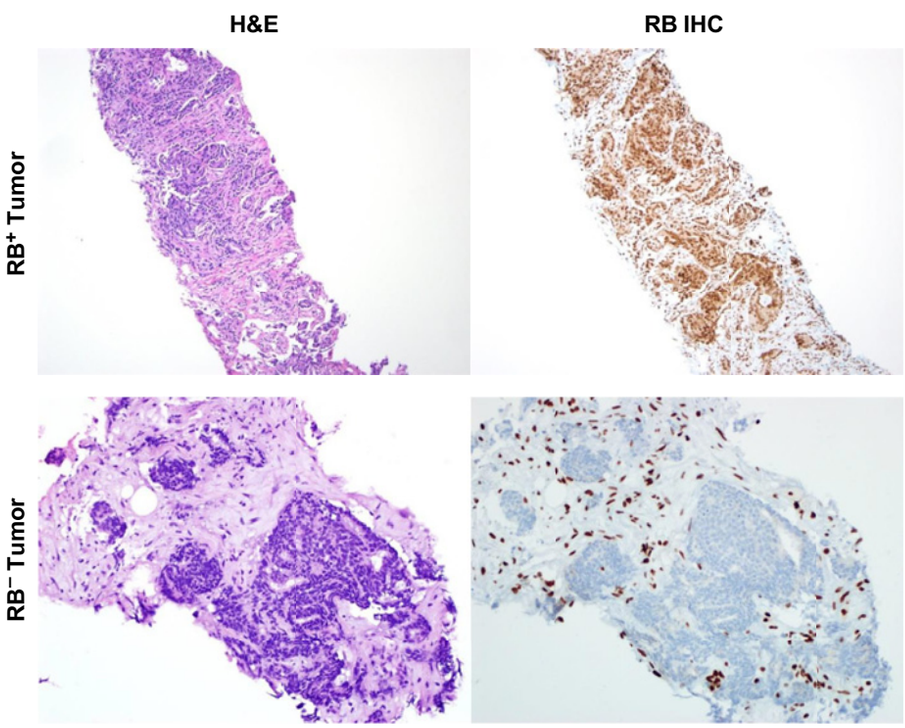

Figure 2.
Representative RB staining in metastatic prostate cancer biopsies. Left, H&E-stained tissues. Right, Typical RB staining in metastatic prostate cancers with (bottom) and without (top) RB loss.

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Table 1. Patient characteristics.

	ADT alone	PD + ADT	*P*
Median age (min–max)	66 (44–81)	68 (47–87)	0.61
Median baseline PSA (min–max)	55.7 (10.9–2,883)	77.2 (6.1–2,123)	0.63
Race			
White	17 (85%)	35 (87.5%)	0.18
Black	1 (5%)	5 (12.5%)	
Other/not reported	2 (10%)	0 (0%)	
ECOG			0.77
0	15 (75%)	27 (67.5%)	
1	5 (25%)	13 (32.5%)	
Primary gleason sum			1.00
6	0 (0%)	1 (2.5%)	
7	5 (25%)	9 (22.5%)	
8–10	11 (55%)	23 (57.5%)	
Unknown	4 (20%)	7 (17.5%)	
Prior treatment			
Radical prostatectomy	9 (45%)	14 (35%)	
Primary radiotherapy	2 (10%)	4 (10%)	
Neoadjuvant/adjuvant systemic therapy	5 (25%)	10 (25%)	
Neoadjuvant/adjuvant radiotherapy	3 (7.5%)	2 (5%)	
Salvage radiotherapy	3 (15%)	4 (10%)	
Other radiotherapy	0 (0%)	5 (12.5%)	
Disease sites at baseline			
Visceral metastases	1 (5%)	2 (5%)	1.00
Bone metastases	14 (70%)	30 (75%)	0.76
Lymph node only	5 (25%)	8 (20%)	0.74
Measurable disease	9 (45%)	27 (67.5%)	0.09
Baseline bone pain	5 (25%)	6 (15%)	0.48
Strata			
Limited disease/ADT initiation prior to biopsy	4 (20%)	8 (20%)	
Extensive disease/ADT initiation prior to biopsy	8 (40%)	16 (40%)	
Limited disease/ADT initiation after randomization	4 (20%)	9 (22.5%)	
Extensive disease/ADT initiation after randomization	4 (20%)	7 (17.5%)	
Median number of cycles of treatment	24.5 (4–47)	24.5 (6–53)	0.98

patients achieving PSA ≤ 4 ng/mL after 7 months of protocol treatment. It was hypothesized that the ADT + palbociclib arm (Arm 2) would have a 20% absolute increase in proportion to 90% (HA: p2 – p1 > 0). The null hypothesis was no difference in proportions (H0: p2 – p1 ≤ 0). All patients randomized were considered evaluable if they received one cycle of treatment or were removed during treatment due to toxicity. With 20 patients randomized to Arm 1 and 40 to Arm 2, there was a 64.2% power to detect a 20% difference in proportions with

Table 2. Adverse events.

Arm	Name	Grade	Number of patients	Percent
Arm 1: ADT	Any grade 3/4 AE		0	0.0%
Arm 2: Palbociclib + ADT	Any grade 3/4 AE		23	57.5%
Arm 2: Palbociclib + ADT	Nonhematologic AE	3	6	15%
Arm 2: Palbociclib + ADT	Hematologic AE	3/4	19	47.5%
Arm 2: Palbociclib + ADT	Abdominal pain	3	2	5.0%
Arm 2: Palbociclib + ADT	Alanine aminotransferase increased	3	1	2.5%
Arm 2: Palbociclib + ADT	Aspartate aminotransferase increased	3	1	2.5%
Arm 2: Palbociclib + ADT	Hyperglycemia	3	1	2.5%
Arm 2: Palbociclib + ADT	Hypertension	3	1	2.5%
Arm 2: Palbociclib + ADT	Left ventricular systolic dysfunction	3	1	2.5%
Arm 2: Palbociclib + ADT	Lymphocyte count decreased	3	1	2.5%
Arm 2: Palbociclib + ADT	Neutrophil count decreased	3	16	40.0%
Arm 2: Palbociclib + ADT	Neutrophil count decreased	4	1	2.5%
Arm 2: Palbociclib + ADT	Platelet count decreased	3	1	2.5%
Arm 2: Palbociclib + ADT	Vomiting	3	1	2.5%
Arm 2: Palbociclib + ADT	White blood cell decreased	3	5	12.5%

Abbreviation: AE, adverse event.

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Table 3. Primary and secondary endpoints.

Endpoint	Arm 1 (ADT only)		Arm 2 (Palbociclib + ADT)		P
	n = 20	%	*n* = 40	%	
PSA response at 28 weeks					
PSA ≤ 4 ng/dL	16	80	32	80	0.87
PSA undetectable (PSA <0.2 ng/mL)	10	50	17	43	0.50
PSA PFS % (95% CI)	80	(55–92)	90	(76–96)	0.72[a]
Best PSA response rate					0.25[b]
CR (PSA <0.2 ng/mL)	13	65	22	55	
PR (PSA ≥0.2–4 ng/mL)	5	25	12	30	
Stable disease (not PR or PD)	1	5	6	15	
Progression (25% increase and an absolute increase of 2 ng/mL)	1	5	0	0	
Measurable disease					0.42[b]
Number evaluable	9	45	27	67.5	
CR	4	44.4	11	40.7	
PR	4	44.4	13	48.2	
SD	1	11.1	2	7.4	
PD	0	0	1	2.7	
Best bone response					0.10
Number evaluable	14	70	30	75	
Stable/improved	14	100	25	83.3	
Progression	0	0	5	16.7	

[a]Log-rank test.
[b]Mid *P*-value Jonckheere–Terpstra exact test.

a one-sided type I error of 0.10 using the mid *P*-value method of the Fisher exact test. Although this power was lower than typical, we felt that the benefit of inclusion of randomization and negative control justified this design. The differences in proportions are reported with the mid *P*-value test of the 2×2 table with a one-sided type I error of 10%. The associated binomial proportions with the corresponding Wilson 80% binomial confidence intervals (CI) are reported by arm. Secondary endpoints of undetectable PSA, PSA response, and radiographic response are reported using counts and proportions by treatment arm with mid *P* values of the Fisher exact test or Jonckheere-Terpstra exact test. Biochemical PFS, clinical PFS, and time to CRPC by treatment arm are described using product limit estimates using Kaplan–Meier methods and log-rank *P* values. CTC correlatives are described at timepoints in relation to treatment. CTC counts per mL were dichotomized into <2 compared with 2 or greater for outcome associations based on published data from this platform (26) with the cut-off point decided *a priori*. Genomic biomarker prevalence is reported in the subset with nonprostate tissue containing tumor. Associations between CTC pretreatment and genomic biomarkers with PSA response were explored with 2×2 tables and Fisher exact test and biochemical PFS with Cox proportional hazards models. Thirteen exploratory biomarkers were assessed; effect size, 95% CIs, and *P* values were presented. The analysis was completed using SAS 9.4 (SAS Institute Inc.).

Results

Trial patients

To identify 60 patients with RB^+ tumors between July 2014 and February 2017, a total of 72 eligible patients were enrolled from seven centers (University of Utah: 27, University of Michigan: 14, City of Hope: 10, Vanderbilt: 9, Thomas Jefferson: 7, John Hopkins: 3, University of Washington: 2) and evaluated for their RB status (Schema: **Fig. 1**). Baseline demographics are shown in **Table 1**. No statistically significant differences in patient characteristics were observed, although there was a nonsignificant trend toward increased measurable disease in the PD + ADT versus ADT arms (67.5% vs. 45%, respectively, $P = 0.09$). All patients underwent metastatic disease biopsy ($n = 47$) or had previously undergone metastatic biopsy ($n = 25$). Forty-one patients had a biopsy of soft-tissue metastasis and 31 had a biopsy of bone lesion; 90% (64/72) had adequate tissue for RB assessment after a maximum of two biopsy attempts. Ninety-seven percent (62/64) retained RB expression and were stratified by early initiation of ADT and extent of disease and randomized. Sixty patients initiated treatment (Arm 1: 20, Arm 2: 40). Patients had median age of 66 (Arm 1) and 68 (Arm 2). Median baseline PSA was 55.7 (range, 10.9–2,882.5) for Arm 1 and 77.2 (range, 6.1–2,123) for Arm 2 ($P = 0.63$). The median number of cycles (cycle = 28 days) on study was 24.5 for Arm 1 and 24.5 for Arm 2. The most common reason for study discontinuation was disease progression (55% in arm 1; 50% in Arm 2). One patient on Arm 2 discontinued palbociclib and bicalutamide after 16 cycles due to an adverse event, development of interstitial lung disease. A total of 4 patients on Arm 2 discontinued treatment for reasons other than progression or adverse events (after 6–32 cycles). All patients were evaluable for the primary endpoint.

RB loss in metastatic HSPC

In total, 64 of 72 biopsied patients had metastatic tissue available for RB assessment. Representative hematoxylin and eosin (H&E) and RB staining is shown in **Fig. 2** for RB^+ and RB-negative (RB^-) patients. Two of 64 (3%) patients demonstrated loss of RB expression which was similar to previously published data (13). Ten primary prostate samples were obtained from RB^+ patients and also retained RB. Histologically, the RB^- tumors demonstrated neuroendocrine features. One of the RB^- patients also underwent genomic and transcriptomic analysis of the metastatic tumor tissue which confirmed presence of *RB1* homozygous deletion as well as *PTEN* loss and presence of *TMPRSS2-ERG* fusion. These findings demonstrate that RB loss is rare in the mHSPC population and suggest that RB loss

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in this setting may be associated with neuroendocrine/small cell differentiation.

Safety

Grade 3/4 adverse events by arm are shown in **Table 2**. No adverse events > grade 2 were observed in Arm 1. Fifty-eight percent of patients in Arm 2 experienced grade 3/4 adverse events and 48% experienced grade 3/4 hematologic events (19/40) with the most common being neutropenia (40%, 16/40). A total of 12.5% (5/40) of patients in Arm 2 had a grade 3 decreased white blood cell count. Other grade 3/4 adverse events occurred in ≤5% of patients.

Primary and secondary endpoints

All patients who initiated treatment were evaluable for the primary and secondary endpoints (**Table 3**). In total, 80% of patients in both arms achieved PSA ≤ 4 ng/mL [Arm 1: 16/20 = 80% (80% CI, 64–91), Arm 2: 32/40 = 80% (90% CI, 70–88), $P = 0.87$]. The rate of PSA ≤ 4 ng/mL in the control Arm 1 was higher than the anticipated rate of 70% based on historical data. Sixty-five percent (13/20) of patients in Arm 1 and 55% (22/40) of patients in Arm 2 achieved an undetectable PSA (< 0.2 ng/mL) a difference which was not statistically significant ($P = 0.50$). To determine whether palbociclib prolonged PSA response, we examined biochemical PFS (**Fig. 3A**). Rate of

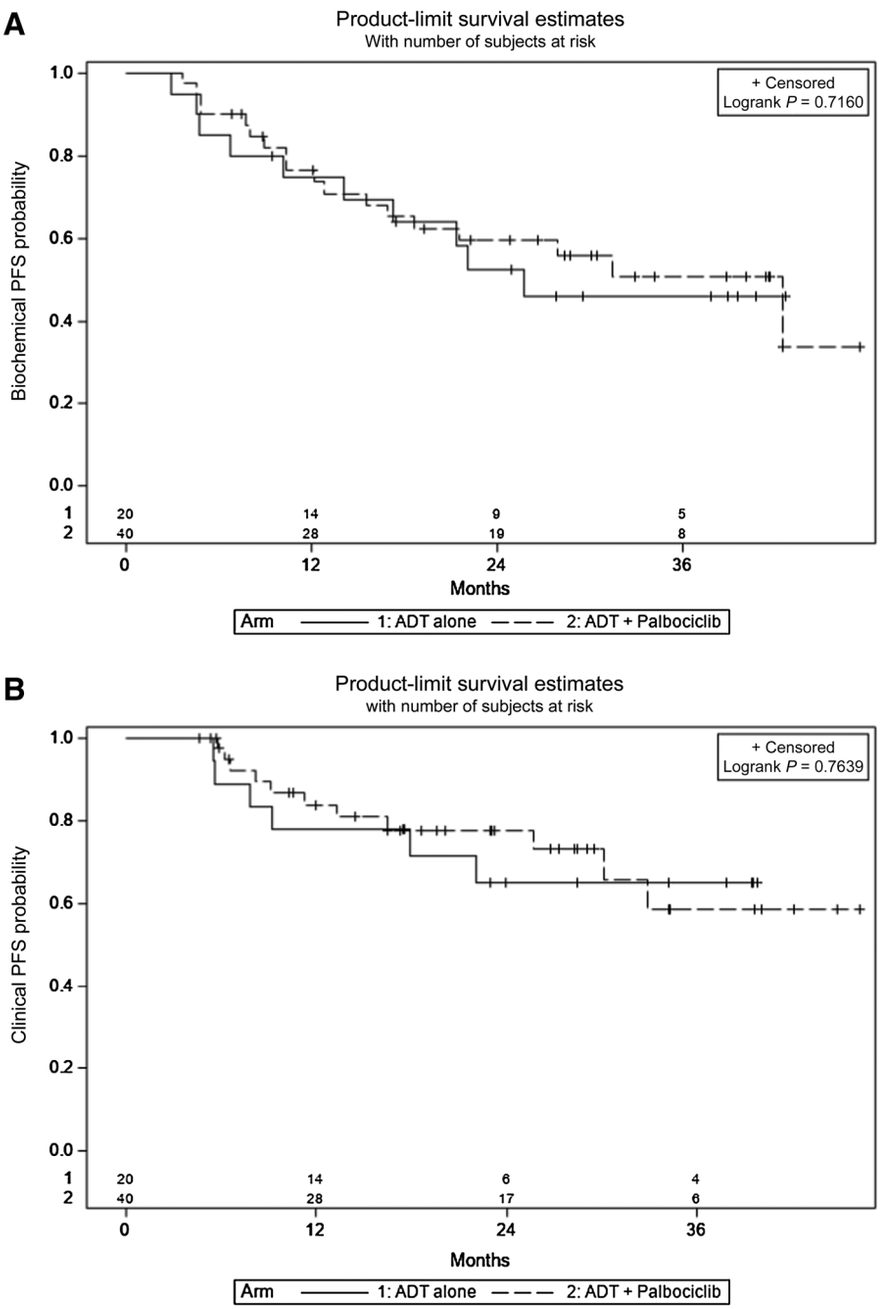

Figure 3.
PFS. There were no statistically significant differences in biochemical PFS (**A**) or clinical PFS (**B**) between ADT and ADT + palbociclib arms.

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Figure 4.
Waterfall plot for radiographic response. Metastatic lesion type is indicated across top of graph. Black bar, ADT + palbociclib; gray bar, ADT alone.

biochemical PFS at 12 months was 69% (95% CI, 44–85) for Arm 1 and 74% (95% CI, 57–85) for Arm 2 ($P = 0.72$) suggesting that palbociclib did not significantly delay PSA progression.

We examined the effect of palbociclib on measurable disease response and changes in bone metastasis burden. In total, 9/20 (45%) in Arm 1 had measurable disease as defined by RECIST and 27/40 (67.5%) of patients in Arm 2 had measurable disease ($P = 0.09$). A total of 30 of 40 (75%) patients in Arm 2 had bone disease compared with 14/20 (70%) in Arm 1 ($P = 0.65$). **Figure 4** presents a waterfall plot of change in RECIST tumor measurements by treatment demonstrating high response rates in both arms. The measurable disease response rate was not different between arms (Arm 1: 89% vs. Arm 2: 89%, $P = 0.78$; **Table 3**). Rates of bone scan improvement/stability (based on PCWG2 criteria) were also not significantly different between arms (Arm 1: 100%, 14/14 vs. Arm 2: 83.3%, 25/30, $P = 0.10$). **Figure 3B** demonstrates the radiographic PFS data for both arms again demonstrating no significant difference between arms ($P = 0.76$). Finally, median time to development of castrate-resistant disease (biochemical/radiographic progression) was similar between arms [Arm 1: 25.8 months (95% CI, 9.2–NR) vs. Arm 2: 27.9 months (95% CI, 12.2–NR); $P = 0.92$]. Together, these data suggest that there was no significant clinical benefit from the addition of palbociclib to primary ADT in newly diagnosed M1 prostate cancer, although the higher rate of measurable disease in Arm 2 suggests that the two arms may not have been balanced.

CTC

CTCs have previously shown promise as surrogate markers for overall survival in mCRPC as well in other malignancies (27). In SWOG S0925, lower baseline CTC also correlated with higher rates of PSA response in patients with mHSPC (28). To explore whether CTC enumeration provided additional information regarding treatment response in patients with mHSPC during treatment with ADT ± palbociclib, CTC counts were monitored at pretreatment, 12 and 28 weeks after starting treatment, and at progression using the EPIC platform. Thirty-three of 45 patients (73.3%) had detectable CTCs at pretreatment blood draw (**Fig. 5A**). There was no significant difference in pretreatment CTC by treatment arm. Pretreatment CTC counts of ≥ 2 ($n = 9$) were associated with decreased PSA CR rates ($P = 0.04$; **Table 4**) and decreased clinical PFS times ($P = 0.031$; **Fig. 5B**) in the entire cohort. Blood samples at progression also showed a statistically significant increase in CTC counts (mean increase 1.9 CTCs (95% CI, 0.1–3.6), paired t test $P = 0.038$; **Fig. 5C**) as compared with week 12 values. There was no statistically significant difference in CTC counts between the treatment arms at any of the assessed timepoints.

Molecular data

A total of 41 metastatic biopsy samples were sequenced. Ten matched primary prostate cancer specimens were also sequenced. **Table 5** summarizes the mutations and chromosomal aberrations identified in this study population. ETS fusions 22/40 (55%), PTEN 17/40 (42.5%), TP53 11/40 (27.5%), WNT pathway (APC, CTNNB1, ZNRF3, RSPO) 9/40 (22.5%), KMT2C 8/40 (20%), SPOP 6/40 (15%), PI3K pathway (PIK3R1, PIK3CB, PIK3CA) 5/40 (12.5%), CHD1 5/40 (12.5%), FOXA1 4/40 (10%), and BRCA1/2 3/40 (7.5%) were the most commonly detected mutations. 8p loss (35/40, 87.5%), 8q gain (18/40, 45%), 13q (18/40, 45%), and 7p gain (6/40, 15%) were the most commonly detected chromosomal aberrations. 8q amplification, CCND1 amplification, and KMT2C and PTEN mutations were observed in metastatic biopsies, but not matched primary prostate cancer samples. Clinically, TP53 (HR, 2.95; 95% CI, 1.21–7.22; $P = 0.01$), PI3K pathway (HR, 3.24; 95% CI, 1.03–10.1; $P = 0.04$) mutations, and 8q gain (HR, 4.68; 95% CI, 1.82–12.0; $P = 0.001$) were associated with reduced time to biochemical progression regardless of treatment arm in this exploratory analysis (**Fig. 6**). Of these molecular markers, only 8q gain, which is associated with MYC amplification, was also significantly associated with ≥2 CTCs ($P = 0.01$) and extensive stage disease ($P = 0.02$; ref. 29).

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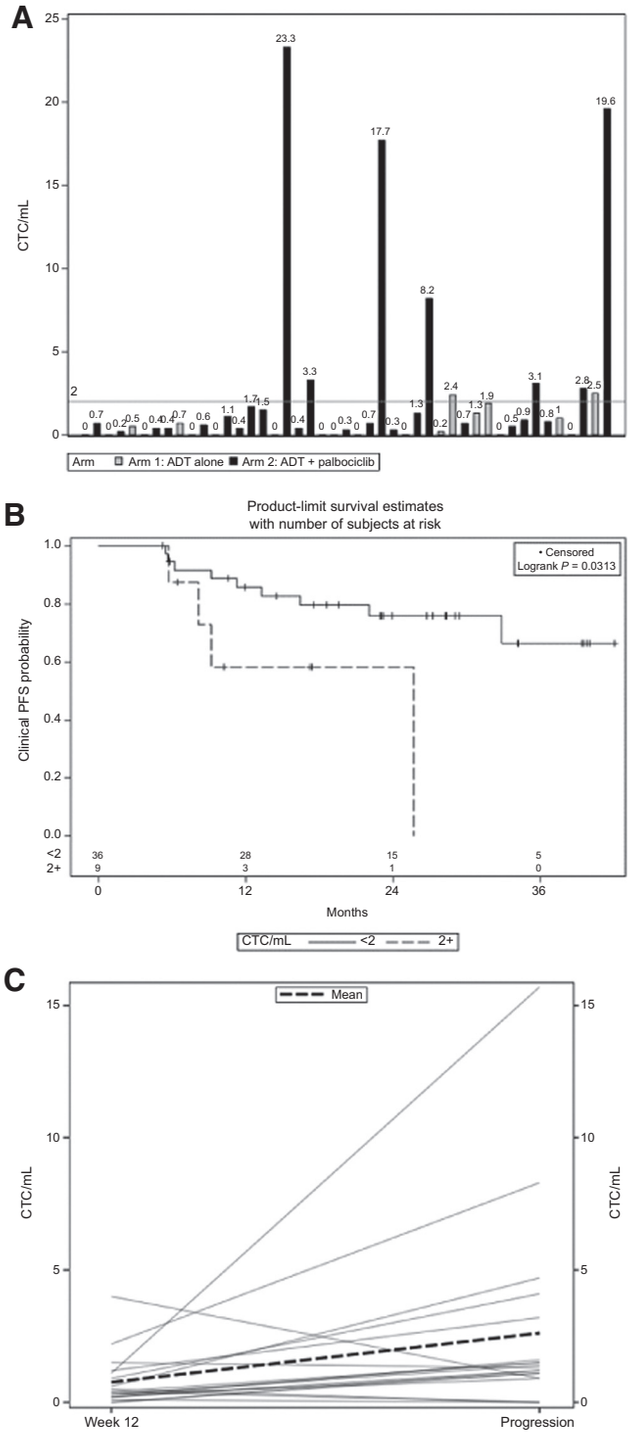

Figure 5.

CTC correlative data. **A,** Pretreatment CTC counts by patient and treatment arm. **B,** Stratification of patients by pretreatment CTC (≥ 2 or < 2 CTC/mL) demonstrated that patients with higher CTC counts have significantly shorter clinical PFS. **C,** CTC counts increase between week 12 and progression for most patients ($N = 19$).

Discussion

Despite decades of progress, mHSPC remains incurable. Preclinical data utilizing prostate cancer models supported the hypothesis that cotargeting CDK4/6 in conjunction with AR could provide benefit to patients with mHSPC (17). Of note, women with ER^+ advanced breast cancer demonstrated increased PFS when the hormonal and CDK4/6 axes are targeted in parallel (16).

The addition of the CDK4/6 selective inhibitor, palbociclib, to ADT did not improve PSA or radiographic response in men with new mHSPC with intact RB expression in this randomized phase II trial. The addition of palbociclib treatment resulted in expected toxicities including neutropenia. While the results of this study do not support the use of CDK4/6 targeted therapies in mHSPC patients, other ongoing studies are examining whether there is a role for these

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Table 4. CTC counts correlated with PSA response.

Pretreatment CTC count/mL	PSA ≤4 at 28 weeks	PSA >4 at 28 weeks	*P*
<2	30 (83.3%)	6 (16.7%)	0.49
2+	7 (77.8%)	2 (22.2%)	

Pretreatment CTC count/mL	PSA CR	PSA PR/SD	*P*
<2	25 (69.4%)	11 (30.6%)	0.04
2+	3 (33.3%)	6 (66.7%)	

Table 5. Molecular alterations in study cohort.

	N (%)
ETS fusion	22 (55.0%)
PTEN	17 (42.5%)
TP53	11 (27.5%)
WNT	9 (22.5%)
KMT2C	8 (20.0%)
SPOP	6 (15.0%)
PIK3 pathway	5 (12.5%)
CHD1	5 (12.5%)
FoxA1	4 (10.0%)
BRCA1/2	3 (7.5%)
AR	2 (5.0%)
CDK12	2 (5.0%)
ZFHX3	2 (5.0%)
KRAS	2 (5.0%)
CCND1	1 (2.5%)
ATM	1 (2.5%)
8p loss	35 (87.5%)
8q gain	18 (45.0%)
13q loss	18 (45.0%)
7p gain	6 (15.0%)

agents in mCRPC alone or in combination with other therapies (NCT02555189, NCT02494921, NCT02905318). It remains possible that CDK4/6 inhibition may offer clinical benefit to patients with more advanced disease or may work synergistically with other agents such as chemotherapy and newer androgen targeted therapies. Moreover, it will be important in future studies to address the efficacy of CDK4/6 targeted agents to effectively suppress kinase activity and engage RB function in prostate cancer.

Various factors may have contributed to the negative results observed here. First, shortly after enrollment started on this study, the CHAARTED data showing survival benefit to those with extensive metastatic disease was presented. After these results were presented, patients who were candidates for docetaxel were encouraged to receive docetaxel. We hypothesize that patients with more extensive or higher-risk disease, which were underrepresented in this study, may have been the population most likely to realize benefit from the treatment intensification strategy presented here. In SWOG 9346, which compared intermittent versus continuous ADT in men with mHSPC, 71% (965/1,345) and 45% (604/1,345) of patients had PSA ≤4 ng/mL and ≤0.2 ng/mL, respectively (18, 19), while in this study, the rates were 80% and 50%, respectively. The comparatively greater PSA responses in our study population with ADT alone may have limited our ability to detect significant improvements in the designated endpoints with intensified therapy. In addition, progression to castration resistance may not depend on CDK4/6 activation, or treatment with palbociclib may drive earlier loss of RB and thus escape from CDK4/6 inhibition.

Correlative studies from this study suggest potentially informative CTC and molecular markers. Similar to S0925, a randomized phase II study in a similar mHSPC population, where higher baseline CTC was associated with decreased PSA response (28), we found that baseline CTCs ≥ 2 were associated with lower PSA response and PFS. Similar to other studies, we found that *RB* and *TP53* loss correlated with neuroendocrine differentiation (30). Molecular analysis of tumor samples from our patient population also revealed that ETS fusions

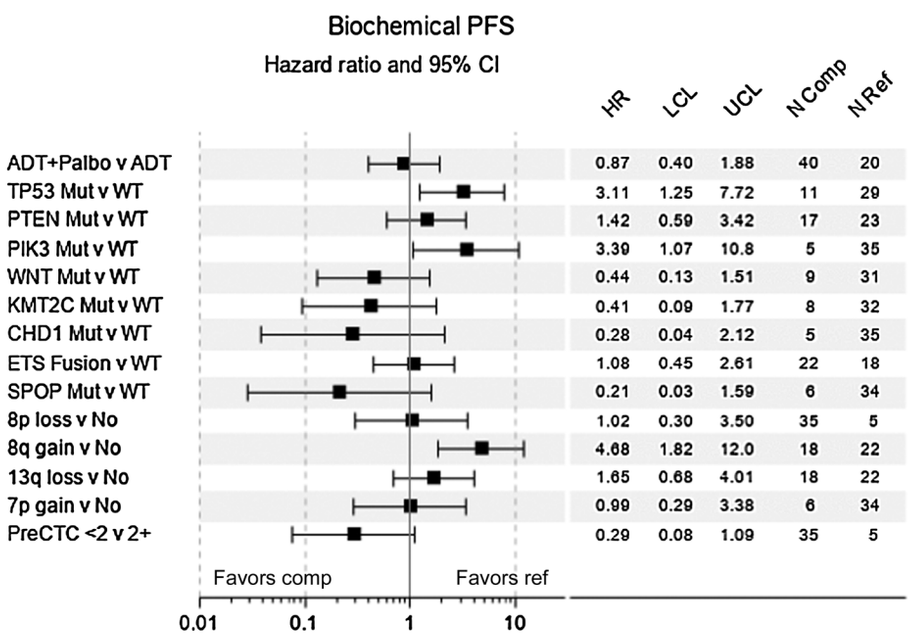

Figure 6.
Forrest plot of biochemical PFS and association with mutation and CTC counts.

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and *PTEN* and *TP53* mutations were the most common molecular events identified in this cohort of mHSPC, similar to other studies (13). We observed that *TP53* mutations are associated with reduced time to PSA progression but also identified 8q gain and *PIK3* mutations as having potential prognostic significance. These results are comparable with other published data for this patient population (31). Taken together, these data further suggest that CTCs and mutations have potential prognostic importance in patients with mHSPC which will require further validation.

This study illustrates the feasibility of biopsy- and biomarker-driven studies in the treatment-naïve mHSPC population. Because most studies of new therapies have focused on the castrate-resistant patient population, few metastatic disease biopsy-driven biomarker-based studies have been attempted in the treatment-naïve mHSPC population. The survival benefit of treatment intensification with abiraterone, enzalutamide, apalutamide, or docetaxel in patients with newly diagnosed mHSPC further raises the bar for development of biomarker-targeted therapies in this clinical space. Our data suggest that molecular profiling of mHSPC and testing of appropriate molecularly targeted therapies in this patient population is feasible, but that targeting RB was not associated with benefit. This does not rule out potential benefit from other appropriately selected targeted therapies. The goal is to better personalize care for patients as much as possible. Efforts toward this goal are in progress specifically in relation to DNA repair defective tumors (NCT03413995, NCT04332744). Thus, while biopsy- and biomarker-driven strategies may not be appropriate for all patients with mHSPC, precision-based therapy is a feasible and attractive approach for those with high-risk disease or DNA repair deficient (DRD) tumors.

Authors' Disclosures

P.L. Palmbos reports grants from Pfizer and Prostate Cancer Foundation during the conduct of the study, as well as other from Immunomedics and F. Hoffmann-La Roche Ltd outside the submitted work. S. Daignault-Newton reports other from Pfizer during the conduct of the study, as well as personal fees from American Urological Association outside the submitted work. S.A. Tomlins reports nonfinancial support from Ventana/Roche during the conduct of the study, as well as personal fees and other from Strata Oncology and grants and personal fees from Astellas outside the submitted work; in addition, S.A. Tomlins has a patent for ETS gene fusions in prostate cancer issued, licensed, and with royalties paid from Ventana/Roche, Hologic/GenProbe, and LynxDX. N. Agarwal reports personal fees from and reports consultancy with Astellas, AstraZeneca, Aveo, Bayer, Bristol Myers Squibb, Calithera, Clovis, Eisai, Eli Lilly, EMD Serono, Exelixis, Foundation Medicine, Genentech, Janssen, Merck, MEI Pharma, Nektar, Novartis, Pfizer, Pharmacyclics, and Seattle Genetics during the conduct of the study. A.K. Morgans reports personal fees from Astellas, AstraZeneca, Advanced Accelerator Applications, Clovis, Dendreon, Blue Earth, Janssen, and Pfizer; grants and personal fees from Bayer; nonfinancial support from Genentech; and personal fees and nonfinancial support from Sanofi, Seattle Genetics, and Myovant during the conduct of the study. W.K. Kelly reports other from Novartis during the conduct of the study. V.K. Arora reports personal fees from Bristol Myers Squibb and grants and personal fees from ORIC Pharmaceuticals outside the submitted work. E.S. Antonarakis reports grants and personal fees from Merck, AstraZeneca, Clovis, Amgen, BMS, Eli Lilly, Bayer, Constellation, and Curium outside the submitted work. M.S. Davenport reports other from Wolters Kluwer outside the submitted work. A.M. Chinnaiyan reports grants from NIH and HHMI during the conduct of the study, as well as personal fees and other from Tempus and other from LynxDx outside the submitted work. K.E. Knudsen reports advisory/consulting relationships with Sanofi, CellCentric, Genentech, and Janssen, unrelated to this work, and in the last 3 years, over the same timeframe received research support from CellCentric and Celgene. M. Hussain reports grants from Pfizer and PCF during the conduct of the study. M. Hussain also reports personal fees from Sanofi/Genzyme, Daiichi Sankyo, BMS, RTP, Janssen, and Merck; grants, personal fees, and other from Bayer and Genentech; personal fees and other from Astellas and Pfizer; and grants and personal fees from AstraZeneca outside the submitted work. No disclosures were reported by the other authors.

Authors' Contributions

P.L. Palmbos: Investigation, writing–original draft, writing–review and editing. **S. Daignault-Newton:** Data curation, formal analysis, validation, methodology. **S.A. Tomlins:** Study pathologist. **N. Agarwal:** Co-investigator on the clinical trial, accruing patients, review and approve manuscript. **P. Twardowski:** Co-investigator on the clinical trial, accruing patients, review and approve manuscript. **A.K. Morgans:** Co-investigator on the clinical trial, accruing patients, review and approve manuscript. **W.K. Kelly:** Co-investigator on the clinical trial, accruing patients, review and approve manuscript. **V.K. Arora:** Co-investigator on the clinical trial, accruing patients, review and approve manuscript. **E.S. Antonarakis:** Co-investigator on the clinical trial, accruing patients, review and approve manuscript. **J. Siddiqui:** Resources. **J.A. Jacobson:** Radiology support. **M.S. Davenport:** Resources, radiology support. **D.R. Robinson:** Resources, genomics analysis and interpretation. **A.M. Chinnaiyan:** Genomics analysis and interpretation. **K.E. Knudsen:** Translation of science. **M. Hussain:** Conceptualization, supervision, investigation, writing–review and editing.

Acknowledgments

Funding provided by Pfizer for the clinical trial.

This work was funded by grants from Pfizer, Movember-PCF Challenge (to M. Hussain), NIH K08 CA201335 (to P.L. Palmbos), and EDRN U01CA214170 and SPORE P50 CA186786 (to J. Siddiqui). The authors would like to thank the patients and their families for participation in this study. Clinical Trial Registration: NCT02059213.

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Received January 6, 2021; revised February 16, 2021; accepted March 12, 2021; published first March 16, 2021.

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