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Clinical Trial

Programmed cell death 1 (PD-1) targeting in patients with advanced osteosarcomas: results from the PEMBROSARC study

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Abstract ***Purpose:*** There are some lines of evidence suggesting a potential role of immunotherapy for treating patients with osteosarcomas.
Patients and methods: This was an open-label, multicentre, phase 2 study of pembrolizumab in combination with metronomic cyclophosphamide in patients with advanced osteosarcomas. All patients received 50 mg b.i.d. of cyclophosphamide one week on and one week off and 200 mg of intravenous pembrolizumab (every 3 weeks). There was a dual primary end-point, encompassing both the non-progression and objective responses at 6 months per Response Evaluation Criteria in Solid Tumours (RECIST), version 1.1. An objective response rate of 20% and/or a 6-month non-progression rate of 60% were determined as reasonable objectives for treatment with meaningful effect. Correlative studies of immune biomarkers were planned from the patients' tumour samples.
Results: Between October 13 2015 and July 3 2017, 17 patients were included. Fifty were assessable for the efficacy end-point. Four patients experienced tumour shrinkage, resulting in a partial response (PR) in one patient (6.7%). The 6-month non-progression rate was 13.3% (95% confidence interval [CI]: 1.7–40.5). The most frequent adverse events were grade I or II nausea, anaemia, anorexia and fatigue. programmed death-ligand 1 (PD-L1) expression rate was low, observed in only 2 cases of 14 with available tumour material. The only patient who experienced PR had a PD-L1–negative tumour.
Conclusion: Programmed cell death 1 (PD-1) inhibition has limited activity in osteosarcomas. Further studies investigating PD-1 inhibitor in combination with agents modulating the microenvironment are warranted.
Trial registration: This study is registered with ClinicalTrials.***gov***, number NCT02406781.

1. Introduction

Historically, sarcomas were the first tumour model for which immunotherapy was suggested as a relevant therapeutic strategy [1].

Osteosarcoma is a rare bone cancer which mainly affects adolescents and young adults. Current standard treatment consisting of surgery plus peri-operative chemotherapy allows about 70% of patients to be cured. However, up to 30% of patients will develop metastatic disease, leading to death in most cases with a median overall survival of less than 12 months. Novel and effective therapies for patients with advanced osteosarcomas are therefore urgently needed.

The programmed cell death 1 (PD-1)/programmed death-ligand 1 (PD-L1) interaction is a major pathway hijacked by tumours to suppress immune control. PD-L1 can be expressed in up to 58% of cases of soft-tissue sarcomas (STS), osteosarcoma and gastrointestinal stromal tumors (GIST) [2,3], and targeting the PD-1/PD-L1 interaction has been associated with impressive anti-tumour activity in a pre-clinical model of osteosarcoma [4].

Pembrolizumab is a potent and highly selective humanised monoclonal antibody that directly blocks the interaction between PD-1 and PD-L1/PD-L2, with demonstrated activity in various cancers [5].

Metronomic cyclophosphamide (CP) has immunomodulatory properties [6] and has shown a synergistic effect on immunostimulation when combined with immunotherapies such as oncolytic adenovirus [7], survivin human leukocyte antigen- I (HLA-I) peptide vaccines [8] or anti–PD-1 antibody [9].

We therefore hypothesised that the association of pembrolizumab and metronomic CP could have synergistic activity with a toxicity profile that benefits patients with advanced STS and osteosarcomas.

2. Methods

2.1. Patients

Patients had to be aged 18 years or older and have histologically confirmed metastatic and/or unresectable osteosarcoma with documented disease progression [as per Response Evaluation Criteria in Solid Tumours (RECIST 1.1)] [10] within 6 months before entry into the study.

2.2. Study design and treatment

This was a single-arm, phase II, multicentre clinical trial based on Simon's two-stage design and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practices. This study was approved by the Institutional Ethics Committee of Institut Bergonié (Comité de Protection des Personnes Sud-Ouest et Outre

Mer III). All patients provided written informed consent before enrolment in the study. Patients received 50 mg of CP orally b.i.d. one week on and one week off and 200 mg of pembrolizumab intravenously at day 8 of a planned 21-day cycle. Patients discontinued treatment if one of the following events occurred: patient's decision to withdraw, unacceptable toxicity, disease progression as per RECIST 1.1, undercurrent illness or changes in the patient's condition preventing further treatment by the judgement of the investigator.

2.3. Response assessment and toxicity

Tumour assessment was carried out every 6 weeks. The response was determined as per RECIST 1.1 [10] guidelines after blinded central imaging review. Toxicities were assessed continuously as per Common Terminology Criteria for Adverse Events, version 4.0.

2.4. Correlative studies

Sections of archival formalin-fixed, paraffin-embedded tumour tissue were analysed by immunohistochemistry (IHC) for expression of PD-L1 (clone E1L3N) by tumour cells (TCs) and immune cells (ICs), as well as for infiltration by CD3-positive (CD3+) T cells (clone 2GV6). The formalin-fixed paraffin-embedded (FFPE) human tumour specimens were cut into section of 3 μm thickness. Antigen retrieval was carried out on a PT-link (Dako) using the EnVision FLEX Target Retrieval Solutions (Dako). Endogenous peroxidase and non-specific staining were blocked with 3% H_2O_2 (10603051; Gifrer) and protein block (X0909; Dako), respectively. Staining was revealed with 3-amino-9-ethylcarbazole substrate (SK-4200; Vector Laboratories). After mounting either with glycergel (C056330-2; Dako) for IHC, the slides were scanned using the Nanozoomer (Hamamastu). Stained slides were analysed using Halo software (Indicalab). For CD3 marker, the density of positive cells/mm^2 in the tumour area was quantified. For PD-L1, percent of positive staining of TCs and ICs was evaluated as described previously [11] by two independent and trained reviewers blinded to the clinical data.

2.5. Statistical analysis

The primary efficacy end-point was a dual end-point encompassing non-progression and objective response at 6 months as per RECIST 1.1.

Simon's optimal two-stage design [12] was used. To distinguish a favourable true 6-month non-progression rate of 60% from a null rate of 40%, and a favourable objective response rate of 20% from a null rate of 5% with 80% power and 5% type I error, 30 assessable patients were needed. After inclusion of the first 15 assessable patients, accrual could continue for a total of 30 patients if at least 9 non-progression or 3 objective responses were observed. At the end of recruitment, at least 20 non-progression or 8 objective responses were needed to conclude that the combination had a meaningful effect.

Secondary end-points included the best overall response as per RECIST 1.1, 1-year progression-free survival (PFS), 1-year overall survival (OS), safety and correlations with the immunological characteristics of the tumours. PFS was defined as the time from the start of treatment to the time of progression or death (from any cause). Patients who were alive and progression-free were censored at the date of last follow-up. OS was defined as the time from the start of treatment to death (from any cause) or the last patient contact.

All enrolled patients who received at least one dose of one of the investigational drugs were eligible for safety analyses. To be assessed for the primary efficacy end-point, a subject had to meet the eligibility criteria and receive at least one dose of CP and one infusion of pembrolizumab. Descriptive statistics were used to characterise patients at study entry and to report toxicities. Statistical significance was achieved at a p-value of <0.05. The data reported here represent the study database as of 11/09/2018. All analyses were conducted using SAS 9.2 software (SAS Institute, Cary, NC). This study is registered with ClinicalTrials.gov, number NCT02406781.

3. Results

Between October 13 2015 and July 3 2017, 17 patients with advanced osteosarcoma were enrolled across six French Sarcoma Group centres. Fifteen of them were assessable for the primary efficacy end-point (Fig. 1). Baseline patient characteristics are listed in Table 1. After a median follow-up of 18.9 months (95% confidence interval [CI] = 8.3–18.9), all the patients discontinued treatment. Discontinuation was related to disease progression in 12 cases (80%) and toxicity for 3 patients (20%). Sixteen patients were included in the safety analysis. At the time of analysis, 74 cycles of pembrolizumab and metronomic CP had been administered, with a median of 3 cycles per patient (range 1–14). The most commonly observed toxicities were grade I or II nausea, fatigue, anorexia and anaemia. Grade III or IV toxicities were rare (observed in 6 patients) and mainly included fatigue, anaemia, lymphopenia and acute renal failure (Table 2).

Of the 15 patients assessable for efficacy analysis, one patient had no tumour imaging evaluation because of rapid clinical deterioration and death within 4 weeks of treatment onset. Four patients had tumour shrinkage (25%) (Fig. 2A). The best response was partial response (PR), stable disease (SD) and progressive disease for 1 (6.7%), 5 (33.3%) and 8 (53.3%) patients, respectively.

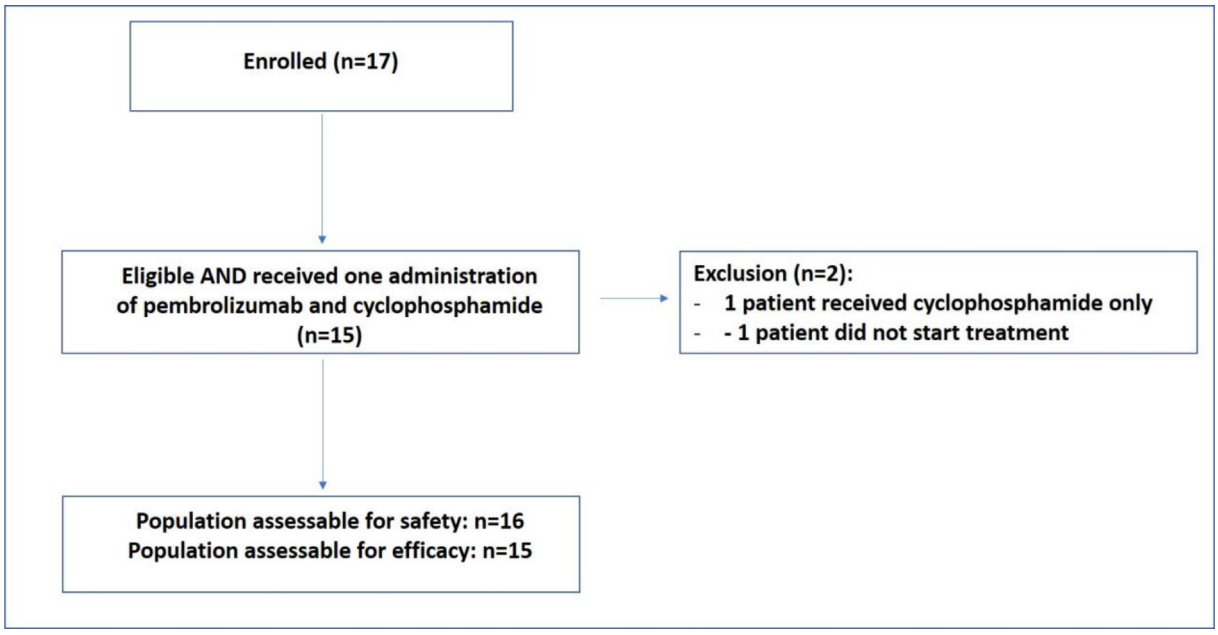

Fig. 1. Flowchart of patient selection for the PEMBROSARC study.

Two patients were progression-free at 6 months. The 6-month non-progression rate was 13.3% (95% CI = 1.7–40.5). The median PFS was 1.4 months (95% CI = 1.0 months–1.4 months), and the median OS was 5.6 months (95% CI = 2.1 months–12.1 months) (Fig. 2B and C).

Fourteen patients had available data on infiltrate densities of immune cells and PD-L1 expression in their tumour samples. PD-L1 expression (≥1%) in TCs and ICs was observed in 2 cases (14.3%) and one (7.1%) case, respectively. The median densities of CD3+ cells was 17.4 cells/mm^2 (range: 0–55.2). Overall, PD-L1 expression in tumour cells was positively associated with CD3+ cell density (mean CD3+ cells density of 40.5/mm^2 versus when PD-L1 positive cases versus 14.7 in PD-L1 negative cases) although this did not reach statistical significance given the limited sample size. Among the 4 patients with tumour shrinkage (including the patients with SD and PR at 6 months), 3 had available tumour material. None of them had expression of PD-L1 on tumour cells or immune cells.

Table 1
Patient characteristics (n = 17).

Gender, n (%)	
Male	10 (58.8%)
Female	7 (41.2%)
Age	
Median, years (range)	41 (18–84)
ECOG PS, n (%)	
0	5 (29.4%)
1	12 (70.6%)
Stage, n (%)	
Locally advanced	14 (82.4%)
Metastatic	15 (88.2%)
Metastatic sites, n (%)	
Lung	11 (64.7%)
Bone	2 (11.8%)
Other	7 (41.2%)
Prior line(s) of chemotherapy for advanced disease, n (%)	
1	17 (100%)
2	7 (41.2%)
>2	6 (35.3%)

ECOG, Eastern Cooperative Oncology Group; PS, performance status.

4. Discussion

Treatment of relapsed osteosarcoma is particularly challenging. Therapeutic options are limited with no drugs registered in this setting, and 5-year OS does

Table 2
Treatment-related adverse events (n = 16).

Adverse event	Grade I and II		Grade III and IV	
	n	%	n	%
Nausea	4	25.0	–	.
Anaemia	3	18.8	1	6.3
Anorexia	3	18.8	.	.
Fatigue	3	18.8	2	12.5
Arthralgia	2	12.5	.	.
Constipation	2	12.5	.	.
Leucopenia	2	12.5	1	6.3
Thrombocytopenia	2	12.5	.	.
Weight loss	2	12.5	.	.
Acute renal failure	.	.	1	6.3
Chronic renal failure	1	6.3	.	.
Diarrhoea	1	6.3	.	.
Dyspnoea	1	6.3	1	6.3
Dysthyroidism	1	6.3	.	.
Lymphopenia	.	.	1	6.3
Neutropenia	1	6.3	.	.
Pruritus	1	6.3	.	.
Vomiting	1	6.3	.	.

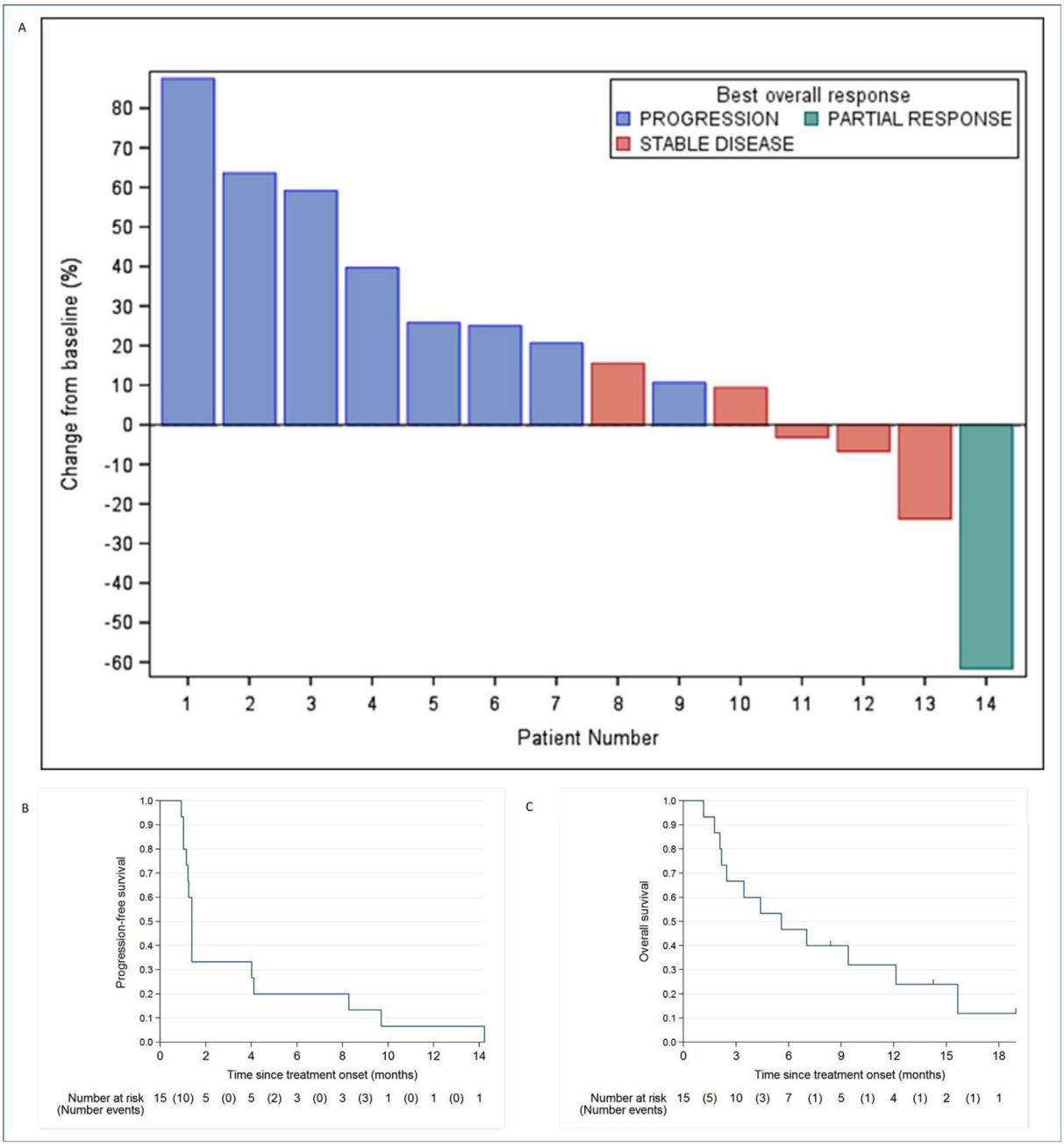

Fig. 2. **Pembrolizumab + low-dose cyclophosphamide efficacy in patients with osteosarcoma.** (**A**) Maximal tumour regression curves (waterfall plots) in patients with centrally reviewed medical imaging (n = 14). (**B–C**) Kaplan-Meier curves of progression-free (**B**) and overall (**C**) survival in the population of patients assessable for efficacy (n = 15).

not exceed 20% [13]. Several lines of evidence suggest that immunotherapy may represent a promising strategy in patients with osteosarcoma. First, osteosarcoma is characterised by a high level of genomic complexity and a high mutational burden that may generate specific tumour neoantigens [14]. Moreover, infiltration by CD8+ lymphocytes is a common feature of osteosarcoma and has been associated with improved outcomes [15,16]. In addition, mifamurtide, a modulator of innate immunity, has been approved in Europe for use in non-metastatic osteosarcoma based on the results of a randomised trial which reported improved survival when this agent was combined with chemotherapy in comparison with chemotherapy alone [17].

Only two patients of 15 (13.3%) had significant clinical benefit (long-term PR and SD, respectively). These results are in line with those of the Sarcoma Alliance for Research through Collaboration SARC028 study which assessed the efficacy of pembrolizumab in patients with

advanced soft-tissue and bone sarcomas. Only one of the 22 patients with osteosarcoma who were included exhibited PR (4%) [18].

The safety profile of the pembrolizumab-cyclophosphamide combination was similar to that observed in the soft-tissue sarcomas and GIST cohorts [11]. Moreover, the addition of low-dose cyclophosphamide to pembrolizumab did not appear to be detrimental in comparison with previous studies investigating pembrolizumab as a single agent in solid tumours [19].

We found low expression of PD-L1 in tumour samples, only 2 cases exhibiting >1% PD-L1+ TCs and only one that was PD-L1+ for ICs. These results are in agreement with those of previously reported studies using validated anti–PD-L1 IHC assays [15,16]. A lack of PD-L1 expression has been correlated with limited benefit from PD-1 blockade in carcinomas [20]. Interestingly, the unique PR observed in our study was in a patient bearing a tumour with no expression of PD-L1 and a relatively low level of lymphocytes infiltration. Such apparent discrepancy may be explained by the fact that the analysis of ICs infiltrates and PD-L1 expression was performed on samples derived from primary tumours. Indeed, a recent study has shown a heterogeneous tumour-immune microenvironment between primary and metastatic lesions of osteosarcomas [16]. The authors showed a significantly higher density of tumour-infiltrating T cells in metastatic osteosarcoma lesions than in primary tumours. Moreover, although the proportion of PD-L1–positive cases was low among primary tumours (13%), this proportion was significantly higher among metastatic samples (48%, $p = 0.004$) [16]. The reasons for such differences in PD-L1 expression are not known, but some reports suggest the role of prior exposure to chemotherapy which may select PD-L1–expressing clones [21–25]. Altogether, these data underscore the need to implement mandatory sequential tumour biopsies in future immunotherapy trials including patients with osteosarcoma to acquire relevant tissues for correlative tumour microenvironment analyses.

Overall, the results of this study which is the first specifically designed to assess the impact of PD-1 inhibition in advanced osteosarcomas indicate that this therapeutic strategy has only modest activity in patients with advanced osteosarcomas. Pre-existing T-cell anti-tumour immunity is an important prerequisite to the anti–PD-1/PD-L1 response [26,27]. Recent pre-clinical data have shown that combining anti–PD-1 inhibitors with agents reprogramming the tumour microenvironment of osteosarcoma by boosting the T-cell infiltration is associated with promising anti-tumour activity [28]. Further clinical trials should explore such strategies and implement appropriate correlative studies to better understand mechanisms underlying sensitivity and resistance of osteosarcomas to immune checkpoint inhibition.

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Conflict of interest statement

A.I. has received research grant from MSD. Other co-authors have no conflict of interest.

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