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CLINICAL TRIAL

PD1 targeting in advanced osteosarcomas patients: 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 osteosarcomas patients.

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 endpoint, encompassing both the non-progression and objective responses at 6 months per Response Evaluation Criteria in Solid Tumours (RECIST) v1.1. 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 endpoint. Four patients experienced tumour shrinkage, resulting in a partial response (PR) in one patient (6.7%). The 6-month non-progression was 13.3% (CI 95% [1.7 – 40.5]). The most frequent adverse events were grade 1 or 2 nausea, anemia, anorexia and fatigue. PD-L1 expression rate was low, observed in only 2 cases out of 14 with available tumor material. The only patient who experienced PR had a PD-L1-negative tumor.

Conclusion: PD-1 inhibition has limited activity in osteosarcomas. Further studies investigating PD1 inhibitor in combination with agents modulating the microenvironment are warranted.

Keywords: osteosarcoma, PD1, immunotherapy, pembrolizumab

Trial registration: This study is registered with ClinicalTrials.gov, number NCT02406781.

INTRODUCTION

Historically, sarcomas were the first tumour model for which immunotherapy was suggested as a relevant therapeutic strategy¹.

Osteosarcoma is a rare bone cancer which mainly affects adolescents and young adults. Current standard treatment consisting of surgery plus peri-operative chemotherapy allows to cure about 70% of patients. However, up to 30% of patients will develop metastatic disease leading to death in the majority of 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 PD-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 STS, osteosarcoma, and 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 humanized 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 immuno-modulatory properties⁶ and has shown a synergistic effect on immuno-stimulation when combined with immunotherapies, such as oncolytic adenovirus⁷, survivin HLA-I peptide vaccines⁸ or anti-PD1 antibody.⁹

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

METHODS

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 prior to entry into the study.

Study design and treatment

This was a single-arm, phase 2, 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.

Response assessment and toxicity

Tumour assessment was carried out every 6 weeks. The response was determined per RECIST 1.1¹⁰ guidelines after blinded central imaging review. Toxicities were assessed continuously per Common Terminology Criteria for Adverse Events v4.0.

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 (TC) and immune cells (IC), as well as for infiltration by CD3-positive (CD3+) T cells(clone 2GV6) The formalin-fixed-paraffin-embedded (FFPE) human tumors specimens were cut at 3µm thick sections. The 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 H₂O₂ 3% (Gifrer, 10603051) and protein block (Dako, X0909) respectively. The primary and secondary antibodies used for IHC are summarized in table X. Staining was revealed with 3-amino-9-ethylcarbazole substrate (Vector Laboratories, SK-4200). After mounting either with glycergel (Dako, C056330-2) for IHC the slides were scanned with Nanozoomer (Hamamastu). Stained slides were analyzed by Halo software (Indicalab). For CD3 marker, the density of positive cells/mm² in tumor area was quantified. For PD-L1, % of positive staining of tumor cells (TC) and immune cells (IC) were scored as described previously¹¹ by two independent and trained reviewers blinded to the clinical data.

Statistical analysis

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

Simon's optimal two-stage design¹² 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. Following 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 endpoints included the best overall response 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 endpoint, 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 characterize 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.

Role of the funding source

This study was sponsored by Institut Bergonie (Bordeaux, France). The funders of the study were not involved in the study design, the data collection and analysis, the interpretation of the data, and the writing of the report. All authors had full access to all the data in the study, made the decision to submit these data for publication, were involved in writing the report, and agreed upon the final content of the report. The corresponding author had final responsibility to submit for publication.

RESULTS

Between October 13 2015 and July 3 2017, 17 patients with advanced osteosarcoma were enrolled across six French Sarcoma Group centres. 15 of them were assessable for the primary efficacy endpoint (Figure 1). Baseline patient characteristics are listed in Table 1. After a median follow-up of 18.9 months (95% 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%). 16 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 1 or 2 nausea, fatigue, anorexia, and anaemia. Grade 3 or 4 toxicities were rare (observed in 6 patients) and mainly included fatigue, anemia, lymphopenia and acute renal failure (Table 2). Of the 15 patients assessable for efficacy analysis, one patient had no tumor imaging evaluation because of rapid clinical deterioration and death within 4 weeks of treatment onset. Four patients had tumor shrinkage (25%) (Figure 2A). The best response was partial response, stable disease and progressive disease for 1 (6.7%), 5 (33.3%) and 8 (53.3%) patients respectively. 2 patients were progression-free at 6 months. The 6-month non-progression rate was 13.3% CI95%= [1.7 – 40.5]. The median PFS was 1.4 months (CI95%, 1.0 months – 1.4 months) and the median OS was 5.6 months (CI95%, 2.1 months –12.1 months) (Figures 2B and 2C)

Fourteen patients had available data on infiltrate densities of immune cells, and PD-L1 expression in their tumour samples. PD-L1 expression ($\geq 1\%$) in tumour cells (TC) and immune cells (IC) was observed in 2 (14.3%) and one (7.1%) cases respectively. The median densities of CD3+ cells was 17.4 cells/mm² (range 0-55.2). Overall, PD-L1 expression in tumor cells was positively associated with CD3+ cell density (mean CD3+ cells density of 40.5/mm² 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 tumor shrinkage (including the patients with SD and PR at 6 months), 3 had available tumor material. None of them had expression of PD-L1 on tumor cells or immune cells.

DISCUSSION

Treatment of relapsed osteosarcoma is particularly challenging. Therapeutic options are limited with no drugs registered in this setting and 5 years OS does not exceed 20%. Several lines of evidence suggest that immunotherapy may represent a promising strategy in osteosarcoma patients. First, osteosarcoma is characterized by a high level of genomic complexity and a high mutational burden that may generate specific tumor neoantigens¹⁴. Moreover, infiltration by CD8+ lymphocytes is a common feature of osteosarcoma and has been associated with improved outcome¹⁵⁻¹⁶. 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 randomized trial which reported improved survival when this agent was combined with chemotherapy in comparison with chemotherapy alone¹⁷.

Only two patients out of 15 (13.3%) had significant clinical benefit (long-term partial response and stable disease 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 partial response (4%).¹⁸

The safety profile of the pembrolizumab-cyclophosphamide combination was similar to that observed in the soft-tissue sarcomas and GIST cohort^{s11}. 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 tumors¹⁹.

We found low expression of PD-L1 in tumour samples, only 2 cases exhibiting >1% PD-L1+ tumor cells, and only one that was PD-L1+ for immune cells. These results are in agreement with 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 partial response 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 immune cells infiltrates and PD-L1 expression was performed on samples derived from primary tumors. Indeed, a recent study has shown a heterogeneous tumor-immune microenvironment between primary and metastatic lesions of osteosarcomas.¹⁶ The authors showed a significantly higher density of tumor-infiltrating T cells in metastatic osteosarcoma lesions compared to primary tumors. Moreover, although the proportion of PD-L1 positive cases was low among primary tumors (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 clones²¹⁻²⁵. Altogether, these data underscore the need to implement mandatory sequential tumor biopsies in future immunotherapy trials including osteosarcoma patients in order to acquire relevant tissues for correlative tumor microenvironment analyses.

Overall, the results of this study which is the first specifically designed to assess the impact of PD1 inhibition in advanced osteosarcomas indicate that this therapeutic strategy has only modest activity in patients with advanced osteosarcomas. Pre-existing T-cell anti-tumor immunity is an important prerequisite to the anti-PD-1/PD-L1 response.²⁶⁻²⁷ . Recent pre-clinical data have shown that combining anti-PD1 inhibitors with agents reprogramming the tumor microenvironment of osteosarcoma by boosting the T cell infiltration is associated with

promising anti-tumor 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

AI has received research grant from MSD.

Other co-authors have no conflict of interest.

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TABLES

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%)

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

	Grade 1,2		Grade 3,4	
	n	%	n	%
Nausea	4	25.0	.	.
Anemia	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	.	.
Diarrhea	1	6.3	.	.
Dyspnea	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	.	.

FIGURES

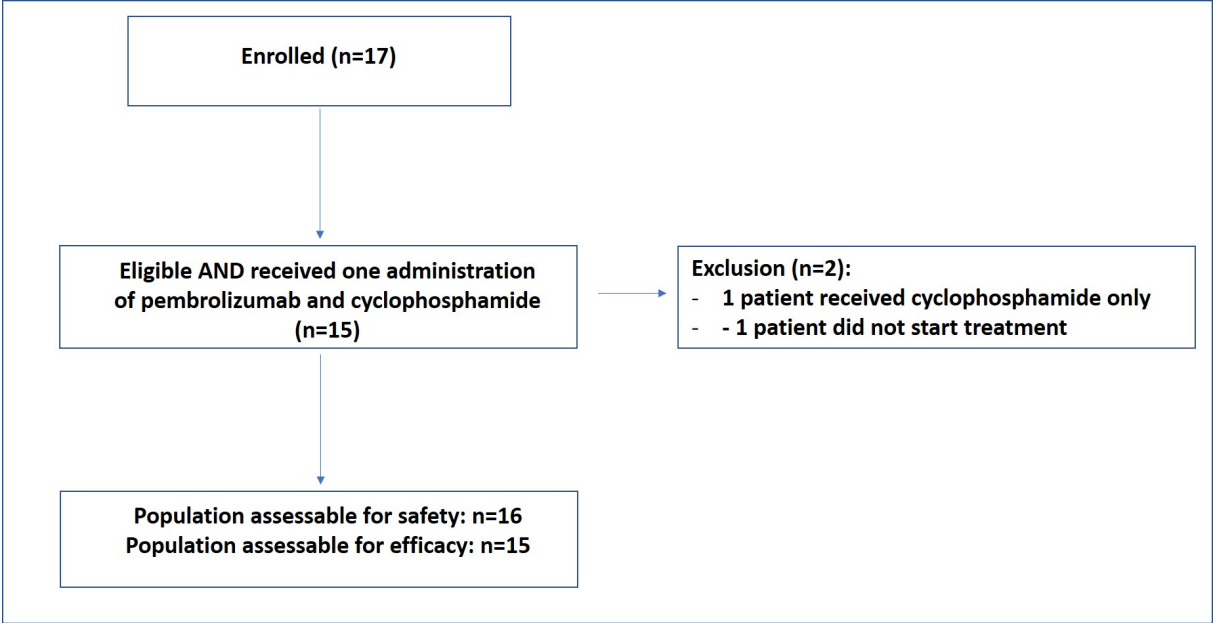


Figure 1. Flowchart of patient selection for the PEMBROSARC study.

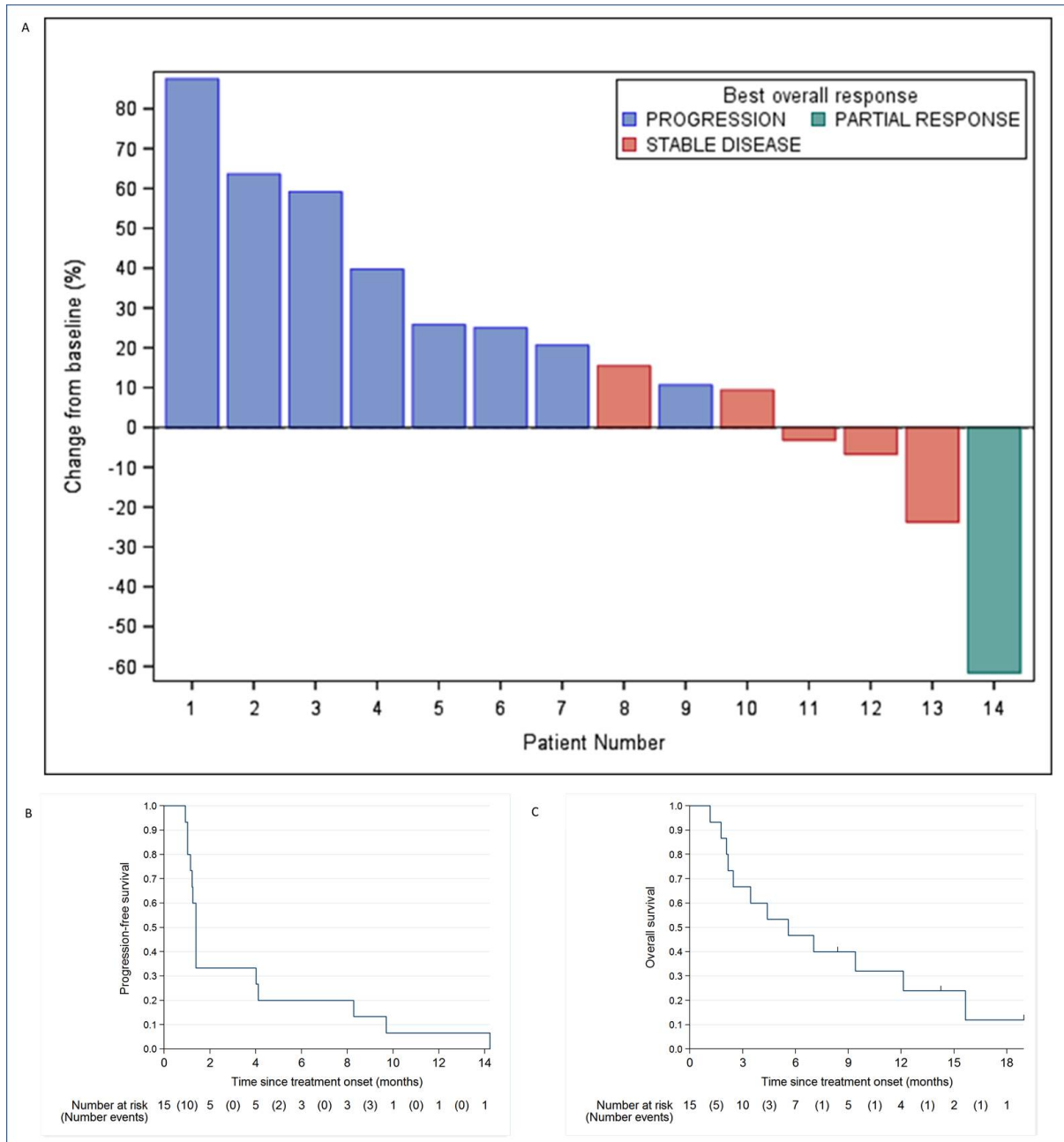


Figure 2. Pembrolizumab + low-dose cyclophosphamide efficacy in osteosarcoma patients (A) Maximal tumor regression curves (waterfall plots) in patients with centrally reviewed medical imaging (n=14) (B-C) Kaplan-Meier curves of progression-free (B) and overall survival (C) in the population of patients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