

# Weekly ascorbic acid infusion in castration-resistant prostate cancer patients: a single-arm phase II trial

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**Background:** Ascorbic acid (AA) has *in vivo* cytotoxic properties at concentrations that can only be achieved through intravenous (IV) administration in humans. Treatment with intravenous AA is widely and increasingly used in complementary medicine despite a lack of clinical evidence for the efficacy of this treatment.

**Methods:** This non-comparative, single-center, phase II trial included patients with chemotherapy-naïve, metastatic castration-resistant prostate cancer (mCRPC) from an outpatient clinic to evaluate the efficacy and safety of IV AA therapy. Patients received weekly infusions of AA (week 1, 5 g; week 2, 30 g; and weeks 3–12, 60 g) followed by efficacy evaluation at 12 weeks. The primary endpoint for efficacy was a 50% reduction in the prostate-specific antigen (PSA) level. The secondary endpoints included changes in health-related quality of life (HRQoL), biomarkers of bone metabolism, inflammation and bone scans. Clinicaltrials.gov identifier: NCT01080352.

**Results:** Twenty-three patients were enrolled in this study, and 20 completed the efficacy evaluation at 12 weeks. The mean baseline PSA level was 43 µg/L. No patient achieved a 50% PSA reduction; instead, a median increase in PSA of 17 µg/L was recorded at week 12. Among the secondary endpoints, no signs of disease remission were observed. In total, 53 adverse events (AEs) were recorded. Eleven were graded as “serious”. Three AEs were directly related to AA, and all of which were related to fluid load.

**Conclusions:** Infusion with 60 g of AA did not result in disease remission. This study does not support the use of intravenous AA outside clinical trials.

**Keywords:** Prostatic neoplasms; cancer; ascorbic acid (AA); complementary medicine; translational medical research

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## Introduction

The use of complementary and alternative medicine (CAM) is becoming increasingly popular for the treatment of patients diagnosed with cancer. Thirty percent of prostate cancer (PC) patients are treated with at least one CAM method (1). Metastatic castration-resistant prostate cancer (mCRPC) is the end stage in a sequence of PC disease landmarks. The initial phase of mCRPC is asymptomatic, with progression only being detectable by biomarkers and imaging techniques. Following the asymptomatic window, a period of increased mortality is observed, with a median survival of 2–3 years.

Treatment with intravenous (IV) ascorbic acid (AA) is widely used in CAM clinics (2), but quality evidence regarding the efficacy and safety of this treatment is lacking. Indeed, two randomized controlled trials (3,4) of oral AA for the treatment of advanced cancers were not able to reproduce the improved overall survival (OS) that was observed in a large case series reported in the 1970s (5,6).

AA shows *in vitro* cytostatic and cytotoxic properties in tumor cell lines, including the NCI60 screening panel, and to have some *in vivo* cytostatic and cytotoxic properties (7,8). The mechanisms underlying these properties of AA have not been fully elucidated, and it remains unclear whether the effect of AA on tumor level, if any, is able to stabilize the disease or cause remission. The cytotoxic concentrations of AA are more than 100-fold higher than those obtainable by oral administration and can only be achieved by IV administration (9), which was not used in the randomized controlled trials (3,4). The average IV AA dose used in CAM for various indications, including cancer, is 28 g every 4 days (2).

The present study investigated the efficacy and safety of weekly high-dose IV AA in mCRPC patients using standardized clinical trial principles (10,11).

## Methods

### Study design

The study was designed as a non-comparative, single-center, phase II trial. Patients were recruited from the outpatient urology clinic at Copenhagen University Hospital Herlev. The study was initially designed with 20-g infusions, but the dose was increased after the enrollment of eight participants (unpublished data), as the predefined plasma concentration range was not obtained with 20-g infusions. All patients continued their normally scheduled visits to ensure the best standard of care. If patients were referred to chemotherapy,

their participation in the present trial was discontinued.

### Patient eligibility

The eligibility criteria included adenocarcinoma of the prostate with at least one metastatic lesion visible by bone or CT scan; ongoing androgen-deprivation therapy with castration-level testosterone (<1.7 nmol/L); disease progression [indicated by prostate-specific antigen (PSA) level or imaging findings] as defined by the Prostate Cancer Clinical Trials Working Group (PCWG-2) (10), no prior chemotherapy; and Eastern Cooperative Oncology Group (ECOG) performance of 0–2. The key exclusion criteria were surgical removal or radiation of the prostate; significant renal impairment; significant cardiac disease; history of oxalate renal stones; hemochromatosis; and glucose-6-phosphate dehydrogenase deficiency.

All patients signed informed consent forms. The trial was approved by the Regional Ethics Committee (H-C-2009-018), the Danish Health and Medicines Authority (2612-3978), and the Danish Data Protection Agency (2007-58-0015/750.19-15). It was registered (Eudra-CT 2008-008692-33/NCT01080352) and followed the current Guidelines for Good Clinical Practice and the Declaration of Helsinki.

### Intervention

Patients received one weekly infusion of AA, as previously described (12). In brief, the initial dose was 5 g, followed by 30 g in week 2 and a final dose of 60 g in week 3. The infusion rate was 1 g/min. The trial target dose of 60 g was continued once per week until the initial efficacy evaluation at 12 weeks. Patients were also given a daily oral dose of 500 mg AA from the first infusion, and it was continued for 26 weeks to avoid a hypothetical rebound deficiency following infusion (13).

### Statistical considerations

Data were analyzed using R v.3.0.3. Data are presented as medians along with a Q1–Q3 interquartile range. Data collected at baseline and at week 12 were compared using a predefined paired non-parametric statistical test due to the study design and the small sample size. A P value <0.05 was considered statistically significant. The number of participants needed to achieve sufficient statistical power was calculated based on the primary efficacy marker using a slightly modified Ahern's study design (14). The minimum

response was determined based on TAX327-controls (15).  $p_0$  was estimated as 5%,  $p_1=30\%$ ,  $\alpha=0.05$  and power =80%. Further investigations were warranted if  $\geq 3$  of the 20 patients met the primary endpoint for efficacy.

### *Trial evaluation (efficacy and safety)*

#### **Drug**

Plasma AA concentrations were measured at the end of the infusion and 30 minutes after the end of the infusion on weeks 1–6, 9, and 12. The predefined goal for plasma AA concentration was >5–10 mM. The analytical procedures have been described previously (16).

#### **Efficacy**

The primary endpoint for efficacy was binary and was defined as  $\geq 50\%$  PSA reduction from baseline to 12 weeks. If achieved, an optional 8-week extension of the AA treatment was offered. The following additional biomarkers from the Halabi (17) and Smaletz (18) prognostic model were collected as markers of potential efficacy: hemoglobin (Hgb), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and albumin levels. All venous blood samples were subjected to standard accredited analysis at the Department of Clinical Biochemistry, Herlev University Hospital immediately after sampling. Biomarkers of bone metabolism were included to provide information on turnover rate, including two markers of bone formation and one marker of bone resorption: bone-specific alkaline phosphatase (bALP; an isoform of ALP), pro-collagen type I N-terminal (PINP), and urinary type I collagen cross-linked N telopeptides (uNTx). bALP was measured in venous serum blood samples, and uNTx was measured in spot urine samples (second morning void), normalized to urine creatinine, and analyzed at the Department of Clinical Chemistry, Aarhus University Hospital. PINP was measured in venous serum blood samples at the Department of Clinical Chemistry, Copenhagen University Hospital Glostrup.

Metastatic burden in bone was evaluated by 99 m-Tc bisphosphonate bone scans at weeks 0, 12, 26, and 52. Bone scans were performed as routine investigations at the institutional Department of Clinical Physiology and were analyzed using a computerized detection system (EXINI bone software) to minimize interpretation bias (19). A link between inflammation and cancer has been suggested by epidemiological and experimental data (20,21). To quantify the inflammatory response and the level of oxidative stress, biomarkers of both parameters were measured, namely

C-reactive protein (CRP), YKL-40, 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxodG), and 8-oxo-guanosine (8-oxoGuo). The CRP level was analyzed at the Department of Clinical Biochemistry, Herlev University Hospital, immediately after sampling. The analytic kit was not highly sensitive; hence, concentrations below 3 mg/L were reported as "<3". The plasma YKL-40 level was analyzed at the Department of Medicine, Copenhagen University Hospital Herlev. Markers of oxidative stress-induced DNA (8-oxodG) and RNA (8-oxoGuo) damage were measured in urine samples collected over a 24-h period, which ended on the day of baseline sampling and on the day of the last infusion; this analysis was performed at the Laboratory of Clinical Pharmacology, Copenhagen University Hospital Rigshospitalet. Performance status was recorded at every visit using the ECOG Performance Status Scale (22). Changes in health-related quality of life (HRQoL) were assessed by the European Organization for Research and Treatment of Cancer (EORTC) self-administered questionnaires QLQ-C30 (v3.0) (23) and QLQ-PR25 (24). Scores were transformed to a 0–100 scale according to the manuals of the questionnaires with the R package "QoLR v1.0".

#### **Safety**

Patients remained under observation by the study personnel during the infusion and for 30 min afterwards. Blood was screened for hemolysis 30 min after the infusion. Every 3 weeks, a "safety blood-chemistry profile" was drawn to examine liver toxicity, kidney function, and electrolyte and hemoglobin levels. All adverse events (AE) were scored using the Common Terminology Criteria for Adverse Events v4.03 (CTCAE) (25). Deviations from the reference values present at baseline were only classified as AEs in cases of progression by one CTCAE grade.

#### **Long-term follow-up**

Follow-up visits were planned at 20, 26 and 52 weeks after trial initiation to monitor the long-term effects of the treatment on potential responders. All of the efficacy markers were evaluated at each follow-up visit. Follow-up was discontinued if the patient was enrolled in another clinical trial or if chemotherapy was initiated.

## **Results**

### *Patient characteristics*

Twenty-three patients were enrolled in this study between

**Table 1** Baseline characteristics of all patients enrolled

Variable (n=23)	Median	Q1	Q3
Age, years	73.8	69.2	79.6
Weight, kg	81.0	74.5	91.5
BMI, kg/m <sup>2</sup>	26.2	24.3	28.4
Creatinine, µmol/L	80	74	88
eGFR, mL/min	84	74	90
PSA, µg/L	43	21	72
Time from diagnosis to trial, months	50.7	28.6	90.5

BMI, body mass index; eGFR, estimated glomerular filtration rate calculated with the MDRD formula; PSA, prostate-specific antigen.

**Table 2** Baseline characteristics of all patients enrolled

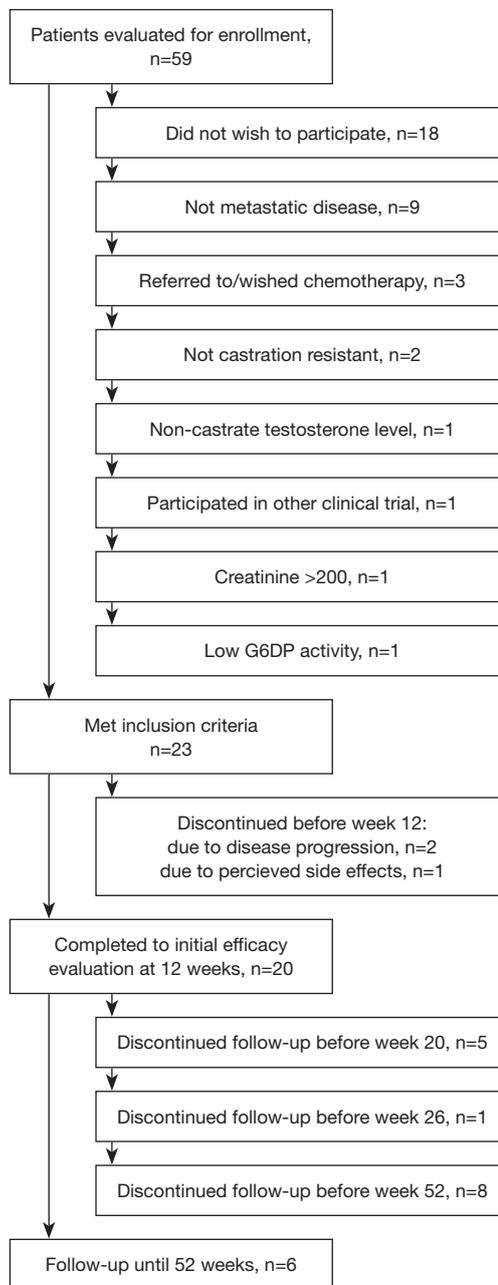
Variable (n=23)	No.	%
Gleason score at diagnosis		
7	3	13
8	9	39
9	10	43
10	1	4
ECOG PS		
0	18	78
1	5	22
Bone metastasis	22	96
Visceral metastasis without bone metastasis	1	4

ECOG PS, Eastern Cooperative Oncology Group Performance Status.

October 2011 and November 2013. All patients were male Caucasians. Detailed baseline characteristics of all the enrolled patients are shown in *Tables 1* and *2*. Prior to enrollment, patients received a least one additional hormone manipulation other than androgen deprivation therapy. Three patients discontinued treatment before the initial efficacy evaluation. Details are provided in *Figure 1*.

### Drug evaluation

With the infusion of 60 g of AA (0.74 g/kg, Q1–Q3: 0.66–0.81), all patients reached an AA plasma concentration within the predefined range. At infusion, the mean

**Figure 1** Trial flowchart of enrollment and discontinuation.

termination plasma AA concentration was 19.3 mM, and it was 15.1 mM after an additional 30 min. The detailed pharmacokinetic data of 10 participants have been published previously (12). The median baseline AA level was 54 µM (Q1–Q3: 45.6–73.5). At week 26, when oral AA supplementation was terminated, the median plasma AA was 88.4 µM (range 45.0–146.6 µM); hence, the patients

**Table 3** Biomarkers for efficacy in all patients evaluated for the extension arm at week 12

Biomarker	Week 0 (n=20)			Week 12 (n=20)			Week 20 (n=15)			Week 26 (n=14)			Week 52 (n=6)		
	Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3
PSA	40	15	67	64*	40	102	78	35	127	71	35	218	72	41	252
Hgb	8.3	7.9	8.7	7.8*	7.5	8.2	7.8	7.4	8.2	8.1	7.4	8.3	7.6	6.4	7.9
LDH	196	180	217	209	188	230	194	181	259	214	172	243	194	184	208
ALP	81	68	99	85*	65	122	84	74	142	89	70	214	93	85	100
Albumin	42	39	43	41	40	45	41	39	43	43	39	44	42	40	45
bALP	29	21	41	29*	22	67	32	22	57	46	21	116	34	28	38
PINP	46.5	17.0	95.0	51.4*	21.4	174	55.1	29.6	136	73.6	28.3	146	43.5	37.6	55.8
uNTx	57.0	20.2	78.8	52.5*	35.3	119	45.8	21.9	97.3	49.9	31.3	83.3	67.0	48.0	140
YKL-40	72	38	107	82	53	143	132	63	155	78	58	140	192	145	242
8-oxo-G	28.8	25.5	39.9	31.4*	28.0	41.3	-	-	-	-	-	-	-	-	-
8-oxo-dG	17.9	15.7	24.4	17.0	15.7	23.3	-	-	-	-	-	-	-	-	-

\*, P<0.05, week 12 compared with baseline. PSA, prostate-specific antigen ( $\mu\text{g/L}$ , ref. <4); Hgb, hemoglobin (mmol/L, ref. 8.3–10.5); LDH, lactate dehydrogenase (U/L, ref. 115–255); ALP, alkaline phosphatase (U/L, ref. 35–105); Albumin (g/L, ref. 34–45); PINP, pro-collagen type 1 N-terminal ( $\mu\text{g/L}$ ); bALP, bone-specific alkaline phosphatase (U/L, ref. 12–44); uNTx, urinary type I collagen cross-linked N telopeptides (nmol/ $\mu\text{mol}$  urine creatinine, ref. 21–83); YKL-40 (ng/mL, ref. 14–155); 8-oxo-dG, 8-oxo-7,8-dihydro-2'-deoxyguanosine (nmol); 8-oxo-G, 8-oxo-guanosine (nmol).

**Table 4** Biomarkers for efficacy in all patients evaluated for the extension arm at week 12

Parameter	N [%]				
	Week 0 (n=20)	Week 12 (n=20)	Week 20 (n=15)	Week 26 (n=14)	Week 52 (n=6)
CRP					
<3	14 [70]	12 [60]	6 [40]	9 [64]	-
3–10	4 [20]	2 [10]	6 [40]	3 [21]	-
>10	2 [10]	6 [30]	3 [20]	2 [14]	-
ECOG PS					
0	17 [85]	17 [85]	12 [80]	10 [71]	4 [33]
1	3 [15]	2 [10]	3 [20]	4 [29]	2 [67]
2	-	1 [5]	-	-	-

CRP, C-reactive protein (mg/L, ref. <10); ECOG PS, Eastern Cooperative Oncology Group Performance Status.

were non-deficient (8).

### Efficacy evaluation

Data from all of the patients who completed the study protocol until the initial efficacy evaluation at 12 weeks are summarized in *Tables 3–6*. No patients achieved the primary

endpoint for efficacy, i.e., a PSA reduction of 50%. Baseline-normalized percentage changes are presented in *Figure 2*. Fifteen patients (75%) displayed a nominal PSA increase, although three of these patients did not meet the PCWG-2 criteria for progression (>25% increase). Nine of 20 patients at week 6 and 10 of 20 patients at week 9 experienced PSA progression. Overall, PSA increased by 17  $\mu\text{g/L}$  (Q1–Q3:

1–37,  $P=0.003$ ). Five patients had a lower PSA at 12 weeks than at the baseline, and the maximum response ( $-27\%$ ) represented an absolute decrease of  $>2 \mu\text{g/L}$ .

No improvement in the secondary efficacy markers was observed. The median ALP level increased ( $P=0.01$ ) and the Hgb level decreased ( $P=0.0001$ ) from the baseline to 12 weeks. The LDH level tended to increase and the albumin level to decrease, implying disease progression. No marker for bone turnover decreased, but minor increases of 14–20% were observed, as shown in *Table 5*. The following results were found for the outcomes, dichotomized as a decrease or increase from the baseline: 9 patients had no response for any bone marker, 7 patients showed a response for 1 biomarker, 2 patients showed a response for 2 biomarkers, and 2 patients showed a decrease in all 3 biomarkers. None of the patients started antiresorptive therapy during the intervention period; 2 patients received 120 mg/4 weeks denosumab prior to and during the trial, and 6/15 patients with follow-up data started denosumab at some point during the follow-up period.

Markers of inflammation and oxidative stress did not change from the baseline, except for a minor increase in 8-oxodG (DNA damage) ( $P=0.03$ ). The dichotomized CRP response with a normal range cut-off ( $>10$ ) did not differ from the baseline to 12 weeks by McNemar's test.

Three patients (19%) showed reductions in BSI and the number of lesions at 12 weeks compared to the baseline; all had one fewer high-probability lesion. Details by week are

**Table 5** Changes in biomarkers for bone metabolism between baseline and week 12

Biomarker	Change	Q1	Q3	Change in %
uNTx	+5.8	-0.2	+26.3	+14
bALP	+4	0	+25	+17
PINP	+4	-3.2	+47	+20

Reported as median, Q1–Q3. PINP, pro-collagen type 1 N-terminal ( $\mu\text{g/L}$ ); bALP, bone-specific alkaline phosphatase (U/L); uNTx, urinary type I collagen cross-linked N telopeptides (nmol/ $\mu\text{mol}$  urine creatinine).

**Table 6** Bone scan index and number of high-probability lesions

Study week	BSI	Q1	Q3	No. of lesions	Q1	Q3
Week 0 (n=17)	0.23	0.05	0.64	2	1	7
Week 12 (n=19)	0.51	0.01	1.62	2	0	5

BSI, bone scan index, or median % of body skeleton infiltrated by bone metastasis, as calculated using EXINI bone software.

shown in *Table 6*. For technical reasons, 3 bone scans were missing from the analysis at baseline, and 1 bone scan was missing at weeks 12 and 26, leaving only 16 paired datasets for evaluation.

### HRQoL and ECOG performance status

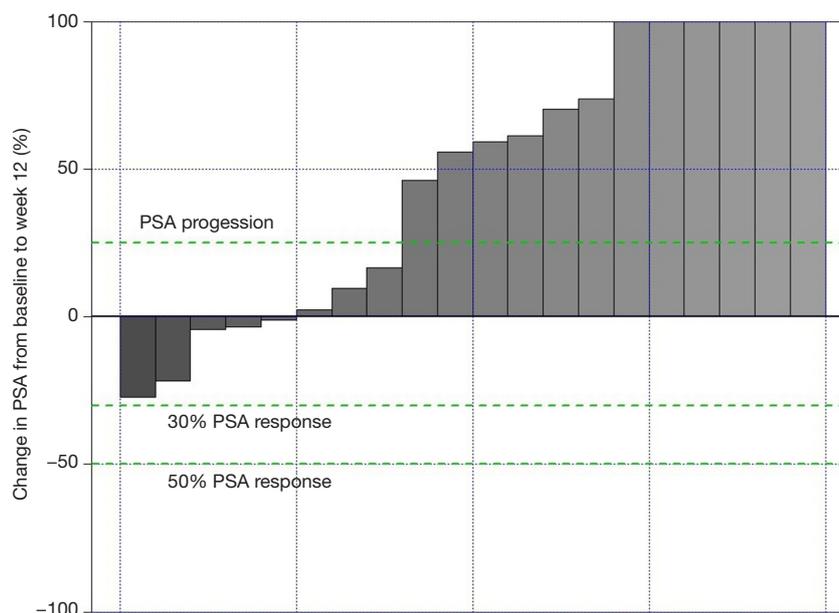
Sixteen patients (80%) had an unchanged ECOG score at week 12; 2 patients had an improved score, and 2 had a worse score, with a drop of 1–2 steps. *Table 7* shows the HRQoL data. Only 1 scale, physical functioning, differed from baseline to week 12, and no trends of improvement were seen for the other scales.

### Safety

A total of 53 AEs were recorded in the trial; 5/23 patients (21.7%) had no AEs, and 4/23 patients (17.3%) had only one AE. Detailed data are shown in *Table 8*. Eleven of the AEs were recorded as serious due to hospitalization, although three of them were elective procedures. One patient discontinued the AA therapy due to side effects (increased lower-urinary-tract symptoms). The most frequent findings were hypertension and anemia. Two episodes of pulmonary embolism were recorded, although one episode was asymptomatic and was discovered by a pre-chemotherapy-evaluation CT scan during the post-trial follow-up.

### Discussion

The present study does not indicate that high-dose IV AA given once per week for 12 weeks induces tumor regression, as evaluated by a significant PSA reduction in patients with mCRPC, although the plasma concentrations of AA did reach the cytotoxic range for cancer cells. This result is in accord with all previous prospective studies, which showed no clinical signs of tumor growth inhibition (26). In the present trial, 12 (52%) of the 23 enrolled patients experienced either biochemical or clinical progression (leading to withdrawal) at 6 weeks, which is a short time to progression compared to a



**Figure 2** Waterfall plot of changes in PSA after 12 weeks of intravenous ascorbic acid. Values were capped at 100%; the maximum increase was +397%. PSA, prostate-specific antigen.

similar cohort studied previously (27).

The criteria for an 8-week treatment extension were based on PCWG-1 recommendations, which imply a survival benefit (11). The use of a  $\geq 50\%$  PSA response as the surrogacy criterion for OS has been questioned. Based on data from TAX327 (28) and SWOG99-16 (29), a  $\geq 30\%$  PSA reduction may improve the accuracy of the response criterion. Irrespective of whether the  $\geq 50\%$  or  $\geq 30\%$  response criterion was applied, no patient achieved a response, as the greatest observed reduction in PSA was 27%.

One of the major limitations of our study is the absence of a control group. A spontaneous 50% PSA decline in mCPRC is rare; it has been previously reported to occur in 1.3% or 3% of cases (27,30). By using PSA as a marker of treatment efficacy, two major criteria are fulfilled: the tumor must produce PSA, and the action of the investigated compound must induce changes that can be detected as changes in PSA. To be included in the present trial, the tumor had to be PSA-producing, but it remains unknown if AA is capable of changing PSA levels per se. Immunotherapy may improve OS without generating a significant PSA response. AA may enhance immune-cell activity (31), but no study has shown any cancer-directed immunotherapy using AA alone. To pursue possible effects of AA not related to PSA expression by tumor cells,

additional biomarkers of the PCWG-2 and two prognostic nomograms were included (10,17,18). No clear trend of improvement was observed for any variable.

The long-term follow-up was primarily designed to identify responders and any potential lasting effects. Because no patient continued to the extension arm, they were candidates for docetaxel, which generates a selection bias for follow-up, with the “least-progressing” patients being left on-study.

Our measure of general oxidative stress-induced DNA and RNA damage was urinary excretion of oxidized nucleosides. It has previously been shown that large oral doses of AA do not reduce intracellular oxidative stress (32), and the data from the present study confirm that, even with the high plasma concentrations of AA obtained by infusion, the overall oxidative stress level did not appear to be affected.

Sufficient data were not collected for the calculation of the PSA doubling time prior to enrollment. Although the use of PSA kinetics as a surrogate endpoint has not been validated, such data could potentially provide information about the slowing of tumor progression (33). Our patient cohort had a high performance status at the baseline that remained stable during the intervention period. Compared to reference material, the QoL scores were high (34). However, the

**Table 7** Health-related quality of life at baseline and at week 12

HRQoL questionnaire and scale	Week 0			Week 12			P
	Median	Q1	Q3	Median	Q1	Q3	
<b>QLQ-C30</b>							
Global health scale							
Global health	83	73	100	83	67	92	0.12
Functional scales							
Physical	93	80	100	83	73	93	0.006
Role	100	83	100	92	67	100	0.15
Emotional	100	92	100	100	83	100	0.09
Cognitive	100	96	100	100	83	100	0.41
Social	100	100	100	100	100	100	0.34
Symptom scales							
Fatigue	11	0	22	11	0	25	0.80
Nausea/vomiting	0	0	0	0	0	0	0.17
Pain	0	0	33	0	0	21	0.67
Dyspnea	0	0	33	0	0	0	0.76
Sleep disturbance	0	0	8	0	0	8	0.78
Appetite loss	0	0	0	0	0	0	0.07
Constipation	0	0	0	0	0	0	0.71
Diarrhea	0	0	0	0	0	0	NA
Financial impact	0	0	0	0	0	0	NA
<b>QLQ-PR25</b>							
Functional scales							
Sexual activity	0	0	17	0	0	0	0.50
Sexual functioning	Not reported, n=3			Not reported, n=2			NA
Symptom scales							
Urinary symptoms	19	13	34	19	13	39	0.41
Bowel symptoms	0	0	17	0	0	8	0.28
Hormonal treatment-related symptoms	17	11	28	17	11	22	0.29
Incontinence aid (n=3)	17	8	25	17	8	25	NA

The P values were calculated using the Wilcoxon signed-rank test. QLQ-C30, quality-of-life core 30; QLQ-PR25, quality of life questionnaire-Prostate Module 25; NA, not available.

present study did not show a trend of improvement in QoL; rather, the opposite was observed. Four previous studies have investigated HRQoL using validated questionnaires, and most of them reported improvements. No study

included a parallel control group or addressed selection bias or the natural course of the disease (35-38). Studies without a control group may overestimate the effect of the intervention because many cancer patients turn to CAMs to

**Table 8** Adverse events

Occurrence	G1	G2	G3	G4	G5	Description
Clinical	4		1			Hypertension
	2	1	1			Urinary tract infection
			3			Transurethral prostate resection
			3			Metastatic affection of medulla spinalis
	2	1				Flu-like symptoms
	2	1				Limb pain
	1	1				Musculoskeletal lesion after minor trauma
			1		1	Pulmonary embolism
	2					Shortness of breath
		1			1	Pneumonia
	1					Diarrhea
	1					Dry eyes
			1			Lower urinary tract symptoms
	1					Osteoporotic fracture
	1					Pre-syncope
	Laboratory/ imaging, asymptomatic	6		1		
3						Aminotransferase elevation
			3			eGFR/kidney function decrease
			1			Leukemia
			1			Atrial fibrillation
1						Bilirubin elevation
1						Hydronephrosis
1						Hypercalcemia
1					Hyponatremia	

All adverse events, sorted based on the grades outlined by the Common Terminology Criteria for Adverse Events. G1, grade 1; G2, grade 2; etc.

satisfy needs other than tumor treatment, including general health improvement, which can potentially contribute to an improved QoL score (39-41).

AA is highly osmotically active and may cause fluid or electrolyte disturbances. However, in the present study,

treatment-induced toxicity was limited and was likely related to the vehicle rather than to AA itself. The natural course of the advancement of mCRPC explains most of the remaining recorded AEs. Cancer is known to increase the risk of thromboembolic events and may explain the two episodes of pulmonary embolism (42). However, as another trial reported pulmonary embolism in 2 of 14 patients (43), we believe that future studies should continue to examine this AE.

We utilized IV infusion of AA, which represents a pulsed delivery with a high peak concentration and rapid clearance within hours. In two recent phase I trials, even higher doses were applied, namely 1.5 g/kg and 110 g/m<sup>2</sup>, inducing peak plasma concentrations of 26 and 37 mM, respectively (37,38). Only half of the plasma concentration of AA is found in the tumor tissue and the surrounding extracellular fluids (44), and tissue hypoxia may further attenuate AA toxicity (45). *In vivo* studies in rodents have typically used daily intraperitoneal administration of 4 g/kg of AA, which generates a stable millimolar plasma concentration profile over time. However, despite continued exposure to presumably therapeutic concentrations of AA, tumor volume was only reduced by 22–53% in treated animals compared to controls (46-48).

Thus, if AA has antineoplastic properties, it appears that the effect of pulsed, periodic IV AA administration may be limited. Whether different AA delivery approaches possess higher therapeutic potential in humans remains to be investigated in future trials.

**Conclusions**

Infusion with 60 g of AA once per week did not induce disease remission in mCRPC patients; therefore, the findings of the present study do not support the use of high-dose IV AA for late-stage PC. At present, the lack of efficacy of this treatment suggests that it should not be used outside clinical trials. A large-scale controlled trial is needed to determine whether high-dose IV AA displays any therapeutic relevance.

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## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

*Ethical Statement:* The trial was approved by the Regional Ethics Committee (H-C-2009-018), the Danish Health and Medicines Authority (2612-3978), and the Danish Data Protection Agency (2007-58-0015/750.19-15). It was registered (Eudra-CT 2008-008692-33/NCT01080352) and followed the current Guidelines for Good Clinical Practice and the Declaration of Helsinki. Written informed consent was obtained from the patient for publication of this manuscript and any accompanying images.

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