Pasotuxizumab, a BiTE® immune therapy for castration-resistant prostate cancer: Phase I, dose-escalation study findings

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Aim: We report results of a first-in-human study of pasotuxizumab, a PSMA bispecific T-cell engager (BiTE®) immune therapy mediating T-cell killing of tumor cells in patients with advanced castration-resistant prostate cancer. **Patients & methods:** We assessed once-daily subcutaneous (SC) pasotuxizumab. All SC patients developed antidrug antibodies; therefore, continuous intravenous (cIV) infusion was assessed. **Results:** A total of 47 patients received pasotuxizumab (SC: n = 31, 0.5–172 µg/d; cIV: n = 16, 5–80 µg/d). The SC maximum tolerated dose was 172.0 µg/d. A sponsor change stopped the cIV cohort early; maximum tolerated dose was not determined. PSA responders occurred (>50% PSA decline: SC, n = 9; cIV, n = 3), including two long-term responders. **Conclusion:** Data support pasotuxizumab safety in advanced castration-resistant prostate cancer and represent evidence of BiTE monotherapy efficacy in solid tumors.
   Clinical trial registration: NCT01723475 (ClinicalTrials.gov)

Future Medicine

Patients with advanced metastatic castration-resistant prostate cancer (mCRPC) have a poor prognosis [1]; although many treatment options are available, treatment effects are transient and survival benefits are limited [2]. Immune checkpoint inhibitors, BiTE® (bispecific T-cell engager) immune therapies and chimeric antigen receptor (CAR) T cells have been approved for certain cancer types over the last decade [3–12]. However, the role of BiTE molecules or CAR T cell approaches in solid cancers is still unclear, including in patients with mCRPC. PSMA is expressed in the epithelial cells of a variety of normal tissues, including the prostate, urinary bladder and proximal tubules of the kidney [13]; however, its expression is much higher in prostate cancer and its metastates [13–15] and, thus, is a compelling therapeutic target [14,16]. Lutetium-177 (^{177}Lu)-PSMA-617, a radionuclide treatment that binds to PSMA, has antitumor activity [16–19], thus validating PSMA as a target for therapy in mCRPC. Additionally, gallium-68 (^{68}Ga)-PSMA-11 is a common positron emission tomographic (PET) imaging tracer used to detect PSMA [20].

Immune checkpoint inhibitors have not shown efficacy in most patients with mCRPC, likely as a result of the heterogeneous and immune suppressive microenvironment in prostate cancer. Early studies with antiprogrammed death 1 (PD-1) antibody monotherapy showed limited efficacy in patients with prostate cancer [21], with recent reports suggesting that only certain subsets of patients benefit [22]. Similarly, a recent study reported that the cytotoxic T-lymphocyte–associated inhibitor ipilimumab may be effective in certain subsets of patients with mCRPC [23]; however, it failed to show significant survival benefit and was associated with higher rates of severe adverse effects in an unselected population [24]. A recent study found that addition of the antiprogrammed death-ligand 1 antibody atezolizumab to the androgen receptor antagonist enzalutamide did not show any benefit in terms of overall survival versus enzalutamide alone [25].

Pasotuxizumab (also known as AMG 212 or BAY 2010112), a 55 kDa BiTE immune therapy, is designed to engage CD3 on T cells and PSMA on prostate cancer cells, thereby activating a patient's own T cells to eliminate PSMA-expressing prostate cancer cells. In preclinical studies, pasotuxizumab was shown to bind to PSMA-expressing human cells and to human T cells and to trigger antigen-dependent target cell lysis, T-cell activation and cytokine release [26]. In human prostate cancer cell lines, pasotuxizumab-directed T cells resulted in cell lysis at half-maximal effective concentrations (EC_{50}) between 0.1 and 4 ng/ml (1.8 and 72 pmol/l) [26]. Moreover, pasotuxizumab delayed tumor growth and led to tumor shrinkage and remission in human prostate cancer xenograft models [26].

This Phase I, open-label, dose-escalation study of pasotuxizumab was undertaken in patients with advanced CRPC. The primary objective was to determine the safety and maximum tolerated dose (MTD) of pasotuxizumab administered by subcutaneous (SC) injection or continuous intravenous (cIV) infusion. Secondary objectives included evaluation of the pharmacokinetics, PSA and radiographic tumor response; exploratory objectives included the evaluation of biomarkers, such as circulating tumor cells (CTCs) and radio-imaging.

Materials & methods
Patients
Men aged ≥18 years with histologically or cytologically confirmed advanced CRPC with treatment failure after ≥1 taxane regimen and who were refractory to abiraterone and/or enzalutamide or refused any other standard therapy were eligible for inclusion in the study. Eligible patients had undergone bilateral orchiectomy or received continuous androgen deprivation therapy and had evidence of progressive disease after discontinuation of anti-androgen therapy (i.e., flutamide, bicalutamide or nilutamide) before study drug treatment; had an Eastern Cooperative Oncology Group performance status of 0–2; had life expectancy of ≥3 months; and had adequate bone marrow, liver and renal function. Key exclusion criteria included confirmed prior or current autoimmune disease, any antitumor therapy or immunotherapy within 4 weeks of the first pasotuxizumab dose (with the exception of denosumab, bisphosphonates and gonadotropin-releasing hormone agonists or antagonists), or history of symptomatic metastatic brain or meningeal tumors (unless the patient had not received definitive therapy for >3 months and had no evidence of tumor growth within 2 weeks of study entry). The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. The study protocol was approved by the institutional review boards/ethics committees at each study site; all patients provided written informed consent.

Study design

This was an open-label, multicenter, Phase I, dose-escalation study (ClinicalTrials.gov, NCT01723475) conducted at five clinical study centers in Germany and Austria and sponsored by Bayer AG, Leverkusen, Germany. It was designed to determine the safety and MTD of pasotuxizumab (primary objectives) and to assess pharmacokinetics, PSA and tumor response (secondary objectives) and biomarkers (exploratory objective) of pasotuxizumab administered either by daily SC injection or cIV infusion. An independent data monitoring committee was established to regularly review safety data. The starting doses for the SC and cIV cohorts were 0.5 and 5 μg daily, respectively.

The MTD was defined as the maximum dose at which <20% of patients in a cohort had dose-limiting toxicities (DLTs) in cycle 1 (i.e., days 1–21). Initially, single-patient SC cohorts were assessed; if no DLTs or treatment-related adverse events (AEs) of grade ≥2 occurred in cycle 1 in the single-patient cohorts, cohorts of three patients with a twofold dose escalation were then enrolled. If any patient in the single-patient cohorts experienced a DLT or a treatment-related AE of grade ≥2 in cycle 1, the cohort was expanded to include two additional patients.

As part of an interim data monitoring committee safety review, it was found that all evaluable patients in the SC cohort had developed antidrug antibodies (ADAs). As a result, the protocol was amended to include topical glucocorticoids to suppress ADA formation. Clobetasol propionate 0.05% cream was applied to each administration site over three 7-day periods for the first two treatment cycles (i.e., during days -7–1 of cycle 1 and days 15–21 of cycles 1 and 2). Methylprednisolone aceponate 0.1% cream was also applied to the site daily for the first three 21-day treatment cycles. In addition, the protocol was amended to require the administration of prophylactic oral and/or IV dexamethasone the evening before the first SC administration of pasotuxizumab, 30 min before administration on day 1 of cycle 1, optionally on days 2 and 3 of cycle 1 and before restarting pasotuxizumab treatment after missing ≥2 SC doses. However, this approach had no effect on the development of ADAs. In August 2016, the development of neutralizing antibodies rendered SC dosing nonviable and the study continued with the cIV cohort only.

In the SC cohort, pasotuxizumab was administered daily by SC injection over a 21-day cycle, with no breaks between cycles. The 2-ml syringes for SC administration were prepared by the local pharmacy and administered either in the clinic by a healthcare professional or at home by the patient. In the cIV cohort, pasotuxizumab was administered using a portable infusion pump and central venous port system. Treatment cycles were administered over a 21-day cycle, but patients received 5 weeks of treatment followed by a treatment-free interval of 1 week (i.e., one treatment-free week at the end of cycles 2 and 4). From cycle 5 onward, patients could receive treatment over 4 weeks followed by a treatment-free interval of 2 weeks. As with the latter SC cohorts, prophylactic oral or IV dexamethasone was administered the evening before the first administration of pasotuxizumab, by IV 30 min before administration on day 1 of cycle 1, optionally oral and/or IV on days 2 and 3 of cycle 1 and before restarting pasotuxizumab after a break in treatment of ≥2 days (i.e., before cycle 3 and every odd treatment cycle thereafter).

At the discretion of the investigators, concomitant therapy was allowed. Permitted concomitant medications included clinically indicated low-dose heparin, antihistamines and antacids were permitted, as were denosumab and bisphosphonates prophylactically or for bone metastases. Paracetamol, metamizole, piritramide and/or short-term high-dose corticosteroids were permitted for the treatment of cytokine-release syndrome (CRS). Concomitant palliative and supportive care for patients with any underlying illness were also permitted.

For each patient, treatment continued until tumor progression, unacceptable toxicity, consent withdrawal, or withdrawal from the study.

Assessments

Adverse events were graded using the Common Terminology Criteria for Adverse Events version 4.03.

To evaluate pharmacokinetic parameters, blood (SC and cIV cohorts) and urine (SC cohort only) samples were collected. Pharmacokinetic measurements were determined using Phoenix® WinNonlin® software (Certara USA, Inc., NJ, USA).

Immunogenicity of pasotuxizumab was evaluated to assess ADA formation. Briefly, antibody formation was initially evaluated with an electrochemiluminescence detection-based bridging immunoassay designed to minimize false negatives. Samples that tested positive were then tested with a confirmatory competitive inhibition assay. Finally, a cell-based bioassay neutralizing assay was used to detect neutralizing ADAs in positive confirmatory samples.

Efficacy was assessed according to the Prostate Cancer Clinical Trials Working Group 2 recommendations [27]. Tumor response for measurable lesions was assessed by computerized tomography (CT) and/or magnetic resonance

imaging according to the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1) [28] at screening, at every fourth cycle, at the end of treatment and as clinically indicated. Nonmeasurable lesions were assessed according to the RECIST 1.1 criteria for nonmeasurable lesions [28]. Bone metastases were assessed by bone scintigraphy. Changes in serum PSA levels were assessed predose on days 1, 8 and 15 of cycle 1, at the beginning of each subsequent cycle and at the end of treatment visit.

PSMA PET/CT imaging was conducted per institutional guidelines outside the study protocol.

Circulating tumor cells were assessed in both the SC and cIV cohorts during treatment. Pharmacodynamic markers, including cytokines, biomarkers of T-cell activation (i.e., CD69$^+$ and PD-1) and enumeration of T lymphocyte subsets were conducted before and during treatment.

Statistical analysis

Analyses of pasotuxizumab safety, tolerability, pharmacokinetics and efficacy were descriptive in nature and presented using summary statistics. The sample size required to adequately determine the MTD depended on the initial dose, rate of dose escalation and observed dose-toxicity and dose-exposure relationships. Based on experience, the chosen sample size of one to nine patients per cohort was considered sufficient to fulfill the study objectives. Patients who received ≥1 dose of pasotuxizumab and had posttreatment safety data available were included in the safety analysis set; patients who completed pasotuxizumab cycle 1 or discontinued pasotuxizumab during cycle 1 as a result of AEs or DLTs were included in the MTD evaluation set. Patients who completed the study without any major protocol violations were included in the pharmacokinetic and pharmacodynamic evaluation set; those with any efficacy data were included in the efficacy analysis set.

Results

Patients

Overall, 68 patients were enrolled between 2 November 2012 and 18 July 2018, including 45 in the SC cohort and 23 in the cIV cohort. Of these, 31 and 16, respectively, received ≥1 dose of pasotuxizumab (breakdown of patients by dose levels is shown in Supplementary Table 1). All treated patients were white, as determined by the investigators and had a median (range) age of 69 (48–82) years. Additional patient characteristics are summarized in Table 1. At study entry, 40/47 patients (85%) overall had stage IV disease, the remainder had stage III disease: 25/31 (81%) in the SC cohort and 15/16 (94%) in the cIV cohort. Overall, 28 patients (90%) in the SC cohort and 15 (94%) in the cIV cohort (Supplementary Table 2) had received ≥1 prior regimen of chemotherapy.

Patients were treated for an overall median time (including treatment interruptions, delays and drug holidays) of 91.0 (maximum, 542.0) days in the SC cohort and 129.0 (maximum, 413.2) days in the cIV cohort. All patients discontinued treatment; the most common reasons were radiologic disease progression (SC cohort, 21/31 [68%]; cIV cohort, 12/16 [75%]) and AEs not related to disease progression (4/31 [13%] and 1/16 [6%], respectively).

Safety

Treatment-emergent AEs were reported for all patients in the SC and cIV cohorts (Table 2); the most common was fever (25/31 [81%] and 15/16 [94%], respectively) followed by injection site reaction (24/31 [77%] and 0/16 [0%]), chills (7/31 [23%] and 11/16 [69%]) and fatigue (11/31 [36%] and 5/16 [31%]). AEs by dosing cohort are summarized in Supplementary Tables 3 and 4. Serious AEs were reported for 22/31 patients (71%) in the SC cohort and 12/16 (75%) in the cIV cohort. Treatment-emergent AEs of grade ≥3 were reported by 27/31 patients (87%) in the SC cohort, with the most common being anemia (39%) and decreased lymphocyte count (26%). Treatment-emergent AEs of grade ≥3 were reported by 13/16 (81%) patients in the cIV cohort, with the most common being decreased lymphocyte count (44%) and infections and infestations, not otherwise specified (31%). In the cIV cohort, ≥1 device-related infection (i.e., port catheter infection) was reported in 7/31 patients (23%) and resulted in ≥1 dose interruption in a total of six patients (19%) overall. These device-related infections were considered serious in six patients (19%) and resulted in ≥1 dose interruption in five of those patients (16%).

Study drug-related AEs were reported by all patients in the SC cohort; serious study drug–related AEs were reported by five patients (16%). There were no deaths from study drug-related AEs. DLTs were experienced by three patients (10%; n = 1 each in the 144.0-µg [grade 3 confusion and grade 2 fever], 172.0-µg [grade 3 injection site reaction] and 172.0-µg + glucocorticoids [grade 3 hypotension and grade 3 fatigue] dose cohorts). Based on safety evaluations, the MTD for the SC dose was determined to be 172.0 µg daily.

Table 1. Baseline demographics and clinical characteristics of patients who received ≥1 dose of pasotuxizumab.			
	SC cohort (n = 31)	cIV cohort (n = 16)	Overall (n = 47)
White race, n (%)†	31 (100)	16 (100)	47 (100)
Ethnicity, n (%)†			
Not Hispanic/Latino	28 (90)	16 (100)	44 (94)
Not reported	3 (10)	0	3 (6)
Age, median (range), y	69 (48–82)	69 (57–78)	69 (48–82)
Clinical characteristics at initial diagnosis, n (%)			
AJCC grading score with Gleason			
Grade 1	1 (3)	0	1 (2)
Grade 2	9 (29)	3 (19)	12 (26)
Grade 3–4	20 (65)	12 (75)	32 (68)
No grade	1 (3)	1 (6)	2 (4)
Gleason score at initial diagnosis			
6	1 (3)	0	1 (2)
7	16 (52)	4 (25)	20 (43)
8	2 (7)	6 (38)	8 (17)
9	11 (36)	5 (31)	16 (34)
Missing	1 (3)	1 (6)	2 (4)
Clinical characteristics at study entry, n (%)			
Histology of prostate cancer			
Adenocarcinoma NOS	29 (94)	13 (81)	42 (89)
Glandular intraepithelial neoplasia (grade III)	1 (3)	3 (19)	4 (9)
Papillary adenocarcinoma	1 (3)	0	1 (2)
Status of primary tumor			
Resected, no residual, or recurrent tumor	11 (36)	7 (44)	18 (38)
Resected, residual, or recurrent tumor	6 (19)	3 (19)	9 (19)
Resected, status of residual tumor unknown	3 (10)	2 (13)	5 (11)
Unresected	11 (36)	4 (25)	15 (32)
TNM stage			
I	0	0	0
II	2 (7)	0	2 (4)
III	3 (10)	1 (6)	4 (9)
IV	25 (81)	15 (94)	40 (85)
Missing	1 (3)	0	1 (2)
ECOG performance status, n (%)			
0	26 (84)	9 (56)	35 (74)
1	5 (16)	7 (44)	12 (26)

†Race/ethnicity were determined by the investigators.
AJCC: American Joint Committee on Cancer; cIV: Continuous intravenous; ECOG: Eastern Cooperative Oncology Group; NOS: Not otherwise specified; SC: Subcutaneous; TNM: Tumor node metastasis.

In the cIV cohort, study drug-related AEs were reported by 15 patients (94%). A serious study drug-related AE (grade 3 fatigue) was reported for one patient (6%) in the 20-μg/d group and was the only DLT observed. Drug-related, nonserious CRS was reported for three patients (19%); two were grade 2 and one was grade 3. No relevant organ toxicities were observed, although treatment-emergent increases in alanine aminotransferase (grade 1, 44%) and aspartate aminotransferase (grade 1, 44%; grade 2, 19%) did occur. There were no study drug-related deaths. Because of a sponsor change, the study was stopped early and the MTD for the cIV cohort was not reached.

Immunogenicity

Of the 31 patients who received ≥1 dose of SC pasotuxizumab, 30 (96.7%) had treatment of sufficient duration to assess formation of ADAs. All 30 patients developed ADAs, with a median onset of 22 days after treatment initiation

Table 2. Adverse events[†].

Adverse event, n (%)	SC cohort (n = 31)	cIV cohort (n = 16)	Overall (n = 47)
Any treatment-emergent AE	31 (100)	16 (100)	47 (100)
Serious	22 (71)	12 (75)	34 (72)
Leading to dose modification[‡]	13 (42)	8 (50)	21 (45)
Leading to permanent discontinuation of study drug	7 (23)	1 (6)	8 (17)
Any study drug–related AE	31 (100)	15 (94)	36 (77)
Worst grade			
1 or 2	16 (52)	5 (31)	21 (45)
3	8 (26)	7 (44)	25 (53)
4	7 (23)	3 (19)	
5	0	0	0
Serious	5 (16)	1 (6)	6 (13)
Leading to dose modification[‡]	5 (16)	1 (6)[§]	6 (13)
Leading to permanent discontinuation of study drug	2 (7)	0	2 (4)
Study drug–related AEs of any grade occurring in >10% of patients during either SC or cIV treatment			
Fever	25 (81)	15 (94)	40 (85)
Injection site reaction	24 (77)	0	24 (51)
Chills	7 (23)	11 (69)	18 (38)
Fatigue	11 (36)	5 (31)	16 (34)
Skin and subcutaneous tissue disorders, other	10 (32)	0	10 (21)
Investigations, other	8 (26)	2 (13)	10 (21)
General disorders and administrative site conditions, other	7 (23)	2 (13)	9 (19)
Lymphocyte count decreased	8 (26)	7 (44)	15 (32)
Pruritus	5 (16)	0	5 (11)
Nausea	4 (13)	1 (6)	5 (11)
Vomiting	4 (13)	1 (6)	5 (11)
Diarrhea	4 (13)	1 (6)	5 (11)
Platelet count decreased	3 (10)	2 (13)	5 (11)
Hypophosphatemia	3 (10)	2 (13)	5 (11)
Cytokine release syndrome	0	3 (19)	3 (6)
Infections and infestations	1 (3)	2 (13)	3 (6)
White blood cell count decreased	1 (3)	2 (13)	3 (6)
Dyspnea	0	2 (13)	2 (4)

[†]AEs were coded according to the Preferred Terms of the Medical Dictionary for Regulatory Activities, version 20.0. Severity of AEs was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03.
[‡]Modifications include delays, interruptions and reduction.
[§]A study drug–related AE of grade 3 fatigue was reported for one patient in the 20-μg/d cohort.
AE: Adverse event; cIV: Continuous intravenous; SC: Subcutaneous.

(Supplementary Table 5). Neutralizing ADAs were detected in 28 of these 30 evaluable patients; these ADAs affected exposure with no associated adverse events. Maximum ADA titers did not correlate with dose. ADAs were sustained through the end of sampling, in other words, ADAs were not transient. Topical glucocorticoid treatment did not have any effect on the development of neutralizing ADAs. Owing to the high rate of ADA development and the lack of response to ADA mitigation measures, further evaluation of the SC route of administration was discontinued. At the time of study discontinuation, ADAs had not been detected in any patients in the cIV cohort (Supplementary Table 6).

Pharmacokinetics

Following SC pasotuxizumab administration (doses of 18–172 μg, including glucocorticoids with the two higher doses), maximum serum concentration (C_{max}) of between 0.18 and 1.70 μg/l was reached between 5.95 and 23.5 h postdose on day 1 of cycle 1 and C_{max} of between 0.196 and 1.83 μg/l was reached between 3.95 and 6.08 h postdose

on day 15 of cycle 1 (Supplementary Table 7). As noted above, ADA development was associated with decreased exposure to the study drug. Accumulation was marginal following repeated dosing and dose proportionality was observed across all dose cohorts. Pasotuxizumab was below the quantification limit (0.44 µg/l) in all urine samples at 0–12 or 12–24 h after the first SC injection in doses ranging from 36 to 172 µg daily.

Following cIV pasotuxizumab administration, the mean plasma steady-state serum pasotuxizumab concentrations increased approximately dose proportionally (Supplementary Figure 1). Steady-state pasotuxizumab serum concentrations were reached within approximately 1 day after initiating pasotuxizumab IV infusion (Supplementary Figure 2).

Efficacy

In the SC cohort, reductions in PSA >50% relative to baseline were observed for the 72- and 172-µg cohorts, but there was no clear relationship between dose and response (Figure 1A). The median (range) best overall PSA response assessed as percentage change in PSA levels from baseline was -24.7% (-87.4%–28.7%; Figure 2B). Reductions in PSA >50% relative to baseline were observed in nine patients (30%) in the SC cohort and were not dose related, as noted above (Figure 1B). PSA responses typically occurred within the first two treatment cycles; however, they were not sustained, most likely because of the development of ADAs. Five of the nine patients with PSA reductions >50% were treated with 172 µg (with and without glucocorticoids); the remaining four patients were in the 18-, 36-, 72- or 144-µg dose cohorts. One patient had an initial unconfirmed response of >50% reduction in liver metastasis in parallel to the reduction in PSA levels of 85% (from 309 µg/l at baseline to a minimum of 45 µg/l).

In the cIV cohort, antitumor activity as indicated by PSA serum level decline was dose dependent, with a median best PSA change from baseline of -22.0, -37.7 and -54.9% for the 20-, 40- and 80-µg/d dose cohorts, respectively, and -20.6% overall (Figure 2A). In the cIV cohort, 14 patients showed a decline in PSA during study treatment (Figure 2B). A >50% decline in PSA was seen in three of nine patients at the higher dose levels: n = 1 each in the 20-µg/d (-75.0%), 40-µg/d (-77.7%) and 80-µg/d (-96.7%) cohorts (Figure 2B). Two patients had a long-term PSA response. Patient 13 (40 µg/d) had a >50% reduction in serum PSA for approximately 50 weeks (baseline, 39.2 µg/l; best PSA response, 9.3 µg/l; Supplementary Figure 3) and had stable disease with 337 days to tumor progression. Patient 15 (80 µg/d) had even more marked reduction in serum PSA (baseline, 196.0 µg/l; best PSA response, 6.4 µg/l), with near-complete regression of lymph node lesions and bone metastases as assessed by PSMA PET/CT and 500 days to tumor progression (see Supplemental Appendix for more detail). Before treatment, accumulation of the PSMA-specific radioligand in the skeleton and pelvic lymph nodes indicated extensive metastatic disease (Figure 3). Within 43 days of beginning treatment, the extent of PSMA-expressing tumor was significantly reduced, with negligible evidence of tumor after 85 days. A profound metabolic and morphologic response in retroperitoneal lymph node metastases was recorded as early as 3 months after treatment initiation. At 16 months, progressive disease with multiple new PSMA-positive osseous lesions was recorded (with ongoing metabolic near-complete remission of lymph node metastases).

In the SC cohort, 18 patients had measurable target lesions at baseline. Of these, no patient had a complete or partial response per RECIST 1.1; five patients (17%) had stable disease. The maximum reduction in tumor size from baseline for 14 patients is shown in Figure 4. Across the 30 evaluable patients in the SC cohort, the median (range) time to tumor progression was 92 (43–505) days.

Of the 11 RECIST-measurable patients in the cIV cohort (Supplementary Table 2), the best overall response according to RECIST 1.1 was stable disease in three patients and noncomplete response/nonprogressive disease in another three patients (Table 3). In the cIV cohort (n = 16), the median (range) time to progression for the 13 patients with progressive disease was 98 (68–500) days and 165, 84, 84, 168 and 292 days in the 5-, 10-, 20-, 40- and 80-µg/d groups, respectively.

Biomarkers

For the SC cohort, decreased lymphocyte count was reported for eight patients (26%). In this cohort, CTC counts after cycles 1, 2 and 4 did not change in a dose-related manner (Supplementary Figure 4). Additionally, CD69+ and PD-1 assessment showed early signs of T-cell activation, with CD69+ activation beginning on day 2 of cycle 1 and continuing through day 8. PD-1 activation began on day 3 of cycle 1 and was less pronounced (Supplementary Figure 5).

For the cIV cohort, decreased lymphocyte count was reported for seven patients (44%); however, recovery of lymphocyte count was complete or almost complete by day 15 (Supplementary Figure 6). The CTC values after
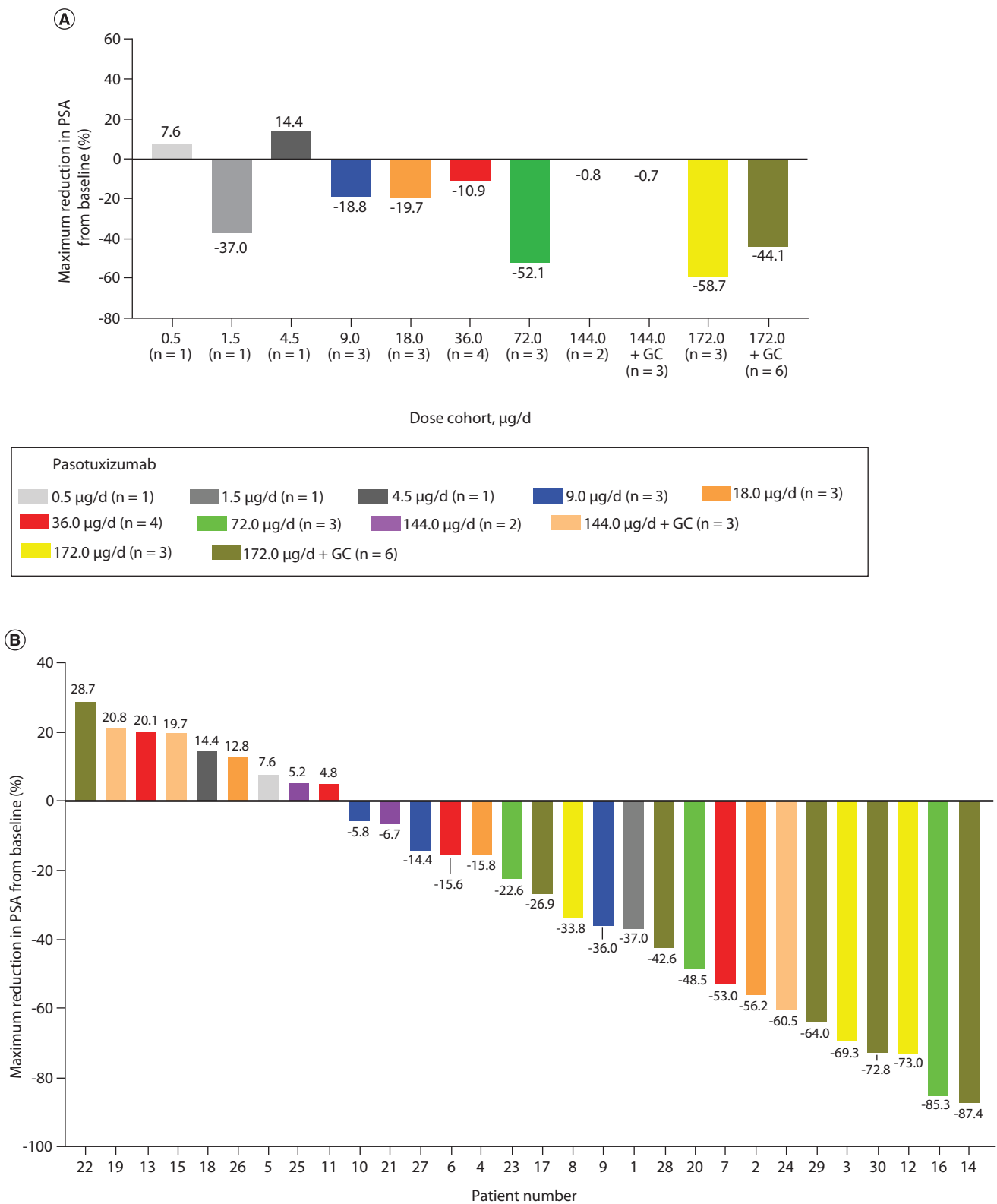
Figure 1.   Best PSA response after pasotuxizumab treatment in the subcutaneous cohort. Best PSA response after treatment initiation for each dose cohort **(A)** and for individual patients **(B)**, shown as percentage change from baseline.
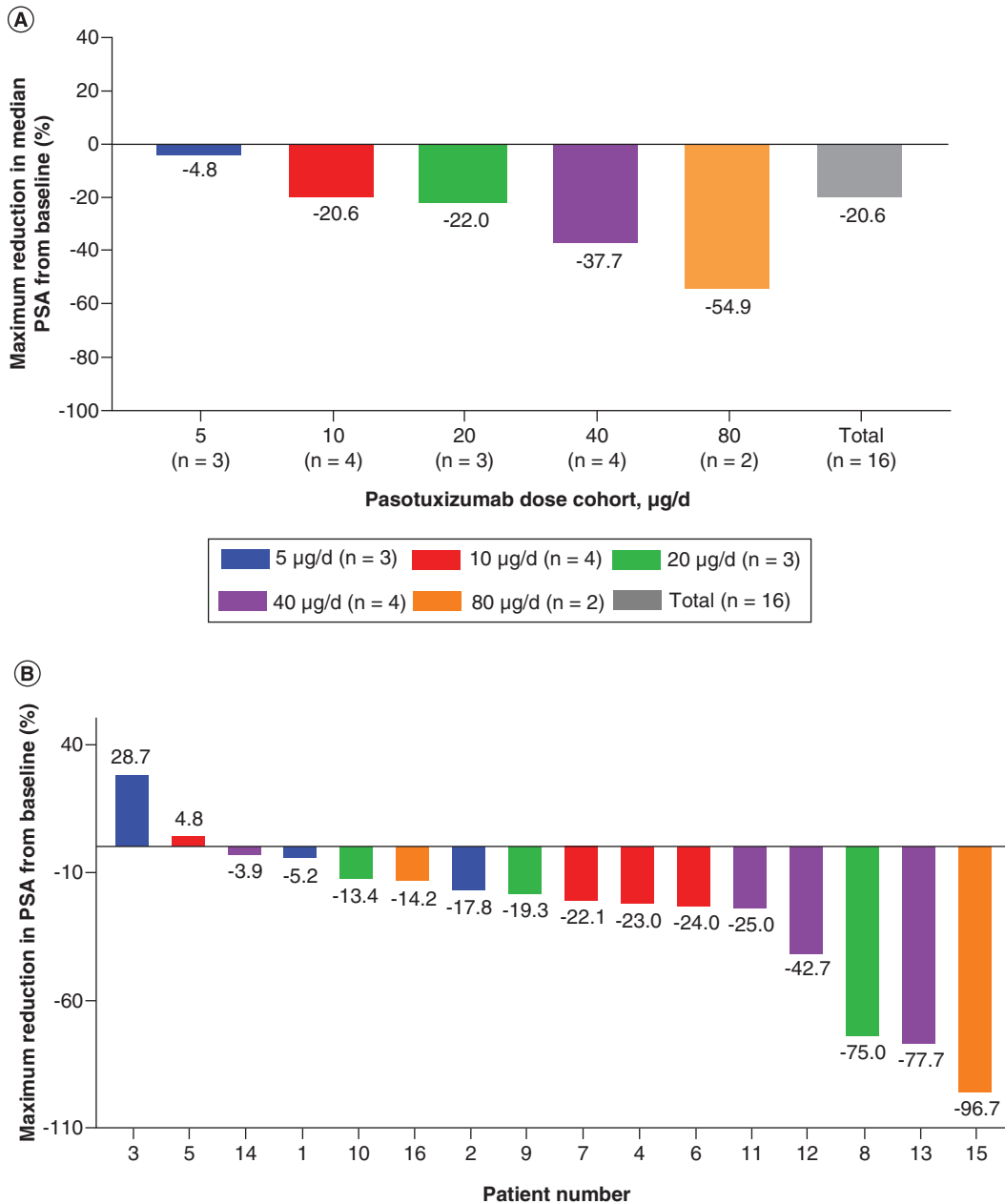GC: Glucocorticoid.

Figure 2. **PSA response after treatment with pasotuxizumab in the continuous intravenous cohort.** Best PSA response after treatment initiation, shown as mean percentage change from baseline for each dose cohort **(A)** and for individual patients **(B)**.

cycles 1, 2 and 4 for the cIV cohort are presented in Figure 5. At doses ≥20 µg/d, a dose-dependent decrease in CTC values was observed posttreatment that was paralleled by increases in CD69^{+} and PD-1 on CD8 T cells (Figure 6A & B). Patient 15, who was treated at the highest dose level and had normalization of lymph node lesions and marked regression of bone metastases, also had a marked CTC response.

Discussion

Overall, pasotuxizumab showed evidence of early efficacy when evaluated in this Phase I, dose-escalation study in patients with advanced CRPC. All patients experienced ≥1 AE of any grade and more than half experienced ≥1 drug-related AE of grade ≥3; fever and injection site reactions were the most common AEs in the SC cohort; with fever and chills the most common in the cIV cohort. The MTD for SC administration of pasotuxizumab was
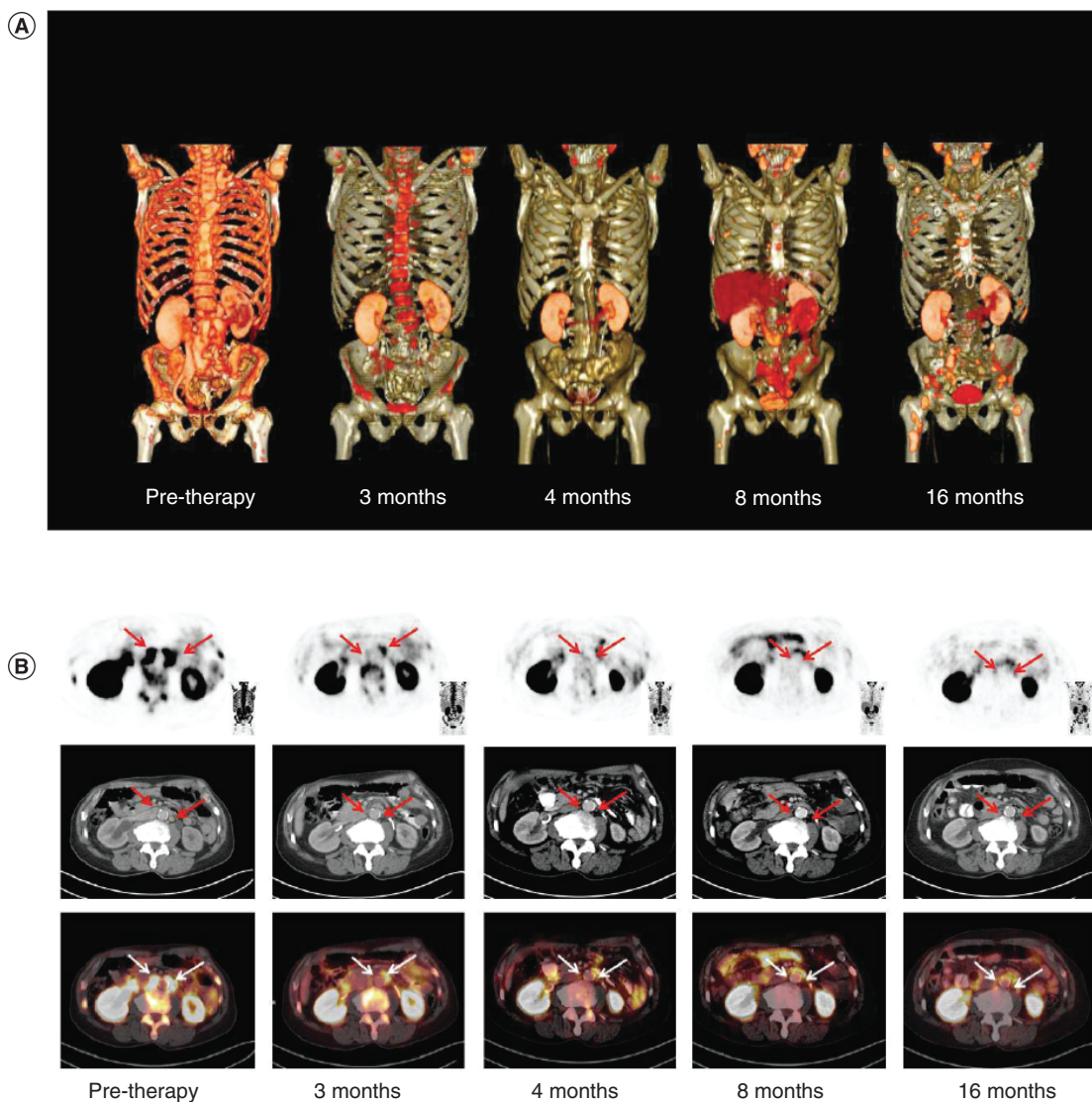
Figure 3. Summary of outcomes in patient 15 after treatment with pasotuxizumab. For patient 15, a compilation of representative PSMA PET/CT 3D whole-body views **(A)** and transaxial ^{68}Ga-PSMA PET (top row **(B)**; inserts, PET maximum intensity projections), CT (middle row **[B]**) and fused PET/CT (bottom row **[B]**) slices at different time points during treatment with pasotuxizumab.
CT: Computed tomography; ^{68}Ga: Gallium-68; PET: Positron emission tomography.

Table 3. Best overall response according to Response Evaluation Criteria in Solid Tumors version 1.1 in the continuous intravenous cohort.

Pasotuxizumab dose, μg/d	Evaluable patients, n	Stable disease, n	Noncomplete response/ nonprogressive disease, n	Progressive disease, measurement proven, n	Nonradiographic progression, n	Not available,[†] n
5	3	0	1	1	0	1
10	4	0	1	1	1	1
20	3	1	0	2	0	0
40	4	1	1	1	0	1
80	2	1	0	1	0	0

[†]Included patients who did not have a postbaseline tumor assessment but who discontinued because of a drug-related toxicity, death, progression by clinical judgment before disease was re-evaluated and were therefore considered evaluable, or who withdrew (patient was considered a nonresponder).
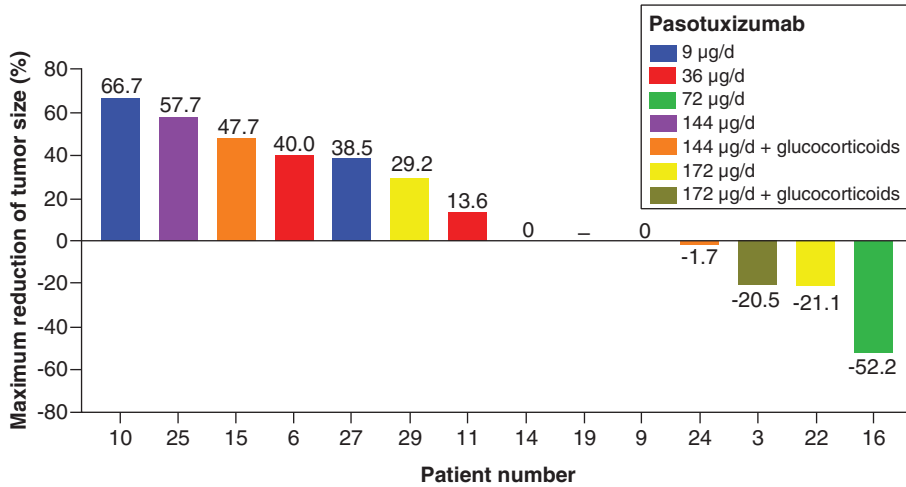
Figure 4.   Best overall tumor response after pasotuxizumab treatment in the subcutaneous cohort.
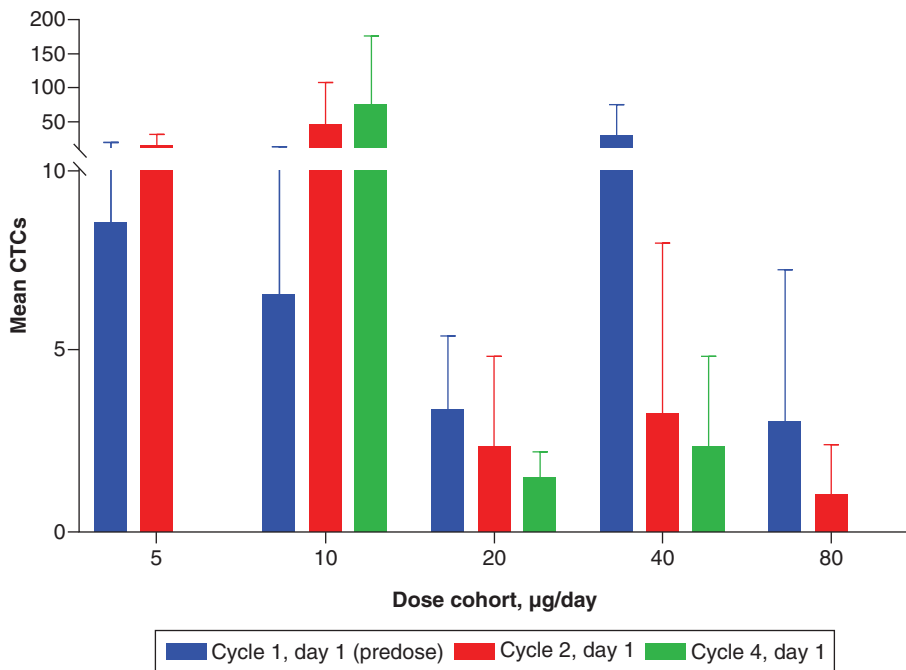
Figure 5.   Mean circulating tumor cell counts by dose cohort in the continuous intravenous cohort. Blood samples for the assessment of CTCs were collected before infusion start (cycle 1, day 1); at cycle 2, day 1; and at cycle 4, day 1. Numbers of CTCs were determined in 7.5 ml of whole blood using the CellSearch® assay that enumerates CD45^{-}, EpCAM^{+} and cytokeratins 8^{+}, 18^{+} and 19^{+} (CellSearch Epithelial Cell Kit/CellSpotter™ Analyzer; Menarini Silicon Biosystems Inc., Huntington Valley, PA, USA). Sample processing and analyses were performed according to the manufacturer's instructions. Data shown are for all patients with valid biomarker data. The mean number of CTCs increased over time in patients receiving pasotuxizumab 5 and 10 μg/d and decreased from cycle 1 through 4 for patients receiving pasotuxizumab 20 and 40 μg/d.
CTC: Circulating tumor cell.

determined to be 172.0 μg daily; however, the cIV cohort was stopped after enrolment of 16 patients and before the MTD was reached because of a change in study sponsorship.

Following SC pasotuxizumab administration, C_{max} was reached 5.95–23.5 h postdose on day 1 of cycle 1 and between 3.95–6.08 h on day 15 of cycle 1. In human prostate cancer xenograft models, the terminal half-life of pasotuxizumab was approximately 8 h [26]. In nonhuman primates, a similar half-life (about 7 h) was calculated
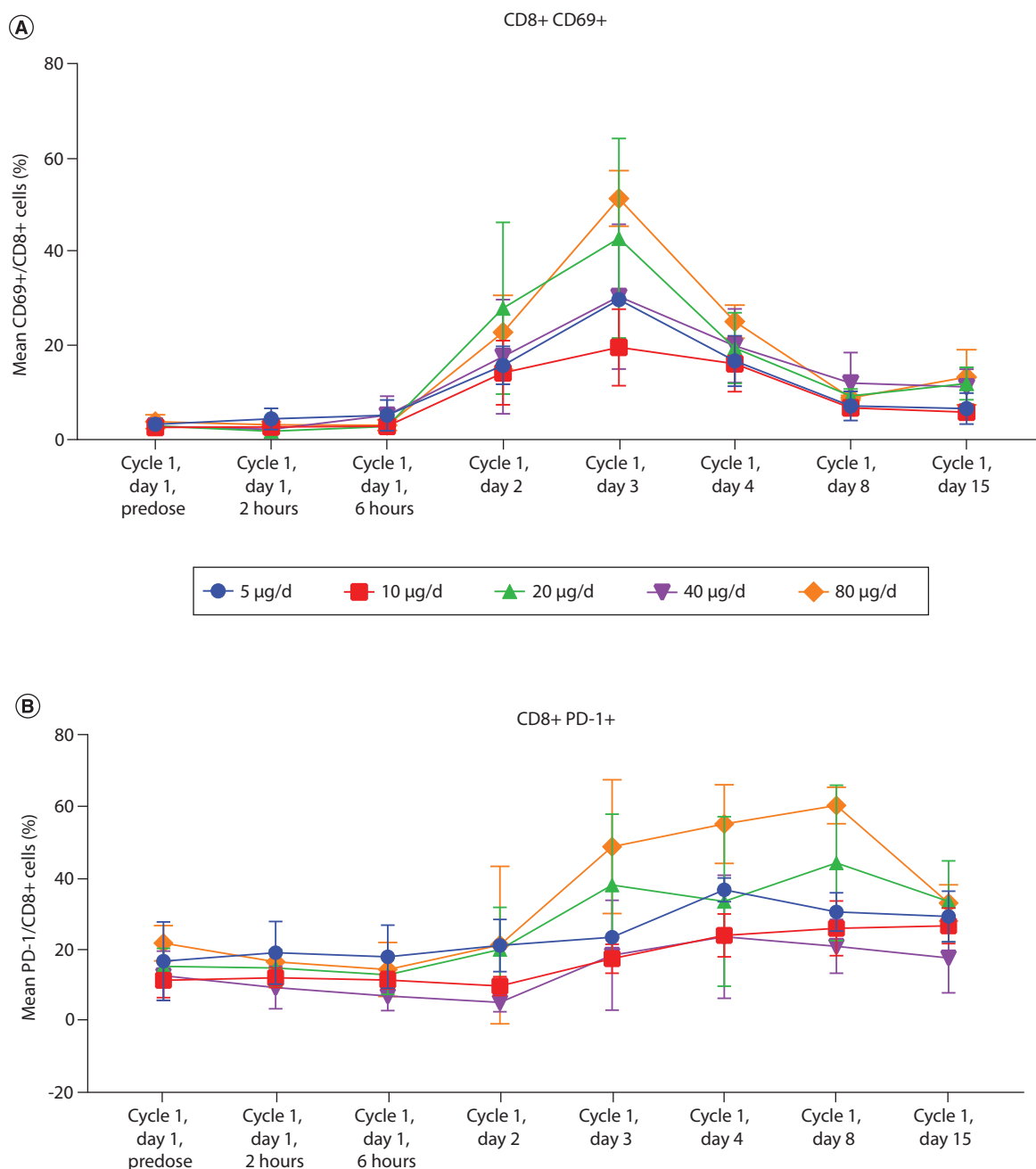
Figure 6.   Flow cytometric analysis of activation of CD8⁺ T cells during cycle 1 in the continuous intravenous cohort.
Activation of T cells as assessed by expression of CD69 **(A)** peaked at day 3 of cycle 1; expression of PD-1 **(B)** was elevated from day 3 to 15 of cycle 1. Presented as the percentage (SD) of CD8⁺ cells in the peripheral blood at each time point expressing CD69 and PD-1.
PD-1: Programmed death 1; SD: Standard deviation.

after SC administration; after IV administration, the half-life was 1 and 3 hours. This pharmacokinetic profile allowed for daily SC administration with marginal accumulation and cIV administration.

All patients who received SC pasotuxizumab for >1 treatment cycle developed ADAs, likely due to the high localized concentrations of pasotuxizumab, uptake of pasotuxizumab by dendritic cells and subsequent presentation to the lymphocytes in the local lymph nodes. In an attempt to avoid the formation of ADAs by antigen-presenting cells localized in the skin and to reduce local skin immune reactions, the protocol was amended to include concomitant administration of topical glucocorticoid, in addition to systemic corticosteroids, to suppress antigen-

presenting cells in new SC cohorts receiving 144 and 172 µg. However, this approach had no effect on the development of ADAs, which did affect exposure but were not associated with adverse events. Therefore, enrollment in the SC cohort was discontinued, and focus was shifted to evaluation of pasotuxizumab administered by cIV infusion. No ADAs were detected in the cIV cohort. As expected this change in the route of administration appeared to have affected ADA development, due in part to the lower local drug concentrations and also because IV administration of biotherapeutics >20 kDa may result in decreased exposure of drug to the lymphatic system than occurs with SC administration [29].

Pasotuxizumab showed signs of clinical response in the SC and cIV cohorts. In the SC cohort, the median best overall PSA response was a decline of 25%, with about a third of patients showing an initial >50% decline in PSA values. PSA levels typically reverted to levels higher than baseline over time, most likely related to the reduced exposure to pasotuxizumab following the development of neutralizing ADAs. Although no patients showed complete or partial responses by RECIST 1.1 criteria, nearly one fifth of patients experienced stable disease, with time without progression ranging from 0 to 11 months in these patients; median time to progression was approximately 3 months across evaluable patients.

In the cIV cohort, moderate lymphocyte redistribution at the start of therapy indicated T-cell engagement by pasotuxizumab and a dose-dependent reduction in CTCs was observed; dose-dependent reductions were observed in serum PSA levels across the doses tested, with about a third of patients in the 20-, 40- and 80-µg/d cohorts showing a >50% decline in PSA. For the two long-term PSA responders, tumor progression was observed 11–17 months after pasotuxizumab initiation.

Of the 11 RECIST-measurable patients in the cIV cohort, the best overall response according to RECIST 1.1 was stable disease in three patients and noncomplete response/nonprogressive disease in another three patients. In patients with progression, median time to progression was 98 days and 165, 84, 84, 168 and 292 days in the 5-, 10-, 20-, 40- and 80-µg/d groups, respectively. Dose-dependent reductions in CTC were observed at doses ≥20 µg/d. One patient treated at the highest dose level had a near-complete remission and experienced clinical benefit with a substantial improvement in quality of life for almost 19 months. The patient experienced grade 3 CRS (cycle 1, day 1 lasting to day 2) with fever ≤40°C, mild vomiting and low-grade diarrhea. Symptoms were managed with IV hydration and antipyretic therapy and were fully reversible without pasotuxizumab interruption.

Pasotuxizumab accumulation was minimal following repeated dosing; dose proportionality was observed across all patient cohorts.

Device-related infections occurred in about a quarter of patients in the cIV cohort, affecting their health and treatment experience, with 86% of those who experienced such infections requiring ≥1 dose interruption. For optimal outcomes, a greater understanding of the underlying cause of these infections is required and management/prevention of these infections will need to be improved. In the cIV cohort, CRS-associated symptoms occurred in three patients within the first few days after treatment start and were manageable and fully reversible; there were no organ-specific toxicities.

Potential in prostate cancer

Patients with mCRPC need more effective treatments. BiTE immune therapies have been found to be of value in the treatment of hematologic cancers and could also be of value in patients with solid tumors, such as prostate cancer [30]. As a BiTE immune therapy, pasotuxizumab is designed to engage a patient's own T cells to PSMA expressing cells [30]. Indeed, the results reported here suggest that pasotuxizumab administered by cIV infusion may provide durable clinical benefit even in extensively pretreated patients with prostate cancer. In the cIV cohort, a dose-dependent decline in PSA levels was observed in 14 patients, providing proof of concept of the value of BiTE immune therapies in prostate cancer. AMG 160, a fully human half-life extended BiTE immune therapy that is administered by short IV infusion, is currently under development for mCRPC [31] and may provide further support for the role of BiTE immune therapy in prostate cancer.

There is great interest and some promising reports for treatment with ^{177}Lu-PSMA-617 [16–19,32,33]. Patients who were previously treated with ^{177}Lu-PSMA-617 were not excluded from this study. Of interest, before receiving pasotuxizumab treatment, the pasotuxizumab long-term responder (patient 15) did not respond to ^{177}Lu-PSMA therapy administered over a period of 6 months. Resistance to ^{177}Lu-PSMA-617 occurs through standard DNA repair mechanisms or multidrug resistance pathways [34], and reduced clinical activity was observed in patients previously treated with chemotherapy [35]. The mechanism of action of pasotuxizumab is based on engaging T-cell-mediated tumor cell killing and is not related to DNA damage or repair. Therefore, pasotuxizumab treatment is

not anticipated to be cross-resistant with standard chemotherapy, radiation therapy, or therapy with radioisotopes; as observed, patient 15 responded to pasotuxizumab despite having no response to ^{177}Lu-PSMA.

This study had a number of limitations. The development of ADAs following SC administration had an effect on the pharmacokinetic profile and the efficacy of the immune therapy. Subsequently, the less immunogenic route of administration, cIV infusion, was evaluated in a relatively small number of patients. Additionally, because of a sponsor change, the cIV study was stopped early, MTD was not reached for the cIV cohort and not all planned assessments were undertaken.

Conclusion

This is the first clinical report showing that a BiTE immune therapy administered as monotherapy can be efficacious in prostate cancer while also demonstrating that BiTE monotherapy can be effective in solid tumors. We consider the early responses observed among patients with advanced CRPC to be encouraging. A continuation of the dose-escalation study that determines the MTD of pasotuxizumab administered by cIV infusion could be informative, especially given the promising results observed in the cIV cohort of the current study [36]. A first-in-human study of AMG 160, a half-life extended BiTE immune therapy that also binds PSMA, is currently underway [37]. The results of that study will further elucidate the potential of BiTE immune therapies to treat CRPC [31].

Summary points

- PSMA is highly expressed in prostate cancer and its metastates compared with normal tissue and has been validated as a target for therapy in metastatic castration-resistant prostate cancer (CRPC).
- Bispecific T-cell engager (BiTE®) immune therapies have been approved for hematologic cancers over the last decade, but their role in solid tumors such as CRPC is unclear.
- Pasotuxizumab (AMG 212 or BAY 2010112), a BiTE immune therapy designed to engage CD3 on T cells and PSMA on prostate cancer cells and to activate a patient's own T cells to eliminate PSMA-expressing prostate cancer cells, has demonstrated preclinical antitumor activity.
- Subcutaneous administration of pasotuxizumab was associated with the development of anti-drug antibodies; antidrug-antibody development was not observed with cIV infusion.
- Dose-dependent decline in PSA levels was observed in the cIV cohort, providing proof of concept of the value of BiTE immune therapies in prostate cancer.
- Two patients had long-term PSA responses; one patient had long-term stable disease and the other patient had near-complete regression of lymph node lesions and bone metastases, with 500 days to disease progression.
- This first-in-human study demonstrated the safety and preliminary efficacy of BiTE monotherapy in the treatment of CRPC.
- Further clinical studies are required to fully elucidate the role of BiTE immune therapies in the treatment of CRPC.

Ethical conduct of research

The authors state that they have obtained institutional review board/ethics committee approval at each study site and that the study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. In addition, written informed consent has been obtained from the participants involved.

Data sharing statement

Open access

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