Clinical Cancer Research

Differential Effect on Bone Lesions of Targeting Integrins: Randomized Phase II Trial of Abituzumab in Patients with Metastatic Castration-Resistant Prostate Cancer

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Abstract

Purpose: Integrins play a critical role in the progression of prostate cancer and its bone metastases. We investigated the use of the pan-αv integrin inhibitor abituzumab in chemotherapy-naïve patients with asymptomatic or mildly symptomatic metastatic castration-resistant prostate cancer.

Experimental Design: PERSEUS (NCT01360840) was a randomized, double-blind phase II study. Men with pathologically confirmed prostate cancer and radiologic progression of bone lesions in the 28 days prior to randomization were assigned to receive abituzumab 750 mg or 1,500 mg or placebo (1:1:1) every 3 weeks in combination with luteinizing hormone-releasing hormone agonist/antagonist therapy. The primary endpoint was progression-free survival (PFS).

Results: The intent-to-treat population comprised 180 patients, 60 in each arm. The primary endpoint of PFS was not significantly different with abituzumab-based therapy compared with placebo [abituzumab 750 mg, 3.4 months, HR = 0.89; 95% confidence interval (CI), 0.57–1.39; abituzumab 1,500 mg, 4.3 months, HR = 0.81; 95% CI, 0.52–1.26; placebo, 3.3 months], but the cumulative incidence of bone lesion progression was lower with abituzumab than with placebo for up to 24 months (cumulative incidence 23.6% vs. 41.1% at 6 months, 26.1% vs. 45.4% at 12 months). Two partial tumor responses were observed (1 abituzumab 1,500 mg and 1 placebo). Approximately 85% to 90% of patients experienced at least one treatment-emergent adverse event (TEAE) in the different arms, but the incidences of serious TEAEs and TEAEs with fatal outcome were similar in the three arms.

Conclusions: Although PFS was not significantly extended, abituzumab appears to have specific activity in prostate cancer–associated bone lesions that warrants further investigation. *Clin Cancer Res; 22(13); 3192–200. ©2016 AACR.*

Introduction

Although metastatic prostate cancer can initially be managed using androgen suppression therapy (1, 2), most patients subsequently progress to castration-resistant prostate cancer (CRPC; ref. 3). Metastases are present in at least 80% of men with CRPC, with bone a preferred metastatic site and skeletal-related events (SRE) such as bone pain, fractures, spinal cord compression, and vertebral collapse being common (4, 5). SREs are associated with increased mortality risk and health care costs, and decreased quality of life (4, 6).

Several treatment options are available for the management of metastatic CRPC (mCRPC), including radiotherapy, hormonal therapies, and cytotoxic agents (4). The cytochrome P17 antagonist abiraterone and androgen receptor blocker enzalutamide, which both improve overall survival (OS), have recently been approved by the Food and Drug Administration (7, 8) and the European Medicines Agency (9, 10) for the treatment of patients with mCRPC (11).

Integrins are heterodimeric cell adhesion molecules (12) involved in cell survival, proliferation, and migration (13). Tight regulation of integrin signaling is necessary for normal cell function, and deregulation is associated with many diseases, including cancer (12); in prostate cancer, deregulated integrin expression has been associated with progression to an advanced stage (14). Most integrin α and β subunits are reported to be downregulated in prostate cancer, whereas α_6 and αv tend to be upregulated (14, 15). αv integrins are known to be important in tissue remodeling associated with wound repair, angiogenesis, and cancer (16); deregulated integrin expression and its effects on apoptosis, cell adhesion, proliferation, and migration appear to influence the progression of prostate cancer to CRPC (15).

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Prior presentation: This trial has been presented in part at the European Society for Medical Oncology 37th Congress in September 2012 (abstract 695TIP); the American Society of Clinical Oncology 50th Annual Meeting in May/June 2014 (abstract 5030); and the European Society for Medical Oncology 39th Congress in September 2014 (abstract 772P).

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Translational Relevance

Bone is the most frequent site of metastasis and disease-related morbidity in patients with metastatic castration-resistant prostate cancer. Integrins, cell adhesion molecules that help to control the survival, proliferation, and migration of normal cells, play a critical role in cancer when deregulated. αv integrins are implicated in prostate cancer invasion and particularly bone metastasis. We examined the efficacy of the pan-αv integrin inhibitor abituzumab in a double-blind phase II study in which men with chemotherapy-naïve, asymptomatic or mildly symptomatic metastatic CRPC were randomized to receive abituzumab or placebo in combination with luteinizing hormone-releasing hormone agonist/antagonist therapy. Overall progression-free survival was not significantly improved with abituzumab-based therapy compared with placebo, but the incidence of bone lesion progression was lower with abituzumab than with placebo. The apparent specific activity of abituzumab against prostate cancer–associated bone lesions warrants further investigation.

Analysis of a large prostate cancer biopsy series from the Charité Hospital (Berlin, Germany) indicated that primary tumors express integrin αvβ5, whereas other αv integrins (αvβ3, αvβ6, and αvβ8) were generally expressed at low levels (Merck KGaA; unpublished data). This observation is supported by another study showing αvβ5 expression by primary prostate tumor cells (17). Other studies have suggested that integrin αvβ5 is expressed in association with tumor invasion and metastasis (16, 18). Furthermore, studies have implicated αv integrins in bone metastasis and bone resorption (14), and the survival of prostate tumor cells in bone (19), the development of osteoblastic bone lesions (20), and in osteoclast-mediated bone resorption in experimental prostate cancer bone metastases (21). These observations suggest that αv integrins are a rational therapeutic target in prostate cancer.

The humanized monoclonal IgG2 antibody abituzumab (EMD 525797) specifically binds to the αv integrin subunit, inhibiting the interaction of αv integrins with ligands in the extracellular matrix (ECM; ref. 22). Preclinical studies confirmed that abituzumab inhibits xenograft tumor growth (23). In a phase I study, single-agent abituzumab had activity in patients with CRPC and bone metastases (24): 18 of 26 patients did not have disease progression (PD) for ≥18 weeks and 2 patients had clinically significant reductions in PSA and pain relief. One of these patients had a confirmed partial response (PR; ref. 24).

On the basis of the role of αv integrins in prostate cancer and the data from the phase I trial, we conducted a phase II trial of abituzumab in combination with the standard of care (SOC) of continuous treatment with a luteinizing hormone-releasing hormone (LHRH) agonist/antagonist to investigate its antitumor activity in chemotherapy-naïve patients with asymptomatic or mildly symptomatic mCRPC.

Patients and Methods

Study design and treatment

PERSEUS was a randomized, double-blind, placebo-controlled, multicenter phase II trial (ClinicalTrials.gov identifier: NCT01360840) carried out at 65 centers in 11 countries (Australia, Belgium, Canada, France, Germany, Netherlands, Poland, Russia, South Africa, Spain, and the United States). Patients were centrally randomized 1:1:1 to continuous treatment with an LHRH agonist/antagonist combined with placebo, abituzumab 750 mg, or abituzumab 1,500 mg every 3 weeks. Use of bisphosphonate therapy was also protocol specified. The study was approved by institutional review boards at each participating institution, and all patients gave informed written consent before enrollment. The study was conducted according to the principles of the Declaration of Helsinki.

Two dose levels of abituzumab were selected in this trial to achieve different levels of target saturation over 3 weeks, because no firm dose recommendation could be made based on an earlier phase I study (24). Pharmacokinetic guidance from this study suggested that abituzumab 750 mg every 3 weeks was likely to achieve serum trough concentrations above the 95% inhibitory concentration (IC_{95}) and to peak at the 99% inhibitory concentration (IC_{99}). Abituzumab 1,500 mg every 3 weeks was selected to provide steady-state serum trough concentrations above the IC_{99} at the end of the 3-week dosing interval.

Patients randomized to placebo who experienced PD were permitted to cross over to open-label treatment with abituzumab 1,500 mg every 3 weeks. Therapy continued until PD or unacceptable toxicity.

Patients

Adults aged ≥18 years with pathologically confirmed adenocarcinoma of the prostate were enrolled. Patients had to have radiologic progression of bone lesions with or without soft-tissue lesions in the 4 weeks (28 days) prior to randomization. Stable and ongoing adequate testosterone suppression was required and demonstrated by castrate levels of testosterone (≤50 ng/dL) for patients who had not undergone surgical castration. Other inclusion criteria included Eastern Cooperative Oncology Group performance status (ECOG PS) 0–1 and acceptable laboratory parameters (including absolute neutrophil count $\geq 1.5 \times 10^9$/L, platelets $\geq 100 \times 10^9$/L, hemoglobin ≥9 g/dL, and total bilirubin ≤1.5 times the upper limit of normal). Bisphosphonate treatment had to start at least 2 days before starting treatment with abituzumab.

Key exclusion criteria included acute pathologic fracture, spinal cord compression, or hypercalcemia at screening; use of nonsteroidal antiandrogens (e.g., flutamide or bicalutamide) within 30 days before treatment; chronic and ongoing treatment with opioids (treatment >10 days); prior chemotherapy, biologic therapy (targeted therapy), or any experimental therapy for mCRPC; radiotherapy to bone lesions and/or orthopedic surgery for pathologic fractures; brain or visceral metastases; and uncontrolled hypertension (systolic blood pressure ≥160 mm Hg and/or diastolic blood pressure ≥100 mm Hg at rest).

Assessments

Tumors were assessed by imaging using thoracic, pelvic, and abdominal CT or MRI scans (if CT contraindicated) at baseline and 12 weeks, then every 6 weeks for four assessments, and subsequently every 12 weeks until PD. Earlier assessment was mandated if symptomatic progression was noted. Patients with bone metastases also underwent bone scintigraphy at these time points. Radiographic imaging for PD was carried out according to

the guidelines of the Prostate Cancer Clinical Trials Working Group (PCWG-2; ref. 25).

Patients who stopped treatment for any reason other than PD underwent PSA and complete tumor assessments every 12 weeks until progression, death, or the end of the study. Response assessment was based on modified RECIST version 1.0, with confirmation 4 to 6 weeks after initial assessment. Suspected PSA responses required confirmatory analysis at the next treatment cycle.

Patients were continuously monitored for treatment-emergent adverse events (TEAE) from the time of first study drug administration until 50 days after the final dose of study drug. Potential causal relationships between the study drug and TEAEs were assessed by investigators. Abnormal laboratory findings (laboratory tests performed before each infusion and at weeks 2–6) and other abnormal investigational findings were not to be reported as TEAEs unless they were associated with clinical signs and symptoms, led to treatment discontinuation, or were considered otherwise medically important by the investigator. Toxicity was graded using National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. Serious TEAEs and related events leading to treatment withdrawal or death were recorded.

All patients were required to provide serum samples for baseline PSA assessment and for subsequent assessments at the beginning of each treatment cycle. All patients also provided formalin-fixed, paraffin-embedded primary tumor material (blocks or biopsy) for assessment of integrin expression during screening. Integrin expression in formalin-fixed, paraffin-embedded tumor material was assessed by IHC using antibodies against pan-αv, αvβ5, and αvβ6 as described previously (26).

In addition, blood samples were provided for predose assessment of circulating tumor cells (CTC) on day 1 of cycles 1, 3, and 5, and of protein and urine biomarkers, including those of bone metabolism, on day 1 of cycles 1, 3, 5, and 7. CTCs were analyzed centrally using the CellSearch Circulating Tumor Cell Kit (Janssen Diagnostics) according to the manufacturer's standard processes. Protein analyses were carried out using IHC, ELISA, and Luminex.

Blood samples for pharmacokinetic analyses were taken from 12 patients in each arm at various time points during cycles 1 to 7. Pharmacokinetic parameters were calculated according to noncompartmental standard methods using KINETICA version 4.1.1 (Innaphase).

Statistical analysis

The primary endpoint was progression-free survival (PFS), defined as the time from randomization to first documented radiographic PD (appearance of $\geq$2 new bone lesions on scintigraphy, confirmed after 6 weeks if asymptomatic or mildly symptomatic; soft-tissue lesion progression according to RECIST 1.0 modified according to PCWG-2; or presence of skeletal events defined as cord compression or fracture) or death from any cause within 12 weeks following the last tumor assessment. A sample size of 165 patients (55 per arm) was planned to observe 110 PFS events (40 in the placebo arm and 35 in each of the abituzumab arms) by the primary analysis cut-off date based on the following assumptions: HR of 0.67 versus placebo; median PFS of 2.76 months with placebo and 4.14 months with abituzumab-based therapy; and a dropout rate of 10%. The sample size was selected to control the standard deviation of the HRs between each of the treatment arms and placebo to a level of 0.23 under the normal approximation assumption.

Secondary endpoints included OS; time to progression (TTP; defined as the time from the date of randomization to the date of objective radiographic PD); overall tumor response; disease control; TTP in soft-tissue lesions, bone lesions, and bone or soft-tissue lesions; PSA response and CTC responses; markers of bone metabolism; and safety. Tumor response in soft-tissue lesions was defined as the presence of at least one confirmed complete response (CR) or confirmed PR. Disease control in soft-tissue lesions was defined as the presence of at least one confirmed CR or confirmed PR or stable disease lasting for $\geq$12 weeks after randomization. Disease control in bone lesions was defined as the appearance of <2 new bone lesions on scintigraphy.

Predefined exploratory endpoints included the association of tumor integrin expression with efficacy and time to derived clinical progression (TTCP; defined as for PFS, but also including time to ECOG PS deterioration and time to initiation or dose increase of medications of interest, including bisphosphonates, analgesics, and anticancer therapy).

The primary analysis of efficacy used the intent-to-treat (ITT) population comprising all patients randomized. The safety analysis population included all patients who received at least one dose of any study drug. HRs for the comparison of each abituzumab arm with the placebo arm were estimated using a Cox proportional hazard model with treatment as the unique covariate. A secondary analysis comparing the pooled abituzumab arms with the placebo arm was performed using a univariate Cox proportional hazards model adjusted for treatment.

To assess the impact of the individual events forming the composite endpoint of PFS, a competing risk analysis was also performed (27).

Secondary endpoints were analyzed as follows: OS was assessed using univariate and multivariate Cox proportional hazards models, whereas PSA and soft tissue, bone, and soft tissue or bone lesion response rates were assessed using descriptive statistics.

For the assessment of the exploratory endpoint of association of tumor integrin expression with patient outcomes, the median histoscore was initially used as a cutoff between high and low expression. The cutoff was then varied around the median to assess whether the median was acceptable for the patient population in the trial.

Statistical analyses were performed using the SAS System (version 9.2 or later).

Results

Patient population

In total, 180 eligible patients were enrolled and randomized between April 2011 and December 2012. Median treatment durations at the primary analysis cut-off date of April 30, 2013 were placebo + SOC, 4.2 (0.5–16.6) months (10 patients remained on treatment at the cut-off date and were censored); abituzumab 750 mg + SOC, 4.1 (0.7–17.1) months (10 patients remained on treatment, 2 others never received study treatment); abituzumab 1,500 mg + SOC, 4.2 (0.7–16.6) months (9 patients remained on treatment; Supplementary Fig. S1). Twenty patients crossed over to open-label treatment; of these, 17 (85.0%) were off-treatment at the primary analysis cut-off date. PD was the primary reason for treatment discontinuation in all arms (Supplementary Fig. S1).

Baseline characteristics of patients in the three treatment arms were generally well balanced (Table 1). Most patients had stage IV

Table 1. Demographic and disease characteristics at baseline: ITT population[a]

Characteristic	Placebo + SOC *n* = 60	Abituzumab 750 mg + SOC *n* = 60	Abituzumab 1,500 mg + SOC *n* = 60	Pooled abituzumab arms *n* = 120
Race, *n* (%)				
Black or African American	2 (3.3)	1 (1.7)	3 (5.0)	4 (3.3)
Asian	0	1 (1.7)	0	1 (0.8)
Native Hawaiian or other Pacific Islander	0	0	1 (1.7)	1 (0.8)
Caucasian	57 (95.0)	58 (96.7)	56 (93.3)	114 (95.0)
Other	1 (1.7)	0	0	0
Median age, years (range)	71.0 (46–88)	69.5 (54–84)	71.0 (53–88)	70.0 (53–88)
ECOG PS[b]				
0, *n* (%)	32 (53.3)	39 (67.2)	34 (56.7)	73 (61.9)
1, *n* (%)	25 (41.7)	18 (31.0)	22 (36.7)	40 (33.9)
Missing, *n* (%)	3 (5.0)	1 (1.7)	4 (6.7)	5 (4.2)
Site of metastases, *n* (%)				
Bone only	51 (85.0)	52 (86.7)	52 (86.7)	104 (86.7)
Bone + liver + other	0	1 (1.7)	0	1 (0.8)
Bone + lung	0	0	1 (1.7)	1 (0.8)
Bone + other	7 (11.7)	3 (5.0)	6 (10.0)	9 (7.5)
Lung	0	1 (1.7)	0	1 (0.8)
Other	1 (1.7)	0	0	0
Missing	1 (1.7)	3 (5.0)	1 (1.7)	4 (3.3)
Tumor grade, *n* (%)				
Well differentiated	5 (8.3)	8 (13.3)	8 (13.3)	16 (13.3)
Moderately differentiated	16 (26.7)	11 (18.3)	13 (21.7)	24 (20.0)
Poorly differentiated	19 (31.7)	24 (40.0)	27 (45.0)	51 (42.5)
Not applicable	14 (23.3)	13 (21.7)	7 (11.7)	20 (16.7)
Missing	6 (10.0)	4 (6.7)	5 (8.3)	9 (15.0)

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; SOC, standard of care.
[a]Unless stated otherwise.
[b]Safety analysis set.

disease at baseline (95.0% in the placebo and abituzumab 1,500 mg groups and 96.7% in the abituzumab 750 mg group), and metastases were localized to or associated with bone in all patients except 2. On the basis of the inclusion criteria (bone lesion progression without SREs, asymptomatic or mildly symptomatic mCRPC), patients had early mCRPC and therefore had not received extensive pretreatment for mCRPC. Patients had stable, ongoing, adequate testosterone suppression [hypogonadal levels of testosterone ($\leq$50 ng/dL)] prior to study entry either due to surgical castration (8 patients in each of the placebo and abituzumab 750 mg groups and 11 in the abituzumab 1,500 mg group) or hormonal therapy. Only approximately 50% of patients in each of the treatment arms had received prior hormonal therapy for mCRPC because all patients were recruited before the approval of abiraterone acetate for the treatment of patients with mCRPC who are asymptomatic or mildly symptomatic after failure of androgen deprivation (FDA approval December 2012, European Medicine Agency approval 2013).

Efficacy

PFS. At the primary analysis cut-off date, 123 patients had PFS events (Table 2). A modest treatment effect of abituzumab compared with placebo, with HRs of 0.89 [95% confidence interval (CI), 0.57–1.39] in the 750 mg arm and 0.81 (95% CI, 0.52–1.26) in the 1,500 mg abituzumab arm (ITT analysis set), was observed (Fig. 1). However, the prespecified effect (target HR <0.67 vs. placebo) was not achieved.

An effect of abituzumab treatment (pooled doses) on bone lesions was observed, with the cumulative incidence of bone lesion progression being lower with abituzumab than with placebo for up to 24 months (cumulative incidence 23.6% vs. 41.1% at 6 months, 26.1% vs. 45.4% at 12 months; Fig. 2). PD was commonly the result of progression of soft-tissue lesions, which was more frequent than bone lesion progression, although differences between abituzumab and placebo were less marked than for bone progression (cumulative incidence 33.4% vs. 24.1% at 6 months, 39.3% vs. 30.5% at 12 months; Table 2). Inhibition of bone lesion formation by abituzumab was therefore identified as the driver of the PFS difference between abituzumab and placebo.

TTP and TTCP results supported the PFS findings. TTP HRs relative to placebo of 0.91 (0.58–1.44) and 0.81 (0.52–1.27) were reported in the 750 mg and 1,500 mg abituzumab arms, respectively; corresponding HRs for TTCP were 1.04 (0.69–1.57) and 0.96 (0.63–1.44; Table 2).

Tumor response. The best overall response in soft-tissue lesions was PR in 2 patients, 1 in the abituzumab 1,500 mg arm and 1 in the placebo arm (Table 2). Baseline PSA and CTC levels were highly variable, and there were no apparent effects of abituzumab on the levels of these markers. Confirmed decreases in PSA levels of $\geq$50% from baseline were noted in 6 (10.0%), 5 (8.3%), and 3 (5.0%) patients in the abituzumab 750 mg and 1,500 mg and placebo arms, respectively. CTC improvement (>5 at baseline to $\leq$5 on-treatment) was observed in 2 (3.3%), 1 (1.7%), and 3 (5.0%) patients, and CTC worsening ($\leq$5 at baseline to >5 on-treatment) was observed in 6 (10.0%), 4 (6.7%), and 4 (6.7%) patients receiving abituzumab 750 mg and 1,500 mg and placebo, respectively. However, patient numbers for these endpoints were small, which precluded further interpretation and correlation.

OS. Survival data (Table 2) were immature at the time of data cutoff, with <25% of patients having died.

Table 2. Efficacy in the ITT population

	Placebo + SOC *n* = 60	Abituzumab 750 mg + SOC *n* = 60	Abituzumab 1,500 mg + SOC *n* = 60	Pooled abituzumab arms *n* = 120
Progression-free survival (PFS)				
Patients with events, *n* (%)	43 (71.7)	41 (68.3)	39 (65.0)	80 (66.7)
Reason for progression, *n* (%)				
Death within 12 weeks after last tumor assessment	2 (3.3)	1 (1.7)	2 (3.3)	3 (2.5)
Bone progression	25 (41.7)	14 (23.3)	14 (23.3)	28 (23.3)
Soft-tissue lesion progression	15 (25.0)	20 (33.3)	21 (35.0)	41 (34.2)
Skeletal-related event	1 (1.7)	6 (10.0)	2 (3.3)	8 (6.7)
Median PFS, months (95% CI)	3.3 (2.8–4.8)	3.4 (2.8–5.6)	4.3 (2.8–6.6)	4.1 (2.8–5.6)
HR (95% CI)	NA	0.89 (0.57–1.39)	0.81 (0.52–1.26)	0.85 (0.58–1.24)
Time to tumor progression (TTP)				
Patients with events, *n* (%)	41 (68.3)	40 (66.7)	37 (61.7)	77 (64.2)
Median TTP, months (95% CI)	3.3 (2.8–5.4)	3.4 (2.8–5.6)	4.6 (2.8–6.9)	4.2 (2.8–5.6)
HR (95% CI)	NA	0.91 (0.58–1.44)	0.81 (0.52–1.27)	0.86 (0.58–1.27)
Time to derived clinical progression (TTCP)[a]				
Patients with events, *n* (%)	49 (81.7)	46 (76.7)	45 (75.0)	91 (75.8)
Median TTCP, months (95% CI)	2.8 (2.7–3.5)	2.8 (2.6–3.4)	2.9 (2.8–5.4)	2.8 (2.7–3.6)
HR (95% CI)	NA	1.04 (0.69–1.57)	0.96 (0.63–1.44)	1.0 (0.70–1.42)
Best overall response in soft-tissue lesions				
Patients evaluable[b]	28	22	24	46
Complete response, *n* (%)	0	0	0	0
Partial response, *n* (%)	1 (3.6)	0	1 (4.2)	1 (2.2)
Stable disease, *n* (%)	16 (57.1)	8 (36.4)	14 (58.3)	22 (47.8)
Progression, *n* (%)	8 (28.6)	10 (45.5)	9 (37.5)	19 (41.3)
Not assessed, *n* (%)	3 (10.7)	4 (18.2)	0	4 (8.7)

Abbreviations: CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; NA, not applicable; SOC, standard of care.
[a]Defined as time from date of randomization to objective radiographic disease progression or death, ECOG PS deterioration, or initiation or dose increase of medications of interest, including bisphosphonates, analgesics, and anticancer therapy, whichever occurred first.
[b]Patients with measurable disease at baseline. Percentages shown are proportions of these numbers.

Safety

Proportions of patients experiencing at least one TEAE, serious TEAEs, and TEAEs with fatal outcome were similar in the three treatment arms (Table 3). The incidence of treatment-related TEAEs was highest in the abituzumab 1,500 mg arm, as was the incidence of TEAEs of grade ≥3 (43.3% vs. 25.0% and 29.3%; Table 3). The abituzumab arms had higher rates of permanent treatment discontinuation than placebo for both TEAEs overall and treatment-related TEAEs (Table 3).

The most commonly reported TEAEs considered by the investigator to be related to study treatment were fatigue (10.3%, 6.7%, and 11.7% of patients in the abituzumab 750 mg and 1,500 mg and placebo arms, respectively) and anemia (1.7%, 11.7%, and 6.7%). Other related TEAEs reported in >5% of abituzumab-treated patients included decreased appetite, nausea, influenza-like illness, and pruritus. One death judged by the investigators to be related to treatment was observed in the placebo arm. No new safety signals were observed.

Integrin and bone biomarker expression

175 primary prostate cancer samples were assessed for pan-αv, αvβ5, and αvβ6 expression by IHC (26). A histoscore of 50 was chosen as the cut-off for the presence or absence of integrin

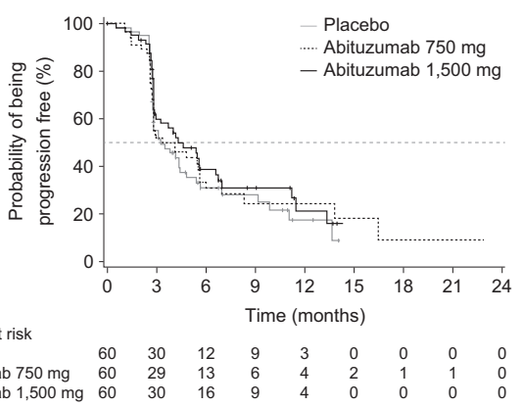

Figure 1.
PFS in the ITT population.

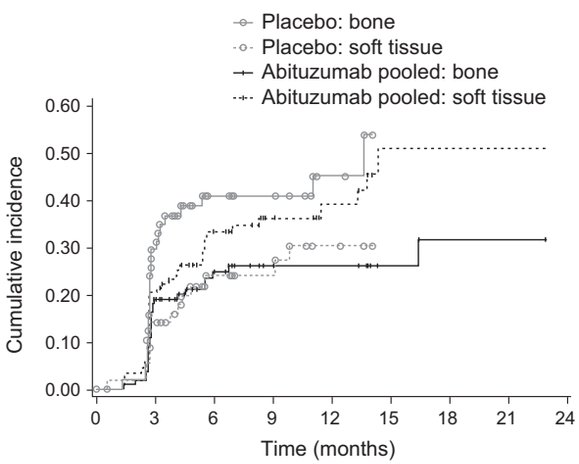

Figure 2.
Cumulative incidence of bone and soft-tissue lesion progression over the course of the study (ITT analysis set).

Table 3. Overview of TEAEs: safety population

	Placebo + SOC *n* = 60	Abituzumab 750 mg + SOC *n* = 58	Abituzumab 1,500 mg + SOC *n* = 60	Pooled abituzumab arms *n* = 118
TEAEs, *n* (%)	55 (91.7)	49 (84.5)	53 (88.3)	102 (86.4)
Treatment-related TEAEs, *n* (%)	27 (45.0)	29 (50.0)	36 (60.0)	65 (55.1)
Grade ≥3 TEAEs, *n* (%)	15 (25.0)	17 (29.3)	26 (43.3)	43 (36.4)
Related grade ≥3 TEAEs, *n* (%)	5 (8.3)	4 (6.9)	9 (15.0)	13 (11.0)
Serious TEAEs, *n* (%)	16 (26.7)	13 (22.4)	14 (23.3)	27 (22.9)
Related serious TEAEs, *n* (%)	3 (5.0)	1 (1.7)	6 (10.0)	7 (5.9)
TEAEs leading to treatment discontinuation, *n* (%)	5 (8.3)	11 (19.0)	8 (13.3)	19 (16.1)
Related TEAEs leading to treatment discontinuation, *n* (%)	1 (1.7)	3 (5.2)	3 (5.0)	6 (5.1)
TEAEs with fatal outcome, *n* (%)	2 (3.3)	2 (3.4)	3 (5.0)	5 (4.2)
Related TEAEs with fatal outcome, *n* (%)	1 (1.7)	0	0	0
Most frequently observed grade ≥3 TEAEs, *n* (%)				
Anemia	2 (3.3)	1 (1.7)	4 (6.7)	5 (4.2)
Back pain	1 (1.7)	1 (1.7)	2 (3.3)	3 (2.5)
Bone pain	1 (1.7)	1 (1.7)	2 (3.3)	3 (2.5)
Fatigue	2 (3.3)	1 (1.7)	0	1 (0.8)
Spinal cord compression	0	2 (3.3)	1 (1.7)	3 (2.5)
Urinary retention	0	2 (3.3)	1 (1.7)	3 (2.5)
Blood ALP increased	2 (3.3)	0	0	0
Dizziness	2 (3.3)	0	0	0

Abbreviations: ALP, alkaline phosphatase; SOC, standard of care.

expression. Pan-αv expression was detected in 90% of cases with evaluable primary prostate cancer tissue. The integrin staining pattern was similar regardless of Gleason grade pattern, and showed a comparable distribution in all treatment arms (data not shown). Integrin αvβ5 expression was detected in approximately 40% of evaluable tumor tissues and αvβ6 was not expressed, confirming previous observations (Merck KGaA; unpublished data; ref. 17).

Prospectively planned analyses of markers of bone metabolism based on pretreatment levels in plasma, serum, or urine (osteopontin, bone-specific alkaline phosphatase, osteocalcin, N-telopeptide of type I collagen, deoxypyridinoline, and C-telopeptide of type I collagen) and change in expression over the course of treatment showed no correlation with PFS or abituzumab therapy.

The planned exploratory plasma protein analysis of pretreatment samples identified 12 biomarker candidate proteins with levels predictive of improved PFS with abituzumab treatment and prognostic for reduced PFS in the control arms (HRs ranged from 0.25 to 0.51; log-rank test $P < 0.05$). Six of these are known to have roles in bone metabolism and tumor growth factor β1 biology: angiogenin, IL1B, mitogen-activated protein kinase kinase 2, mitogen-activated protein kinase 11, TNF receptor superfamily member 17, and leptin receptor (Fig. 3).

Pharmacokinetics

At an abituzumab dose of 750 mg and 1,500 mg, serum abituzumab concentrations exceeded the IC_{95} and IC_{99}, respectively, at the end of the 2-week dosing interval in most patients. Exposure was dose-related and clearly distinguished the two abituzumab groups.

Discussion

In this study of patients with CRPC with bone metastases, abituzumab therapy showed a modest effect on PFS, although the prespecified HR of <0.67 versus placebo was not attained with either dose. However, the cumulative incidence of bone progression was lower with abituzumab than with placebo, with inhibition of new bone lesions driving the PFS difference between abituzumab and placebo. TEAEs were as expected on the basis of phase I data, and there were no new safety signals and no abituzumab treatment-related deaths.

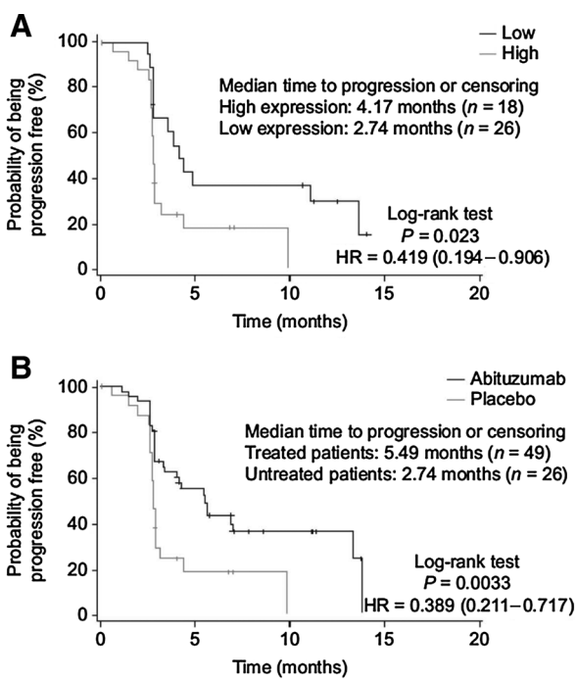

Figure 3.
Role of leptin receptor, a prognostic and predictive biomarker candidate for abituzumab treatment. A, low leptin receptor levels in pretreatment patient plasma (below the median of the studied population) associated with reduced PFS. B, low leptin receptor levels in pretreatment plasma associated with increased PFS with abituzumab-based therapy.

The treatment of metastases of prostate cancer remains problematic due to lack of understanding regarding what triggers metastasis, the reasons for the preferential homing to bone, and the mechanisms involved in the osteoblastic bone phenotype that is often seen in prostate cancer metastases (13). Most current therapies target intrinsic tumor features (e.g., androgen dependence and processes contributing to unrestricted growth), but fail to account for factors potentially involved in the homing of tumor metastasis, notably the host microenvironment and factors relevant for homing to the bone (13).

With the growing appreciation of the complexity of advanced prostate cancer, new therapeutic strategies are being developed to disable the multiple signaling networks that are responsible for tumor maintenance (15). αv integrins have been identified as a critical prostate cancer regulatory subnetwork. Integrin signaling has effects on apoptosis, cell adhesion, proliferation, and migration (28–30). These mechanisms are especially relevant to prostate cancer because prostate tumor cells have an ECM environment that differs from that of normal cells, and changes in integrin profiles may contribute functionally to the growth and establishment of primary and metastatic foci (31, 32).

As discussed, changes in integrin α and β subunit expression have been described in prostate cancer, and their role in PD has been postulated (14). Tumor metastasis is dependent on the ability of cancer cells not only to migrate and invade, but also on their ability to grow at sites distant from the primary tumor. Both of these processes can be mediated by integrins (14). Integrins pan-αv and αvβ5 are expressed on primary prostate tumor cells, as shown in this study, in an earlier study, and in a series of prostate tumor samples from Charité Hospital (Berlin, Germany; Merck KGaA; unpublished data), and may be associated with tumor invasion and metastasis (16).

The growth of prostate cancer metastases in bone involves an intimate interaction between tumor cells and the bone microenvironment (15). Having survived detachment from the primary ECM, tumor cells must establish themselves in new sites; tumor integrins have been shown to be involved in binding to bone ECM and the subsequent establishment of micrometastases (33). αv integrins are involved in tumor cell–bone stroma interactions, with integrin αvβ3 activation on tumor cells appearing to be essential for the recognition of key bone-specific matrix proteins (20), and integrin αvβ5 involved in the growth and survival of prostate metastases in bone (19, 33). These observations suggest that inhibition of αv integrins by abituzumab may be an explanation for the observed activity of abituzumab against metastatic bone lesions.

Prostate cancer bone metastases promote both osteolytic and osteoblastic activity (34), and integrins have been implicated (14). Integrins αvβ5 and αvβ3 are involved in bone recognition by osteoclasts and their binding to bone (21, 33). Integrin αvβ3 also regulates osteoclast adhesion to and migration on osteopontin (21, 33), which is important for osteoclast polarization and bone resorption. This suggests that abituzumab may inhibit osteoclasts, thereby preventing their lytic activity with concomitant inhibition of bone metastasis and SREs, as observed in this trial.

Integrin expression by osteoblasts is less well defined, with differences in expression depending on the model studied (35). This may reflect the heterogeneity of this cell population. However, integrin αvβ3 has been shown to be expressed by osteoblasts (20, 35). Prostate cancer cells lacking integrin αvβ3 expression have been reported to promote bone loss, whereas the presence of tumor-specific integrin αvβ3 promotes bone deposition (20). Thus, integrin αvβ3 inhibition by abituzumab has the potential to inhibit osteoblast activity, another factor that suggests that abituzumab might be expected to reduce the development and/or progression of prostate cancer bone metastases. An additional putative mechanism underlying the influence of abituzumab on bone metastases in prostate cancer is inhibition of angiogenesis due to integrins αvβ5 and αvβ3 in these lesions (16).

These observations suggest that the underlying mechanism of action of abituzumab against bone lesions such as those in the patients in the current study involves inhibition of αv integrins in host cells such as osteoblasts and osteoclasts as well as in prostate cancer cells that have metastasized to bone. In contrast, abituzumab-based therapy did not influence the incidence of soft-tissue lesion progression in this trial. There are a number of possible reasons for this, including lack or low expression of αv integrins by prostate tumor cells that metastasize to soft tissue and reduced dependence of these cells on αv integrins due to signaling pathway redundancy.

However, it is important to note that progression of bone tumors was assessed in this study using scintigraphy, which measures the combined effects of osteoblastic and osteolytic bone activity. Although prostate cancer produces mainly osteoblastic metastases, SREs are mainly due to osteolytic activity (13). The incidence of SRE in this study was 6.7% in the pooled abituzumab cohorts compared with 1.7% with placebo. This might suggest that abituzumab has a more pronounced inhibitory effect on osteoblasts, which could explain the observed decrease in bone lesion progression and suggest that the effect on osteolytic bone metastases and therefore SREs could be less significant.

Other integrin inhibitors that have been studied in clinical trials in prostate cancer include cilengitide, intetumumab, and MK-0429 (36–40). These agents target various αv integrins: cilengitide, integrins αvβ3 and αvβ5 (37); intetumumab, αv integrins (38); and MK-0429, integrin αvβ3 (40). Both cilengitide and intetumumab were well tolerated, but there was no evidence of activity in phase II trials of these agents (36–39). MK-0429 caused a reduction in bone turnover, leading the authors to hypothesize that it may have a role in the treatment of metabolic bone disease (40). This supports our observation of an effect of abituzumab on bone lesions and suggests that further trials in this setting may be warranted. However, there does not appear to have been any further development of any of these agents. Careful consideration of the design of specific studies of the inhibitory effects of abituzumab on bone lesions would be needed, particularly in relation to relevant biomarker/pharmacodynamic analyses and which drugs are used in combination with abituzumab.

In relation to combination therapy, the use of concomitant bisphosphonate therapy, the primary treatment used in clinical practice to prevent SREs, was specified in the protocol of this trial. This was based on available guidelines at the time the trial was initiated, including those from the National Comprehensive Cancer Network and European Association of Urology. Available evidence suggests that bisphosphonates inhibit osteoclast activity at least in part by downregulating integrin αvβ3, disrupting its role in the cytoskeletal rearrangements of osteoclasts associated with resorption (41, 42). Therefore, it might be expected that the inhibitory effects of abituzumab on αv

integrins would complement those of bisphosphonates. Given the higher rate of SREs observed with abituzumab than with placebo, this does not appear to be the case. We have been unable to suggest a hypothesis to explain this observation, but this potential interaction will need to be taken into account if future studies are performed.

The key limitation of this trial is that patients were not selected for therapy based on tumor integrin αv expression. As no cut-offs for the level of integrin αv expression associated with tumor inhibition are available, determining which patients should be eligible based on this criterion was not possible. Rather, we chose to determine whether integrin αv expression by the primary tumor was associated with abituzumab activity. This was not the case, potentially due to the specific roles of αv integrins in different metastatic sites, as discussed.

A further limitation is that integrin profiling of patient material collected from bone lesions, which are the major sites of metastasis in prostate cancer patients, was not performed. This would undoubtedly have added additional value to the translational and biomarker program in this trial. However, the patient burden linked to the collection of this material as well as technical issues with defining a robust decalcification protocol meant that this approach was not pursued.

In conclusion, this placebo-controlled randomized trial did not demonstrate the expected level of activity in terms of PFS improvement with abituzumab-based therapy. However, the data suggest that abituzumab has specific effects against prostate cancer–associated bone lesions, and there is a biologic rationale for these effects. As the management of bone lesions in patients with prostate cancer represents a specific therapeutic challenge and unmet need, our observations warrant further investigation.

Disclosure of Potential Conflicts of Interest

M. Hussain reports receiving research funding from Merck KGaA. S. Le Moulec is a consultant/advisory board member for Astellas, Bayer, and Sanofi. K. Miller reports receiving a commercial research grant from Novartis; has received speakers bureau honoraria from Janssen, Merck KGaA, and Novartis; and is a consultant/advisory board member for Astellas, Astra-Zeneca, Bayer, BMS, Ferring, Janssen, Merck KGaA, Roche, and Sotio. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

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Development of methodology: M. Hussain, R. Bruns, J. Straub, K. Miller
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Hussain, S. Le Moulec, K. Miller
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Hussain, S. Le Moulec, C. Gimmi, R. Bruns, J. Straub, K. Miller
Writing, review, and/or revision of the manuscript: M. Hussain, S. Le Moulec, C. Gimmi, R. Bruns, J. Straub, K. Miller
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R. Bruns, J. Straub
Study supervision: M. Hussain

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