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ORIGINAL ARTICLE

Phase I trial of antigen-targeted autologous dendritic cell-based vaccine with in vivo activation of inducible CD40 for advanced prostate cancer

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Abstract This phase I trial reports the safety and activity of BPX101, a second-generation antigen-targeted autologous antigen presenting cell (APC) vaccine in men with metastatic castration-resistant prostate cancer (mCRPC). To manufacture BPX101, APCs collected in a single leukapheresis were transduced with adenoviral vector Ad5f35 encoding inducible human (ih)-CD40, followed by incubation with protein PA001, which contains the extracellular domain of human prostate-specific membrane antigen. The ih-CD40 represents a modified chimeric version of the dendritic cell (DC) co-stimulatory molecule, CD40, which responds to a bioinert membrane-permeable activating dimerizer drug, rimiducid (AP1903), permitting temporally controlled, lymphoid-localized, DC-specific activation. Eighteen men with progressive mCRPC following ≤ 1 prior chemotherapy regimen were enrolled to evaluate three doses of BPX101 (4×10^6, 12.5×10^6 and 25×10^6 cells) administered intradermally every 2–4 weeks followed by rimiducid (0.4 mg/kg) intravenous (IV) infusion 24 h after each BPX101 dose. There were no dose-limiting toxicities. Immune upregulation as well as anti-tumor activity was observed with PSA declines, objective tumor regressions and robust efficacy of post-trial therapy. This novel antigen-targeted and in vivo activated immunotherapy platform may warrant further development as monotherapy and as a component of rational combinations.

Keywords Dendritic cell vaccine · PSMA · Rimiducid · Immune response · Metastatic · Castration-resistant prostate cancer

Abbreviations

AE	Adverse event
CT	Computerized tomography
CTC	Circulating tumor cell
CTCAE	Common Toxicity Criteria for Adverse Events
DLTs	Dose-limiting toxicities
FKBP	FK506-binding protein
HbsAg	Hepatitis B surface antigen
HTLV	Human T-cell lymphotropic virus
ih	Inducible human
IHC	Immunohistochemistry
KPS	Karnofsky Performance Score
LPS	Lipopolysaccharide
MCP	Monocyte chemoattractant protein
mCRPC	Metastatic castration-resistant prostate cancer
MTD	Maximum tolerated dose
PAP	Prostatic-acid phosphatase
PCWG	Prostate Cancer Working Group
PFS	Progression-free survival
PR	Partial response

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PSA	Prostate-specific antigen
PSMA	Prostate-specific membrane antigen
RANTES	Regulated on activation, normal T cell expressed and secreted
RECIST	Response Evaluation Criteria in Solid Tumors

Introduction

Multiple agents are now available for the systemic therapy of metastatic castration-resistant prostate cancer (mCRPC) including chemotherapeutic agents (docetaxel, cabazitaxel), androgen pathway inhibitors (enzalutamide, abiraterone acetate), immunotherapy (sipuleucel-T) and radiopharmaceuticals (radium-223) [1–9]. Unfortunately, these agents demonstrate substantial cross-resistance and each of them yields extensions of median overall survival (OS) of only 2–4 months.

The role of immunotherapy in mCRPC was validated by the increment provided in two randomized trials evaluating sipuleucel-T, an autologous antigen presenting cell (APC) vaccine targeting prostatic-acid phosphatase (PAP) with co-stimulation by granulocyte–macrophage-colony stimulating factor (GM-CSF) [10, 11]. Sipuleucel-T has extended survival without early benefits in men with mCRPC [10]. Although the toxicities were mild, sipuleucel-T did not yield early benefits in terms of objective tumor regressions, pain responses or prostate-specific antigen (PSA) declines. Given the improved survival and excellent toxicity profile of sipuleucel-T, the continued development of this APC vaccine strategy for the generally elderly mCRPC population is rational.

CD40 is a potent co-stimulatory molecule that sustains dendritic cell (DC) activation, although the activation of CD40 in the lymph node microenvironment appears a key factor [12]. Spencer et al. were able to eradicate established solid tumors in syngeneic mice using DCs transduced with an inducible (i)CD40 followed by exposure to the activating agent rimiducid [13]. In addition, iCD40 synergized with the adjuvant, lipopolysaccharide (LPS), to induce high levels of maturation markers and cytokines (e.g., IL-6, IL-12) in DCs [14]. Rimiducid has previously been shown to be safe as a 2-h intravenous (IV) infusion in humans and achieves biologically active concentrations in doses between 0.01 and 1.0 mg/kg, with plasma levels declining to 7% of the maximum 2 h after infusion [15]. The inducible human (ih)-CD40 was designed to multimerize and get activated following administration of rimiducid, permitting DC-specific activation in the lymph node. A related platform has been used to inducibly regulate apoptosis of genetically modified T-lymphocytes used to treat recurrent leukemia by introducing the gene for caspase-9 linked through a SGGGSG linker to a modified human FK506-binding protein (FKBP)-12 carrying an F36V mutation, which allows conditional dimerization by rimiducid [16, 17].

Prostate-specific membrane antigen (PSMA) is an excellent membrane antigen to target in mCRPC, given its specificity for prostate cells and increasing overexpression with stage and androgen deprivation, as well as overexpression in tumor neovasculature [18, 19]. We constructed an autologous APC vaccine, BPX101, harboring the PA001 protein, containing the extracellular domain of human PSMA and the modified chimeric version of transgene ih-CD40 introduced by adenoviral vector, Ad5f35. A phase I trial was conducted to evaluate the safety, pharmacokinetics, immune responses and preliminary efficacy of three doses of BPX101 plus a fixed dose of the activating agent, rimiducid, in patients with mCRPC.

Methods

Trial design

The classic 3 + 3 design was employed with three patients per cohort, with expansion to six patients in the event of dose-limiting toxicities (DLTs). In the induction phase, patients received BPX101 by intradermal (ID) injection every 2–4 weeks for up to six doses followed by rimiducid IV 24 h after each dose. Patients with no evidence of objective cancer progression after 12 weeks of induction therapy were offered maintenance therapy every 8 weeks up to five doses of BPX101 and rimiducid. Patients underwent clinical, laboratory, radiographic and immune response monitoring periodically (see below). Progression of disease was defined by the Prostate Cancer Working Group (PCWG)-2, Response Evaluation Criteria in Solid Tumors (RECIST)-1.1 and symptomatic progression and not by PSA progression alone [20, 21].

Patient eligibility criteria

Patients were required to have progressive mCRPC and exposure to no more than one prior chemotherapeutic, biologic or combination treatment regimen for mCRPC. Visceral metastasis and narcotics for pain were permitted. Karnofsky Performance Score (KPS) $\geq$70%, castrate testosterone level $\leq$50 ng/dL and adequate hematologic, hepatic and renal function were required. Negative serology tests for human immunodeficiency virus (HIV)-1 and 2, human T-cell lymphotropic virus (HTLV-1), hepatitis B surface antigen (HBsAg) and hepatitis C virus (HCV) were necessary. The presence of central nervous system metastasis, prior exposure to radiopharmaceuticals and the need for corticosteroids were key exclusion criteria.

BPX101 vaccine and rimiducid preparation

Approximately, 1×10^9 peripheral blood mononuclear cells (PBMCs) were collected during a single ~4 h outpatient leukapheresis procedure of up to 12 L of blood volume. A second leukapheresis procedure was performed only if insufficient cells were available from the first leukapheresis. The cells underwent a 6-day process conducted in a central good manufacturing practice (GMP) cell processing facility (MD Anderson Cancer Center) employing a previously described procedure [22, 23]. Briefly, following elutriation, CD14+ PBMCs were isolated on a magnetic separation column and characterized by flow cytometry, and then exposed to GM-CSF (Amgen, Thousand Oaks, CA) and interleukin (IL)-4 (R&D Systems, Minneapolis, MN) in bags to mediate differentiation of PBMCs to mature DCs, transduced with an adenoviral vector (Ad5f35) encoding ihCD40, incubated with 10 μg/mL of PA001 protein (a recombinant extracellular domain of PSMA) for 3 h at 37 °C at a concentration of 5×10^6 cells/mL (this methodology has been previously reported to have a loading efficiency of ≥90%) and pre-activated with rimiducid and LPS to enhance DC maturation and antigen presentation as previously described [13, 14, 22]. The resulting vaccine cells were washed and cryopreserved in individual doses. Cells were diluted with PlasmaLyte-A/HSA/DMSO to achieve individual target doses of 4, 12.5, or 40×10^6 viable DCs, divided into five or eight aliquots of 200 μL each. The maximum dose was chosen as the highest level of DCs that could be obtained from a standard ~12 L leukapheresis, which can generate up to 5.4×10^8 DCs. Prior to administration, BPX101 vaccine was stored frozen at −70 °C, thawed immediately prior to use in a 35–39 °C water bath, then stored at 2–8 °C, and administered as soon as possible after thawing. Rimiducid (Formatech Inc.) was formulated as a 5 mg/mL solution in a 25% solution of the non-ionic solubilizer Solutol HS 15. Rimiducid for Injection was stored at 2–8 °C and protected from excessive light and heat and removed from the refrigerator the night before the patient was dosed and stored at a temperature of approximately 21 °C overnight. The solution was prepared within 30 min of the start of the infusion in glass or polyethylene bottles or non-DEHP bags and stored at approximately 21 °C prior to dosing.

Therapy

Each dosing event consisted of BPX101 vaccine administration via five to eight ID injections. Treatment in the induction phase began at 4×10^6 cells (Cohort 1), then 12.5×10^6 cells (Cohort 2), and then 25×10^6 cells (Cohort 3) every other week for up to six doses. BPX101 was administered as a 1 mL dose for cohorts 1 and 2 and as a 1.6 mL dose for Cohort 3, in 200 μL increments in the dorsal forearm, upper arm and upper leg, alternating between upper arm and dorsal forearm, and between the sides with each vaccine booster for Cohort 1 and 2; and in the dorsal forearm, upper arm and upper leg alternating between the sides with each vaccine booster for Cohort 3. Patients in Cohort 4 were treated with 25×10^6 cells every 4 weeks for up to three doses. Each injection was administered at least 2 cm apart. Thirty minutes prior to administration of the vaccine, patients were pre-medicated with acetaminophen (1000 mg) and diphenhydramine (25–50 mg) orally. Patients were observed for 30 min following the injections for untoward adverse effects. Patients who had no evidence of objective progressive disease after 12 weeks in cohorts 1–3 were offered the opportunity to receive the maintenance phase of therapy with dosing of BPX101 every 8 weeks for up to five times. 24 ± 4 h after each vaccination, a single dose of the activating/dimerizing agent, rimiducid, was administered at a fixed dose of 0.4 mg/kg via IV infusion over 2 h. Castration was maintained with a luteinizing hormone release hormone (LHRH) agonist or antagonist unless the patient was surgically castrated by orchiectomy.

Clinical and laboratory assessments

Patients underwent a history and physical examination, routine blood counts and chemistries including liver function tests every 4 weeks. Circulating tumor cells (CTCs) were enumerated periodically in the first 12 weeks using the commercially available Veridex platform [24]. Radiographic examination including a computerized tomography (CT) scan of the chest, abdomen and pelvis and technetium bone scan was performed every 12 weeks or earlier if clinically warranted. Lesions were objectively measured using the Prostate Cancer Working Group (PCWG)-2 criteria and Response Evaluation Criteria in Solid Tumors (RECIST)-1.1 [20, 21]. Safety was monitored throughout the trial using Common Toxicity Criteria for Adverse Events (CTCAE) version 4.03.

Pharmacokinetics and immune response studies

Plasma samples were collected for determination of rimiducid concentrations during the first two doses in weeks 1 and 3 only. Plasma was collected pre-dose (0 h), 15 and 30 min, and 1, 2, 4 and 8 h following the initiation of rimiducid IV infusion. Blood samples were processed to obtain plasma, frozen and shipped to a bioanalytical facility (KCAS, LLC) for analysis using liquid chromatography and tandem mass spectrometry and a recently validated, GLP-compliant analytical method. The mean plasma levels were determined for each time point along

with variance, standard deviation and coefficient of variation. The results were obtained separately for weeks 1 and 3. Data were subjected to single-factor ANOVA analysis to assess if there were any statistical differences for weeks 1 and 3. The mean maximum plasma rimiducid concentration (C_{max}) values were also calculated for weeks 1 and 3.

Immune response monitoring

Serial immunological response was assessed from blood samples for cytokines associated with T and B cell response as well as maturation of neutrophils and macrophages (IFN-γ, TNF-α, RANTES, GM-CSF, MIP-1α, MIP-1β, MCP-1, IL-6, IL-10) prior to and 1 week after each vaccination. Centrifuged (1500 g) plasma samples were aliquoted and stored in liquid nitrogen for later batch testing. Undiluted samples were analyzed in duplicate using the Milliplex Human Cytokine/Chemokine Panel kit (Millipore, Inc.). Data was analyzed using Bio-Plex software (Bio-Rad Laboratories, Inc.). IL-6 was measured by ELISA assay. A punch biopsy of the skin vaccine injection site was performed at a BPX101 injection site 48–72 h after the third dose (week 7). The skin biopsies and prostate tumor biopsy tissue when available underwent immunohistochemistry (IHC) for PSMA and lymphocyte subsets (CD4, CD8, CD20).

Statistical considerations

Three patients were planned for each dose cohort of this phase I trial with expansion to include six patients based on the occurrence of DLTs. A DLT was defined as any study drug-related grade ≥3 hematologic or non-hematologic adverse event (AE) as defined by the Common Terminology Criteria for Adverse Events (CTCAE, version 4.0) that occurred within the first 4 weeks of treatment. The maximum tolerated dose (MTD) was defined as the highest dose level at which no more than one out of six patients experienced a DLT. Summarization of data was descriptive in nature and based on point estimates and two-sided 95% confidence intervals. Where applicable, *p* values were used in an exploratory manner. For calculations of duration of response, progression-free survival (PFS) and OS, Kaplan–Meier statistics were used to analyze these data and point estimates of the median event rate and 95% confidence interval of the median were to be provided. PSA declines were reported. Version 9.2 of the SAS statistical software package was used to provide all statistical analyses.

Results

Patient characteristics

Eighteen patients were recruited between June 2009 and June 2012: cohorts 1 and 2 had 3 patients each, and cohorts 3 and 4 had six patients each. The final analysis was performed in October 2012. The median age was 72 years (range 58–86) and 17 patients were Caucasian (94.4%) and 1 was African-American. The median PSA was 50 ng/mL (range 2–1070) and the sites of metastases were typical of mCRPC (Table 1). Ten patients (55.5%) had progressed following prior docetaxel.

Toxicities

No patients experienced a DLT or discontinued treatment due to an adverse event (AE) (Table 2). Most AEs were grade 1 and included injection site erythema (100.0%), fatigue (33.3%), injection site induration (33.3%), myalgia (27.8%), anemia (22.2%), diarrhea (22.2%), upper respiratory tract infection (22.2%), hypocalcemia (22.2%) and arthralgia (22.2%). Of the seven grade 3 AEs seen in six patients, two (elevated AST and intestinal obstruction) were considered unrelated to therapy. The remaining five grade 3 AEs (syncope, hematuria, urinary tract infection, fatigue and pulmonary embolism) were considered unlikely related. There were no dose-dependent increase of toxicities or grade 4 AEs observed. Notably, one high-dose Cohort 3 subject experienced a single acute grade 2 cytokine release reaction during infusion of rimiducid after the second vaccination, but continued induction without further drug-related adverse events after the addition of premedication. Two subjects went off protocol prior to the end of induction due to progression.

Efficacy

Overall, 33.3% of patients experienced disease progression or died, with a mean PFS of 269.5 days and mean OS of 477 days. The mean OS among chemo-naive patients was 530 days, and among post-docetaxel patients 304.9 days. Overall, 85.7% of patients had at least stable disease, and 14.3% had progressive disease by RECIST 1.1 and PCWG-2 criteria after completion of therapy at week 13. Evidence for anti-tumor activity was noted in both docetaxel-naïve and post-docetaxel patients. Partial responses (PRs) by RECIST 1.1 were observed in two patients: One patient had six measurable metastatic lesions in the lungs, which were eliminated by the end of the 12 weeks induction phase of treatment (Fig. 1a); another patient attained

Table 1 Patient characteristics

	Cohort 1 ($N = 3$)	Cohort 2 ($N = 3$)	Cohort 3 ($N = 6$)	Cohort 4 ($N = 6$)	Overall ($N = 18$)
Age at first injection					
Mean	75.0	73.3	75.2	68.7	72.7
Median	72.0	74.0	74.5	67.5	72.0
SD	5.20	7.00	7.60	9.90	8.00
Min, max	72, 81	66, 80	67, 86	58, 84	58, 86
Age at prostate cancer diagnosis					
Mean	63.7	70.0	65.7	59.2	63.9
Median	59.0	68.0	65.0	59.0	63.5
SD	10.80	8.20	9.50	7.40	8.90
Min, max	56, 76	63, 79	55, 81	48, 69	48, 81
Race, *n* (%)					
White	3 (100.0)	3 (100.0)	6 (100.0)	5 (83.3)	17 (94.4)
Asian	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Native American	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Black or African-American	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	1 (5.6)
Hispanic	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Other	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Body mass index (kg/m^2)					
Mean	27.9	25.4	29.2	29.4	28.4
Median	27.4	24.1	29.0	29.6	28.9
SD	1.60	3.70	4.90	3.60	3.90
Min, max	27, 30	23, 30	23, 37	24, 34	23, 37
Baseline PSA (ng/mL)					
Mean	109.9	48.8	329.4	87.8	165.5
Median	11.1	46.5	40.9	60.8	50.0
SD	175.70	19.20	483.40	78.90	298.00
Min, max	6, 313	31, 69	2, 1070	4, 200	2, 1070
Duration of prostate cancer (years)					
Mean	11.1	3.6	9.4	9.1	8.6
Median	13.2	2.7	10.2	9.7	9.1
SD	6.10	2.60	4.50	4.10	4.70
Min, max	4, 16	2, 6	4, 16	4, 14	2, 16
Clinical subtype of disease, *n* (%)					
3	1 (33.3)	0 (0.0)	1 (20.0)	3 (100.0)	5 (35.7)
4	0 (0.0)	2 (66.7)	3 (60.0)	0 (0.0)	5 (35.7)
5	2 (66.7)	1 (33.3)	1 (20.0)	0 (0.0)	4 (28.6)
Unknown	0	0	1	3	4
Clinical sites of disease, *n* (%)					
Bone	0 (0.0)	1 (33.3)	2 (40.0)	0 (0.0)	3 (21.4)
Nodal	1 (33.3)	0 (0.0)	1 (20.0)	3 (100.0)	5 (35.7)
Visceral	1 (33.3)	0 (0.0)	1 (20.0)	0 (0.0)	2 (14.3)
Two or more sites	1 (33.3)	2 (66.7)	1 (20.0)	0 (0.0)	4 (28.6)
Unknown	0	0	1	3	4
Clinical stage of prostate cancer, primary tumor, *n* (%)					
X	3 (100.0)	2 (66.7)	0 (0.0)	0 (0.0)	5 (31.3)
T4A	0 (0.0)	1 (33.3)	1 (20.0)	0 (0.0)	2 (12.5)
2B	0 (0.0)	0 (0.0)	0 (0.0)	1 (20.0)	1 (6.3)
2C	0 (0.0)	0 (0.0)	1 (20.0)	2 (40.0)	3 (18.8)
3	0 (0.0)	0 (0.0)	1 (20.0)	0 (0.0)	1 (6.3)

Table 1 continued

	Cohort 1 ($N = 3$)	Cohort 2 ($N = 3$)	Cohort 3 ($N = 6$)	Cohort 4 ($N = 6$)	Overall ($N = 18$)
3A	0 (0.0)	0 (0.0)	0 (0.0)	1 (20.0)	1 (6.3)
3B	0 (0.0)	0 (0.0)	0 (0.0)	1 (20.0)	1 (6.3)
4	0 (0.0)	0 (0.0)	1 (20.0)	0 (0.0)	1 (6.3)
4A	0 (0.0)	0 (0.0)	1 (20.0)	0 (0.0)	1 (6.3)
Unknown	0	0	1	0	1
Clinical stage of prostate cancer, regional lymph nodes, *n* (%)					
X	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	1 (5.6)
0	0 (0.0)	0 (0.0)	1 (16.7)	1 (16.7)	2 (11.1)
1	3 (100.0)	0 (0.0)	2 (33.3)	3 (50.0)	8 (44.4)
2	0 (0.0)	2 (66.7)	2 (33.3)	2 (33.3)	6 (33.3)
3	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	1 (5.6)
Clinical stage of prostate cancer, distant metastases, *n* (%)					
0	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	1 (5.6)
1	3 (100.0)	3 (100.0)	6 (100.0)	5 (83.3)	17 (94.4)
Narcotics use					
Yes	1 (33.3)	0 (0.0)	1 (16.7)	5 (83.3)	7 (38.9)
No	2 (66.7)	3 (100.0)	5 (83.3)	1 (16.7)	11 (61.1)

Percentages are based on non-missing values. For clinical subtype: 3 = nodal spread and no evident bone or visceral disease, 4 = bone disease with or without nodal disease and no evident visceral spread, 5 = visceral metastases with or without spread at other sites

PSA prostate-specific antigen

a PR with continued regression of abdominal lymphadenopathy in the context of rising PSA over a duration of 1 year (Fig. 1b). There were two shifts from >5 to ≤5 CTCs/7.5 mL and six shifts from ≤5 to >5 CTCs/7.5 mL. At the end of the induction phase of therapy within 12 weeks, 6 of 17 (35.3%) evaluable patients had a decline in PSA and 1 patient exhibited a >30% decline (specifically, 46% decline from 330 to 170 ng/mL) (Fig. 2). Another patient had an 85% PSA decline (from 1070 to 169 ng/mL) after 12 weeks. Additionally, 52.9% of all patients experienced a ≥25% increase in PSA doubling time.

Notably, responses to post-BPX101 therapy appeared robust. In one patient, the 12 weeks scans showed mild central necrosis of liver and lung lesions. Thereafter, he received a combination of docetaxel, estramustine and carboplatin, which led to a rapid PSA decline to <0.2 ng/mL and complete response of all visceral metastases (Fig. 1c). In addition, two subjects exhibited a significant PSA response to docetaxel and dose-reduced cabazitaxel (without prednisone), respectively, despite previous progression on docetaxel. Robust PSA responses were also observed with abiraterone acetate administered post-BPX101 in two patients.

Pharmacokinetics

Rimiducid concentrations were measured during the first two doses in weeks 1 and 3 collected from 12 patients pre-dose, 15 and 30 min, and 1, 2, 4 and 8 h following IV infusion. The mean plasma time course profiles of rimiducid were similar for weeks 1 and 3. The mean plasma concentrations increased during the 2-h IV infusion reaching a plateau at 2 h of 617 ± 286 ng/mL in week 1 and 599 ± 327 ng/mL in week 3. After completion of infusion, rimiducid levels decreased rapidly, reaching 17.3 ± 11.1 ng/mL in week 1 and 21.4 ± 17.3 ng/mL in week 3 at 8 h after starting the infusion. C_{max} levels were 628 ± 280 ng/mL for week 1 and 639 ± 284 ng/mL for week 3.

Immune response studies

Clinical evidence of Immune response to BPX101 was observed with injection site erythema and induration (Fig. 3a). Tumor tissue CD4+ T cells, CD8+ T cells and CD20+ B cell infiltration surrounding PSMA-expressing necrotic cells was demonstrated by IHC in a tumor sample of a patient who underwent transurethral resection of the prostate during intervention for hematuria after starting therapy (Fig. 3b). Skin biopsies from the injection site were assayable in five cases and all displayed lymphocytic immune cell infiltration: three patients displayed CD8+ T lymphocyte responses, accompanied by high-level peripheral blood IFNγ production, and two patients also elaborated other aforementioned pro-inflammatory cytokines evaluated according to the protocol (data not shown).

Table 2 Adverse events of any grade

System organ class preferred term	Cohort 1 ($N = 3$) N (%)	Cohort 2 ($N = 3$) N (%)	Cohort 3 ($N = 6$) N (%)	Cohort 4 ($N = 6$) N (%)	Overall ($N = 18$) N (%)
Patients reporting at least 1 AE	3 (100.0)	3 (100.0)	6 (100.0)	6 (100.0)	18 (100.0)
Blood and lymphatic system disorders	0 (0.0)	0 (0.0)	2 (33.3)	2 (33.3)	4 (22.2)
Anemia	0 (0.0)	0 (0.0)	2 (33.3)	2 (33.3)	4 (22.2)
Ear and labyrinth disorders	2 (66.7)	0 (0.0)	0 (0.0)	1 (16.7)	3 (16.7)
Vertigo	2 (66.7)	0 (0.0)	0 (0.0)	1 (16.7)	3 (16.7)
Gastrointestinal disorders	2 (66.7)	0 (0.0)	2 (33.3)	1 (16.7)	5 (27.8)
Diarrhea	1 (33.3)	0 (0.0)	2 (33.3)	1 (16.7)	4 (22.2)
Intestinal obstruction	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	1 (5.6)
Nausea	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	1 (5.6)
Pelvic pain	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)
Vomiting	1 (33.3)	0 (0.0)	0 (0.0)	1 (16.7)	2 (11.1)
General disorders and administration site conditions	3 (100.0)	3 (100.0)	6 (100.0)	6 (100.0)	18 (100.0)
Axillary pain	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)
Catheter site hematoma	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	1 (5.6)
Catheter site pain	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	1 (5.6)
Fatigue	3 (100.0)	1 (33.3)	1 (16.7)	1 (16.7)	6 (33.3)
Influenza-like illness	0 (0.0)	0 (0.0)	1 (16.7)	1 (16.7)	2 (11.1)
Infusion site extravasation	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	1 (5.6)
Injection site erythema	3 (100.0)	3 (100.0)	6 (100.0)	6 (100.0)	18 (100.0)
Injection site hematoma	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)
Injection site induration	3 (100.0)	0 (0.0)	3 (50.0)	0 (0.0)	6 (33.3)
Injection site pain	0 (0.0)	0 (0.0)	1 (16.7)	1 (16.7)	2 (11.1)
Injection site pruritus	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	1 (5.6)
Injection site reaction	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (11.1)
Pain	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	1 (5.6)
Pyrexia	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	1 (5.6)
Immune system disorders	0 (0.0)	0 (0.0)	1 (16.7)	1 (16.7)	2 (11.1)
Cytokine release syndrome	0 (0.0)	0 (0.0)	1 (16.7)	1 (16.7)	2 (11.1)
Infections and infestations	2 (66.7)	0 (0.0)	4 (66.7)	1 (16.7)	7 (38.9)
Oral candidiasis	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	1 (5.6)
Upper respiratory tract infection	2 (66.7)	0 (0.0)	2 (33.3)	0 (0.0)	4 (22.2)
Urinary tract infection	0 (0.0)	0 (0.0)	2 (33.3)	1 (16.7)	3 (16.7)
Injury, poisoning, and procedural complications	3 (100.0)	1 (33.3)	1 (16.7)	1 (16.7)	6 (33.3)
Contusion	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	1 (5.6)
Excoriation	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)
Fall	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (11.1)
Incision site erythema	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (11.1)
Incision site edema	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (11.1)
Periorbital hematoma	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	1 (5.6)
Procedural nausea	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	1 (5.6)
Procedural pain	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	1 (5.6)
Investigations	1 (33.3)	2 (66.7)	3 (50.0)	0 (0.0)	6 (33.3)
Aspartate aminotransferase increased	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)	2 (11.1)
Blood sodium decreased	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	1 (5.6)
Hemoglobin decreased	0 (0.0)	1 (33.3)	1 (16.7)	0 (0.0)	2 (11.1)
Hepatic enzyme increased	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	1 (5.6)
International normalized ratio increased	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)

Table 2 continued

System organ class preferred term	Cohort 1 ($N = 3$) N (%)	Cohort 2 ($N = 3$) N (%)	Cohort 3 ($N = 6$) N (%)	Cohort 4 ($N = 6$) N (%)	Overall ($N = 18$) N (%)
Metabolism and nutrition disorders	2 (66.7)	1 (33.3)	2 (33.3)	0 (0.0)	5 (27.8)
Dehydration	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	1 (5.6)
Hypocalcemia	2 (66.7)	1 (33.3)	1 (16.7)	0 (0.0)	4 (22.2)
Musculoskeletal and connective tissue disorder	2 (66.7)	3 (100.0)	4 (66.7)	4 (66.7)	13 (72.2)
Arthralgia	1 (33.3)	1 (33.3)	2 (33.3)	0 (0.0)	4 (22.2)
Back pain	0 (0.0)	1 (33.3)	0 (0.0)	1 (16.7)	2 (11.1)
Foot fracture	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	1 (5.6)
Groin pain	0 (0.0)	1 (33.3)	1 (16.7)	0 (0.0)	2 (11.1)
Myalgia	2 (66.7)	1 (33.3)	0 (0.0)	2 (33.3)	5 (27.8)
Pain in extremity	1 (33.3)	0 (0.0)	1 (16.7)	0 (0.0)	2 (11.1)
Nervous system disorder	2 (66.7)	1 (33.3)	1 (16.7)	1 (16.7)	5 (27.8)
Dizziness	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (11.1)
Headache	0 (0.0)	1 (33.3)	1 (16.7)	1 (16.7)	3 (16.7)
Syncope	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)
Psychiatric disorders	0 (0.0)	1 (33.3)	2 (33.3)	0 (0.0)	3 (16.7)
Anxiety	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	1 (5.6)
Depression	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	1 (5.6)
Insomnia	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	1 (5.6)
Renal and urinary disorders	2 (66.7)	0 (0.0)	1 (16.7)	2 (33.3)	5 (27.8)
Bladder pain	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	1 (5.6)
Bladder spasm	1 (33.3)	0 (0.0)	1 (16.7)	0 (0.0)	2 (11.1)
Flank pain	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	1 (5.6)
Hematuria	1 (33.3)	0 (0.0)	1 (16.7)	0 (0.0)	2 (11.1)
Hydronephrosis	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	1 (5.6)
Proteinuria	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)
Respiratory, thoracic, and mediastinal disorders	0 (0.0)	0 (0.0)	1 (16.7)	1 (16.7)	2 (11.1)
Oropharyngeal pain	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	1 (5.6)
Pulmonary embolism	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	1 (5.6)
Vascular disorders	1 (33.3)	1 (33.3)	0 (0.0)	1 (16.7)	3 (16.7)
Hypertension	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)
Orthostatic hypotension	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	1 (5.6)
Phlebitis	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	1 (5.6)

TEAE treatment-emergent adverse events

Spikes of varying extent for most cytokines measured in the plasma were seen in the majority of subjects evaluated. A pattern of cytokine spiking 2- to 13,000-fold across all patients, 1 week after each dose, and returning to baseline the following week was observed, generally trending higher after later vaccinations in the maintenance phase. In general, patients in the high-dose cohort exhibited the highest magnitude of spiking, and an apparent correlation with tumor burden with cytokine spiking in the low- and mid-dose cohorts was less apparent at the high dose. For example, one patient in the low-dose cohort showed cytokine spikes averaging 4-fold following each induction phase vaccine dose, which increased to between 6- and 56-fold after the final three maintenance phase injections (Fig. 3c). For the same subject, IL-6 had declined following the induction phase to <1 pg/mL and thereafter spiked between 1680- and 13,000-fold after each of the last three maintenance doses. Another high-dose subject who experienced near complete regression of lung metastases with otherwise stable disease showed cytokine spikes averaging 150-fold during the induction phase with some spikes >1000-fold. Cytokine spikes were not associated with toxicities and there was a trend for correlation between clinical and PSA responses and spiking of serum cytokines.

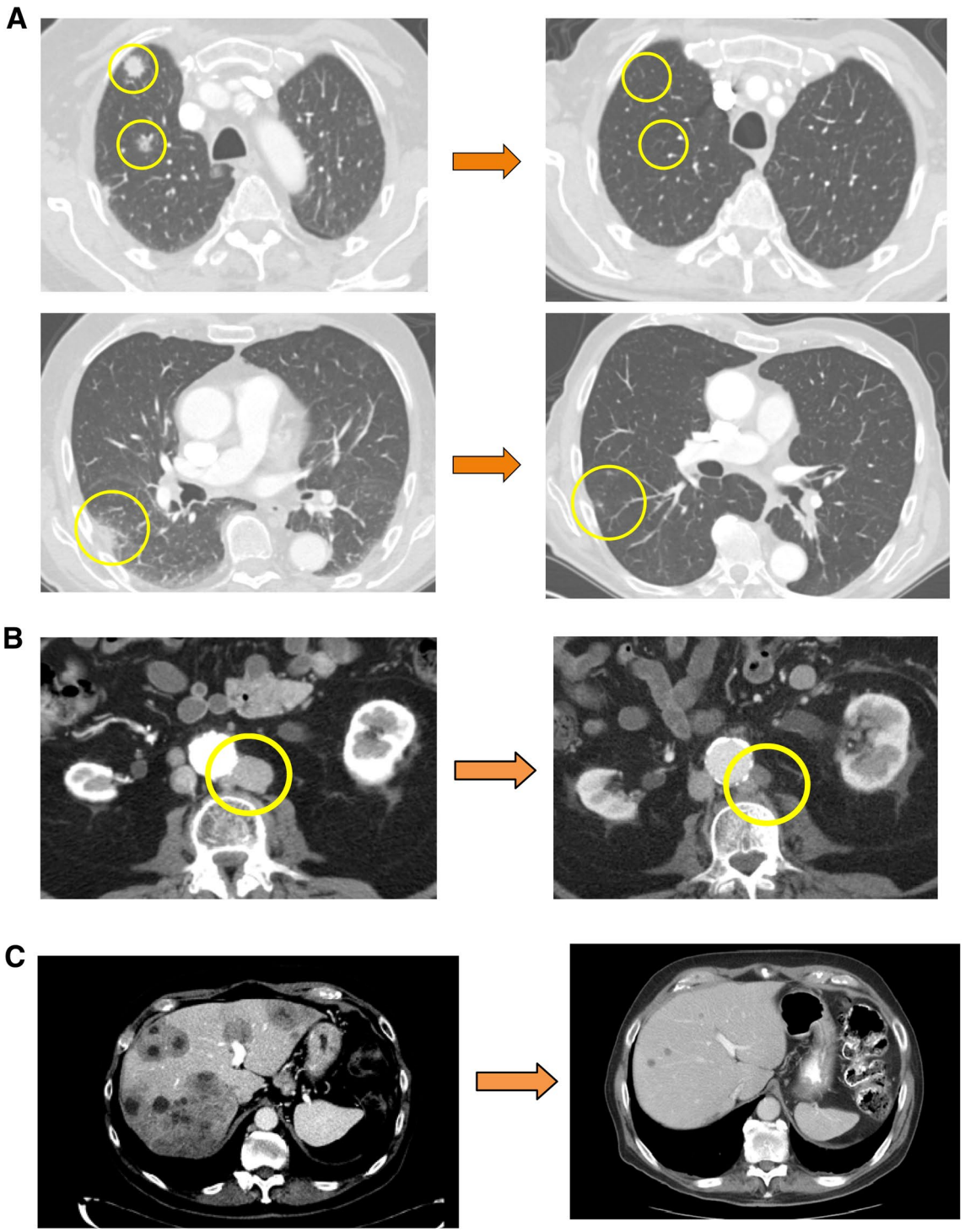

Fig. 1 Objective measurable disease responses with BPX101. **a** Complete regression of measurable lung metastases in one patient receiving BPX101. **b** Partial response of a retroperitoneal lymph node in another patient receiving BPX101 and **c** complete regression of bulky liver metastases with combination docetaxel-based chemotherapy following completion of BPX101 therapy

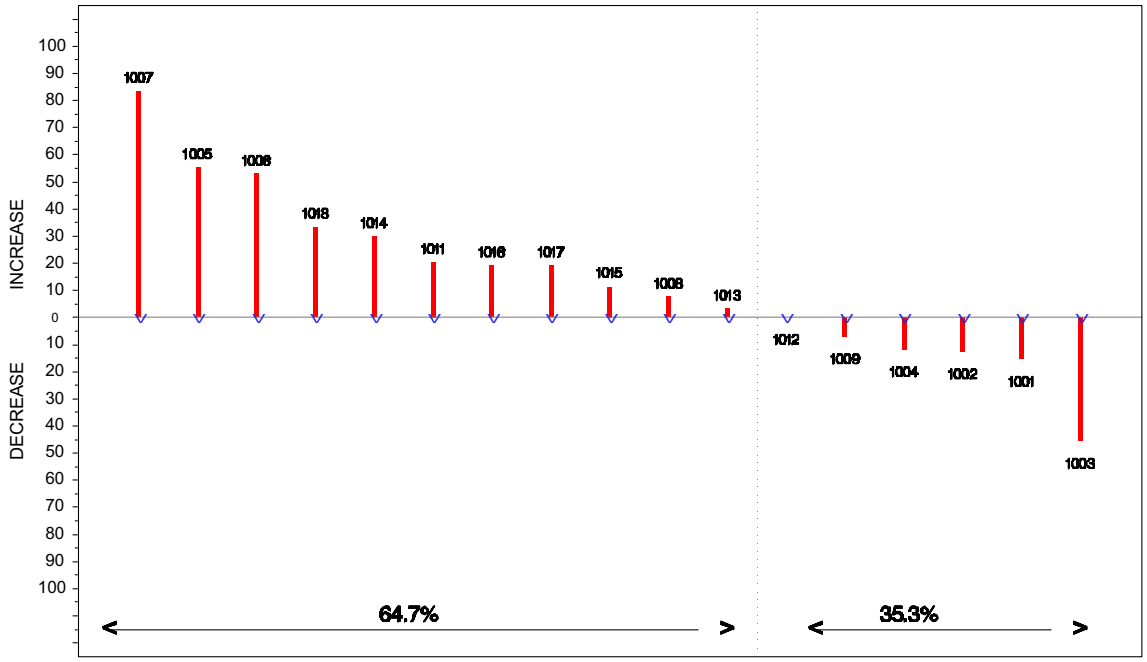

Fig. 2 Maximum PSA change within 12 weeks in evaluable patients ($n = 17$). Six of 17 (35.3%) evaluable patients exhibited a PSA decline of any level and one patient had a >30% decline

Discussion

This phase I trial demonstrates that BPX-101 can be reliably manufactured and safely administered with no DLTs at doses of up to least 25×10^6 cells, followed by intravenous rimiducid given 24 h after each dose. Immune responses were observed and contrary to data with sipuleucel-T therapy, which demonstrated improved survival without short-term responses, monotherapy with BPX101 plus rimiducid yielded objective measurable disease responses, PSA declines and potentially improved activity of post-vaccine therapy. Furthermore, T-cell responses were demonstrated both at the skin injection site and intratumorally, with progressively increasing serum spikes of multiple cytokines following each injection. More interestingly, a trend for correlation between objective and PSA responses and the magnitude of serum cytokine spikes following vaccination was observed suggesting these cytokine changes may be indicative of anti-tumor immune responses.

Historically, immunotherapy for mCRPC has demonstrated mixed success. GM-CSF has demonstrated modest disease-modulating activity in advanced prostate cancer [25, 26]. Older vaccines such as GVAX (cell line engineered to secrete GM-CSF) failed to improve outcomes [27, 28]. Then, sipuleucel-T was demonstrated to improve OS in a landmark phase III trial [10]. Although there was initial skepticism regarding the benefit conferred by sipuleucel-T, subsequent presentations have shed insights regarding prostate cancer-specific immune responses generated by this agent, which appeared to correlate with improved clinical outcomes [29, 30]. Ongoing phase I/II trials are evaluating the combination of sipuleucel-T with agents that augment T-cell response such as cytotoxic T-lymphocyte antigen (CTLA)-4, programmed death (PD)-1 and indoleamine 2,3-dioxygenase (IDO)-1 inhibitors.

Subsequent data following the emergence of sipuleucel-T have been somewhat disappointing. Ipilimumab, a monoclonal antibody targeting CTLA-4, a T-lymphocyte checkpoint, showed a trend for improved OS in post-docetaxel patients, and improved survival in a subset of those with lower tumor burden [31]. Unfortunately, the subsequent phase III trial of ipilimumab in docetaxel-naïve mCRPC patients did not improve OS. A phase I trial reported no activity for nivolumab, a PD-1 inhibitor, in 17 patients with mCRPC [32]. Nevertheless, pembrolizumab, another PD-1 inhibitor, is undergoing phase II evaluation (Keynote-199 trial) and other immunotherapeutic agents are undergoing phase III evaluation including prostvac-TRICOM, a pox-virus-based co-stimulatory molecule-assisted antigen-targeted vaccine, and DCVAC, a second-generation APC vaccine pulsed with killed LNCaP tumor cells [33, 34]. Other approaches such as DNA and peptide vaccines and activation of pro-drug in tumors engineered to express the herpes thymidine kinase gene appear preliminarily promising [35–38].

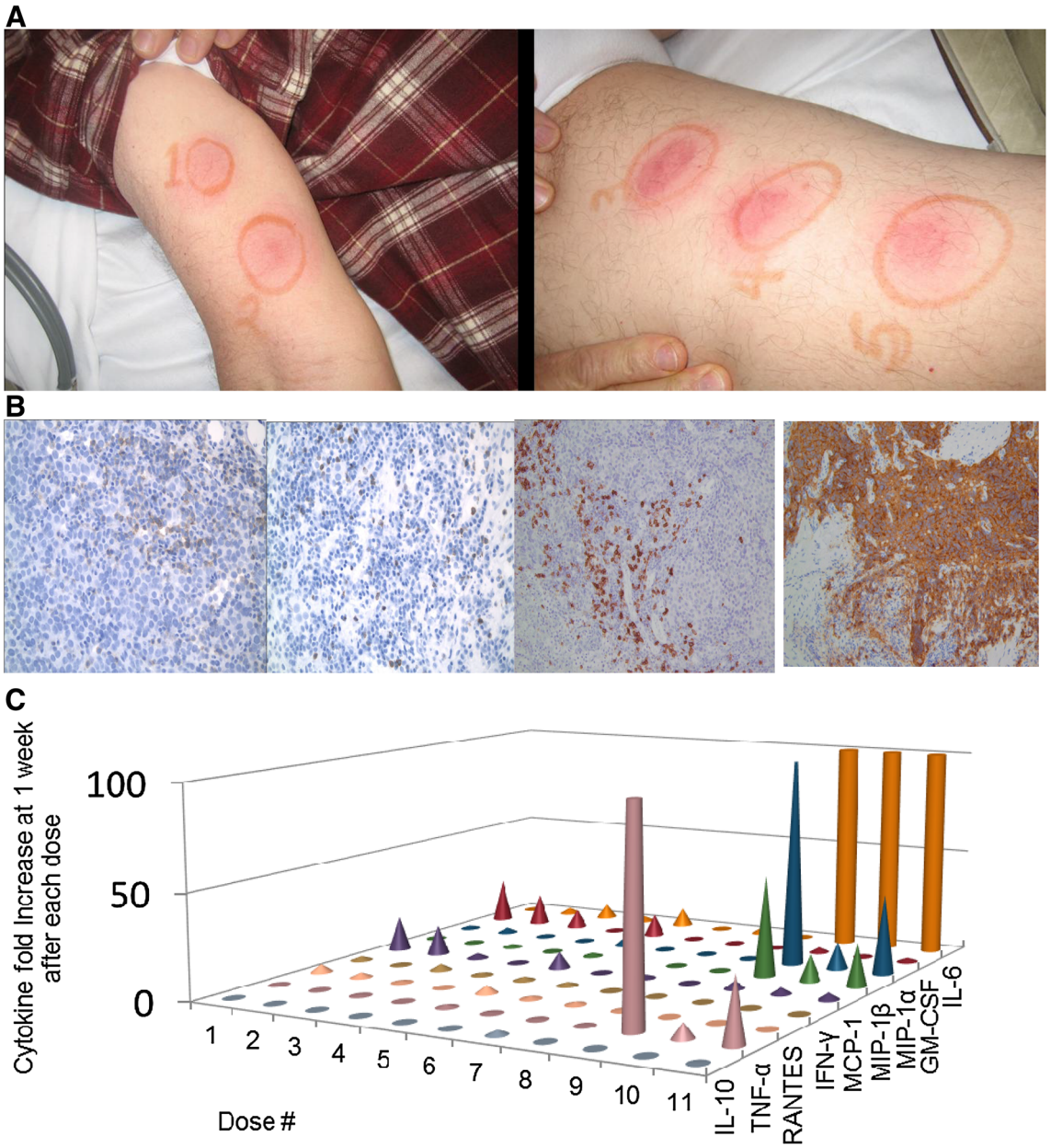

Fig. 3 Immune response to BPX101. The figure shows **a** injection site erythema and induration in a patient, **b** tumor immunohistochemistry showing CD4+ T cells (*left* extreme panel), CD8+ T cells (*left-middle*) and CD20+ B cell infiltration (*right middle*) surrounding PSMA-expressing tumor cells (*right* extreme) in a sample from transurethral resection of the prostate and **c** showing cytokine spikes in a patient in the low-dose cohort

This phase I trial is limited by the small sample size. Immune responses were documented mostly in terms of pro-inflammatory serum cytokine spikes. T-cell responses in the tumor and injection site were documented in a subset of patients, but control biopsies before vaccine administration were not performed. However, the specificity of the immune response to prostate antigens is unclear. The cytokines were selected to capture an immune response following vaccine activation, but are essentially non-specific in nature. The cytokines observed to spike may be associated with a cellular immune response (e.g., TNFα, IFN-γ, RANTES) or an antibody response (e.g., IL-6, IL-10). However, cytokines have pleiotropic functions and even have a detrimental impact on cancer control. For example, IL-6 and IL-10 have both pro-inflammatory and anti-inflammatory functions. GM-CSF, MIP-1α, MIP-1β and MCP-1 are involved in granulocyte and macrophage maturation. Optimized enzyme-linked immunospot (ELISPOT)

assays to detect PSMA-specific T-cell responses in peripheral blood, tumor or injection site could not be refined and developed, and hence were not performed. Although objective measurable tumor responses and PSA declines were noted with BPX101 plus rimiducid, a larger trial is necessary to validate these results. Furthermore, while responses to post-BPX101 agents appeared robust, it is difficult to discern an increment over what would have been observed without preceding BPX101 therapy without a randomized trial. Finally, the cost of manufacturing APC vaccines is a challenge. In this context, the BPX101 vaccine was manufactured by employing a single session of leukapheresis, followed by the use of the cryopreserved and stored vaccine product (unlike sipuleucel-T, which requires leukapheresis before each dose of vaccine). The requirement to return the following day for infusion of the activating agent imposes a slight inconvenience. The small incidence of a cytokine release reaction following rimiducid needs to be addressed, but may be anticipated to be alleviated by appropriate routine premedication before rimiducid.

In summary, temporally controlled, lymphoid-localized, DC-specific activation of ihCD40 by rimiducid in a PSMA-targeted APC vaccine, BPX101, demonstrated safety, immune response and anti-tumor activity in both chemonaive and post-chemotherapy patients with mCRPC. Further development of this promising therapeutic modality may be warranted including combinations with T-cell checkpoint inhibitors and anti-androgen agents. Further refinement of the vaccine platform should also be developed in conjunction with measures to reduce the costs of production. In preclinical studies, unified adenoviral vectors encoding PSMA along with composite ihMyD88/CD40 obviated LPS requirements and exhibited potent anti-tumor potential in a streamlined process [39]. Indeed, a second-generation vaccine, BPX-201, constructed using DCs transduced with adenoviral vector bearing genes for iMyD88/CD40 and PSMA followed by activation in vivo by rimiducid is being evaluated in a phase 1 trial (NCT01823978).

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Compliance with ethical standards

Conflict of interest G. Sonpavde: Consultant for Bayer, Sanofi, Pfizer, Novartis, Eisai, Janssen, Amgen, Astrazeneca, Merck, Genentech, Argos, Agensys; research support to institution from Bellicum, Bayer, Onyx, Celgene, Boehringer-Ingelheim, Merck, Pfizer; author for Uptodate; speaker for Clinical Care Options. J.D. McMannis, Y. Bai, M. Seethammagari, J.M.C. Bull, V. Hawkins, T. Dancsak, N. Lapteva, J.M. Levitt: funding to institution by Bellicum Pharmaceuticals, Inc. D.M. Spencer, A. Moseley, K.M. Slawin: employed by and shareholders of Bellicum Pharmaceuticals, Inc.

Research involving human participants The protocol was approved by the institutional review boards (IRBs) at the University of Texas, Houston, Texas.

Informed consent All participants provided written informed consent. The trial was registered at ClinicalTrials.gov under the identification NCT00868595.

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