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Use of PD-1 Targeting, Macrophage Infiltration, and IDO Pathway Activation in Sarcomas
A Phase 2 Clinical Trial

Maud Toulmonde, MD; Nicolas Penel, MD, PhD; Julien Adam, MD, PhD; Christine Chevreau, MD; Jean-Yves Blay, MD, PhD; Axel Le Cesne, MD; Emmanuelle Bompas, MD; Sophie Piperno-Neumann, MD; Sophie Cousin, MD; Thomas Grellety, MD; Thomas Ryckewaert, MD; Alban Bessede, PhD; François Ghiringhelli, MD, PhD; Marina Pulido, MSc; Antoine Italiano, MD, PhD

IMPORTANCE There is a strong rationale for treating sarcomas with immunotherapy.

OBJECTIVE To assess the efficacy and safety of programmed cell death protein 1 (PD-1) targeting in combination with metronomic chemotherapy in sarcomas.

DESIGN, SETTING, AND PARTICIPANTS This was an open-label, multicenter, phase 2 study of 4 cohorts of patients with advanced soft-tissue sarcoma (STS), including leiomyosarcoma (LMS), undifferentiated pleomorphic sarcoma (UPS), other sarcomas (others), and gastrointestinal stromal tumor (GIST). All patients received 50 mg twice daily cyclophosphamide 1 week on and 1 week off and 200 mg of intravenous pembrolizumab every 3 weeks.

INTERVENTION OR EXPOSURE Pembrolizumab in combination with metronomic cyclophosphamide.

MAIN OUTCOMES AND MEASURES There was a dual primary end point, encompassing both the nonprogression and objective responses at 6 months per Response Evaluation Criteria in Solid Tumours (RECIST) v1.1 for LMS, UPS, and others and 6-month nonprogression for GIST. An objective response rate of 20% and/or a 6-month nonprogression rate of 60% were determined as reasonable objectives for treatment with meaningful effect. Correlative studies of immune biomarkers were planned from patient tumor and plasma samples.

RESULTS Between June 2015 and July 2016, 57 patients were included (median [range] age, 59.5 [18.5-84.0] years; 24 women [42%]); 50 patients were assessable for the efficacy end point. Three patients experienced tumor shrinkage, resulting in a partial response in a single solitary fibrous tumor. The 6-month nonprogression rates were 0%, 0%, 14.3% (95% CI, 1.8%-42.8%) for LMS, UPS, and others, respectively, and 11.1% (95% CI, 2.8%-48.3%) for GIST. The most frequent adverse events were grade 1 or 2 fatigue, diarrhea, and anemia. The only patient who experienced partial response was the only one with strong programmed cell death 1 ligand 1–positive staining in immune cell. Strong infiltration by macrophage expressing the inhibitory enzyme indoleamine 2,3-dioxygenase 1 (IDO1) was observed in the majority of cases. Moreover, a significant increase in the kynurenine to tryptophan ratio was observed in patient plasma samples during the study treatment.

CONCLUSIONS AND RELEVANCE We found that PD-1 inhibition has limited activity in selected STS and GIST. This may be explained by an immunosuppressive tumor microenvironment resulting from macrophage infiltration and IDO1 pathway activation.

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Supplemental content

Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Antoine Italiano, MD, PhD, Early Phase Trials and Sarcoma Units, Department of Medical Oncology, Institut Bergonié, 229 Cours de l'Argonne, Bordeaux, France (a.italiano@bordeaux.unicancer.fr).

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

The programmed cell death protein 1 (PD-1)/programmed cell death 1 ligand 1 (PD-L1) interaction is a major pathway hijacked by tumors to suppress immune control. In up to 58% of cases of soft-tissue sarcoma (STS), osteosarcoma, and gastrointestinal stromal tumors (GIST), PD-L1 can be expressed,[2,3] and targeting the PD-1/PD-L1 interaction has been associated with impressive antitumor activity in a preclinical 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 and/or programmed cell death 1 ligand 2, 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 peptide vaccines,[8] or anti-PD-1 antibody.[9]

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

Key Points

Question Is programmed cell death protein 1 (PD-1) targeting a relevant strategy for patients with soft-tissue sarcomas (STS)?

Findings This phase 2 clinical trial assessed the combination of the selective PD-1 inhibitor pembrolizumab in combination with low-dose cyclophosphamide in 4 cohorts of patients with soft-tissue sarcoma (STS). Only 3 of 50 patients assessable for efficacy experienced tumor shrinkage, and analyses of tumor samples showed strong infiltration by M2 macrophages expressing the immunosuppressive enzyme inhibitory enzyme indoleamine 2,3-dioxygenase (IDO).

Meaning Given the importance of macrophage infiltration and the IDO1/kynurenine pathway in patients with STS, further strategies are warranted to assess the combination of anti–PD-1/programmed cell death 1 ligand 1 with colony-stimulating factor 1 receptor inhibitors and/or IDO inhibitors in patients with selected sarcoma subtypes.

Methods

This was a single-arm, phase 2, multicenter clinical trial based on the Simon 2-stage design with 4 cohorts of patients: cohort 1, unresectable leiomyosarcoma (LMS); cohort 2, undifferentiated pleomorphic sarcoma (UPS); cohort 3, other sarcoma (others); and cohort 4, gastrointestinal stromal tumors (GIST). 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 enrollment in the study. Trial protocol is available in Supplement 1.

Patients received 50 mg of CP orally twice daily 1 week on and 1 week off and 200 mg of pembrolizumab intravenously at day 8 of a planned 21-day cycle. Details regarding eligibility criteria, design, and statistical analysis are provided as eMethods in Supplement 2.

Ninety-five percent, 2-sided exact binomial confidence intervals were computed for the response rates. Progression-free survival and overall survival were estimated using the Kaplan-Meier method. Median follow-up was calculated using the reverse Kaplan-Meier method. For correlative biomarker analyses, the nonparametric Wilcoxon 2-sample test, the Kruskal-Wallis test, and the Wilcoxon signed-rank test for paired data were used for comparisons of continuous variables. For categorical variables, either the χ^2 test or Fisher exact test was used. To investigate associations between the expression of biomarkers, the Spearman rank-order correlation was used. To assess the association between the exploratory markers for categorical variables, the χ^2 test (expected frequencies ≥5) or Fisher exact test was used (expected frequencies <5). To assess the association between the exploratory markers for continuous variables, the exact Wilcoxon 2-sample test was used when comparing 2 independent samples (sample size ≥10 and no assumptions of normality distributions or equality of distributions). For more than 2 independent samples, the Kruskal-Wallis test was used.

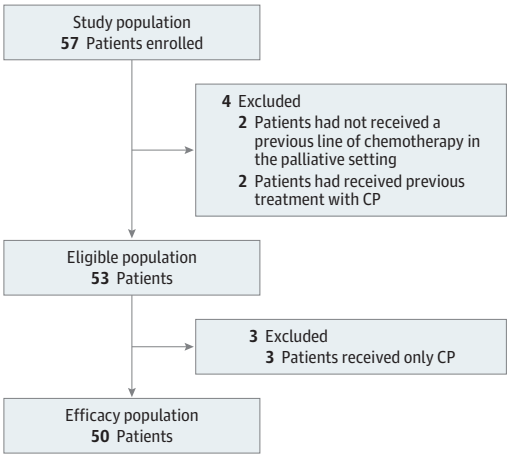
In the case of paired data, the Spearman rank-order correlation was used, which is a nonparametric measure of association based on the ranks of the data values. The Spearman correlation coefficient is a statistical measure of the strength of a monotonic relationship between paired data. Its interpretation is similar to that of Pearson, meaning that the closer the coefficient is to plus or minus 1, the stronger the monotonic relationship is. A Spearman correlation coefficient between 0.60 and 0.79 indicates a strong relationship and 0.40-0.59 indicates a moderate relationship. Statistical significance was achieved at a P value of less than .05. The data reported here represent the study database as of January 16, 2017. All analyses were conducted using SAS 9.2 software (SAS Institute).

Results

Between June 19, 2015, and July 8, 2016, 57 patients with advanced sarcoma (LMS, UPS, GIST, and other) were enrolled across 7 French Sarcoma Group centers. Four patients did not meet the eligibility criteria, and 50 patients were assessable for the primary efficacy end point (**Figure 1**). Baseline patient characteristics are listed in the **Table**. After a median follow-up of 6.8 months (95% CI, 5.3-9.4), 3 patients (5%) were still on treatment (1 each in the UPS, other, and GIST cohorts), and 54 patients (95%) discontinued treatment. Discontinuation was related to disease progression in 45 cases (79%) (15, LMS; 9, UPS; 13, other; and 8, GIST), toxic effects for 4 patients (7%) (all 4, UPS), death for 4 patients (7%) (1, UPS; 2, other; and 1, GIST), and investigator decision for 1 patient (2%).

All 57 patients were included in the safety analysis. At the time of analysis, 206 cycles of pembrolizumab and metronomic CP had been administered, with a median (range) 2 (0-17) cycles per patient. The most commonly observed toxic

Figure 1. CONSORT Diagram for Inclusion of 57 Patients



All 57 patients in the initial study population were included in the safety analysis. CP indicates cyclophosphamdide.

effects were grade 1 or 2 fatigue, diarrhea, and anemia. Grade 3 or 4 toxic effects were rare (observed in 10 patients) and mainly included fatigue, oral mucositis, and anemia (eTable 1 in Supplement 2).

Of the 50 patients assessable for efficacy analysis, 3 were progression-free at 6 months (1 patient with a solitary fibrous tumor, 1 with an endometrial stromal sarcoma, and 1 with a GIST), indicating that the first-stage objective was not satisfied for any of the cohorts. The best response was progressive disease for 31 patients (10, LMS; 7, UPS; 8, other; and 6, GIST) and stable disease for 16 patients (3, LMS; 5, UPS; 5, other; and 3, GIST). One objective response was observed in a patient with an initially progressive solitary fibrous tumor (**Figure 2**). The 6-month nonprogression rate was 0%, 0%, and 14.3% (95% CI, 1.8%-42.8%) for LMS, UPS, and other, respectively, and 11.1% (95% CI, 2.8%-48.3%) for GIST. For LMS, UPS, other, and GIST, the median progression-free survival was 1.4 months (95% CI, 1.2-1.4), 1.4 months (95% CI, 1.1-4.0), 1.4 months (95% CI, 0.9-4.0), and 1.4 months (95% CI, 0.9-5.3), respectively; and the median overall survival was 9.2 months (95% CI, 2.4-15.9), 5.6 months (95% CI, 3.2-16.1), 7.1 months (95% CI, 2.0-16.3), and not reached at the time of analysis for LMS, UPS, other, and GIST, respectively.

Overall, PD-L1 expression (≥1%) in tumor cells and immune cells was observed in 6 (12%) and 19 (40%) cases, respectively (eFigure 1 and eTable 2 in Supplement 2), and PD-L1 expression in immune cells was 23%, 64%, 29%, and 43% in LMS, UPS, other, and GIST, respectively. Only 1 patient had PD-L1 expression in immune cells greater than 10%, and this was the only responding patient (eFigure 2 in Supplement 2).

Forty-nine patients had available data on infiltrate densities of immune cells in their tumor samples. The median (range) density of cytotoxic T (CD8)-positive cells was 48 (2-1447) cells/mm^2; CD68-positive/CD163-negative cells, 0.8 (0-10) cells/mm^2; and CD163-positive cells, 2.4 (0.25) cells/mm^2. Strikingly, the median (range) ratio of CD163/(CD68+CD163) cell density was 0.8 (0-1) cells/mm^2, reflecting the predominance of CD163-positive macrophages of the protumor M2 phenotype

Table. Characteristics of 57 Patients

Characteristic	No. (%)
Sex	
Male	33 (58)
Female	24 (42)
Age, median, (range), y	59.5 (18.5-84.0)
ECOG PS	
0	21 (37)
1	33 (58)
ND	3 (5)
Histological subtype	
Leiomyosarcoma	15 (27)
UPS	16 (28)
Others[a]	16 (28)
GIST	10 (18)
Stage	
Locally advanced	9 (16)
Metastatic	48 (84)
Prior line(s) of chemotherapy	
0	2 (3)
1	13 (23)
2	22 (39)
>2	20 (35)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; GIST, gastrointestinal stromal tumors; PS, performance scale; UPS, undifferentiated pleomorphic sarcoma.

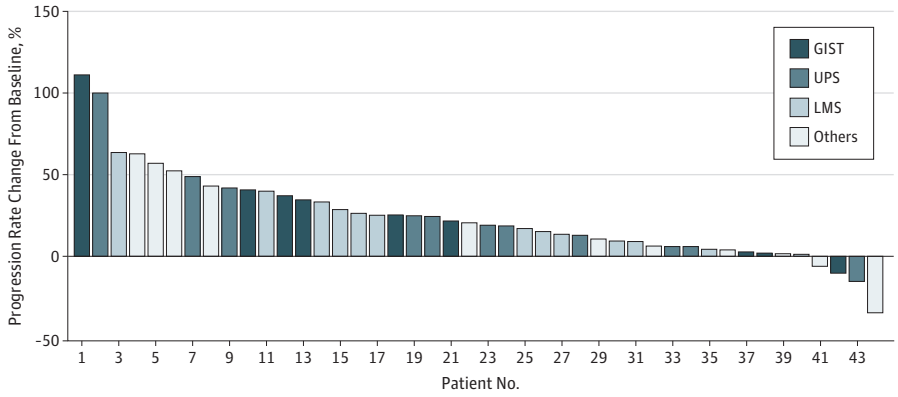
[a] Other tumors included 3 endometrial stromal sarcomas, 2 dedifferentiated liposarcomas, 2 myxofibrosarcomas, 2 extraskeletal chondrosarcomas, 1 pleomorphic liposarcoma, 1 Ewing sarcoma, 1 round cell liposarcoma, 1 myxoid liposarcoma, 1 synovial sarcoma, 1 solitary fibrous tumor, and 1 angiosarcoma.

(eFigure 2 in Supplement 2). Thirty-one percent of the patients in the LMS cohort had a CD163 to (CD68+CD163) cell ratio above the median; as did 73% of the UPS cohort; 46%, other; and 38%, GIST. The UPS cohort had the highest CD163 to (CD68+CD163) cell ratio at 0.96 (interquartile range [IQR], 0.77-0.99) followed by other (0.80 [IQR, 0.66-0.85], GIST (0.68 [IQR, 0.53-0.87]), and LMS (0.54 [IQR, 0.34-0.84]) (P = .01). We also found inhibitory enzyme indoleamine 2,3-dioxygenase 1 (IDO1) was expressed in immune cells in 69%, 73%, 29%, and 63% of LMS, UPS, other, and GIST, respectively, and that this was largely by macrophages (eFigure 3 and eTable 3 in Supplement 2).

Overall, PD-L1 expression in immune cells was significantly positively associated with CD8-positive cell density (Wilcoxon, 662; mean rank ratio score of 34.8 vs 16.6 when PD-L1 was expressed; P < .001), and IDO1 expression in immune cells (Fisher exact test, 0.002; P = .003). We found a significant correlation between CD8-positive and CD163-postive cell densities (Spearman [r_s], 0.6; P < .001), IDO1-positive and CD163-positive cell densities (r_s, 0.5; P = .001), and IDO1-positive cell density and the CD163 to (CD163+CD68) ratio (r_s, 0.5; P = .001).

Because there are no reference values for the densities of CD8, CD68, or CD163 staining in STS, we compared our data with data from an independent cohort of 47 patients with resected non-small-cell lung cancer, a type of cancer in which anti-PD-L1 targeting has demonstrated activity, using the same

Figure 2. Change in Progression Rate for 44 Patients



GIST indicates gastrointestinal stromal tumor; LMS, leiomyosarcoma; others, other sarcomas; UPS, undifferentiated pleomorphic sarcoma.

staining and image analysis methods. Interestingly, we found that the CD8-positive cell density was significantly higher in the lung carcinoma cohort than in the STS cohort (median [IQR], 422 [183-609] vs 48 [10-135]; $P < .05$).

Finally, we observed a statistically significant increase in the kynurenine to tryptophan ratio between plasma samples collected before the first infusion of pembrolizumab at cycle 1, day 8 and at cycle 2, day 8 and cycle 3, day 8 (median increase of 34.6; Wilcoxon, 162; $P < .001$) (eFigure 4 in Supplement 2). Increases in the kynurenine to tryptophan ratio between cycle 1, day 8 and cycle 3, day 8 were also correlated with a higher density of IDO1 expression in pretreated tumor samples (Wilcoxon, 18.5; $P = .04$).

Discussion

Pre-existing T-cell antitumor immunity has been hypothesized as a prerequisite to the anti-PD-1/PD-L1 response.[10,11] Our current analysis demonstrates that tumor-infiltrating CD8-positive effector lymphocytes are significantly reduced in the vast majority of tumor samples from patients with STS and GIST compared with those from patients with non-small-cell lung cancer. Moreover, we found low expression of PD-L1 in tumor samples, none exhibiting more than 10% PD-L1-positive tumor cells, and only 1 that had more than 10% PD-L1-positive immune cells. These results are in agreement with previously reported studies using validated anti-PD-L1 immunohistochemical assays.[3,12] A lack of PD-L1 expression has been correlated with limited benefit from PD-1 blockade in carcinomas.[13] Interestingly, the unique partial response observed in our study was in the patient bearing a unique tumor with more than 10% PD-L1-positive immune cells. Furthermore, this patient's tumor had mild IDO1-positive immune cells, a CD68-positive cell density below the median (0.57 cells/mm^2), and a very high CD8-positive cell density (439 cells/mm^2).

We also observed that a high proportion of STS and GIST tumors were prominently infiltrated by CD163-positive macrophages that favor the M2 phenotype known to play a role in immune suppression. Importantly, we also observed that these tumor-associated macrophages expressed IDO1. This suggests that this pathway could preferentially contribute to the immune-suppressive phenotype of these cells and could be an important mechanism of the primary resistance to PD-1 inhibition observed in this study.[14]

Kynurenine, which is notably produced by IDO1, is a key metabolite of tryptophan that can promote selective expansion of regulatory T cells.[15] Interestingly, we also report a statistically significant increase in the kynurenine to tryptophan plasma ratio in patients during treatment. These findings reinforce the role of the IDO1/kynurenine pathway in the resistance to PD-1/PD-L1 targeting in STS and GIST.[16]

Limitations

One of the main limitations of the study is that it included heavily pretreated patients. In future sarcoma immunotherapy trials, the greater immune competence and better prognosis of less heavily pretreated patients both argue for considering patients who have not necessarily exhausted all standard treatments.

Conclusions

This study indicates that the activity of PD-1 targeting in advanced STS and GIST is limited. In preclinical studies, while immune checkpoint inhibitors were insufficient in controlling tumor growth, combining macrophage targeting through colony stimulating factor 1 receptor inhibition resulted in superior tumor control.[17] Given the importance of macrophage infiltration and the IDO1/kynurenine pathway in STS as suggested by our data, further strategies are warranted to assess the combination of anti-PD-1/PD-L1 with therapies targeting these immunological features, such as colony stimulating factor 1 receptor inhibitors and/or IDO inhibitors in selected sarcoma subtypes.

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Author Affiliations: Department of Medical Oncology, Institut Bergonié, Bordeaux, France (Toulmonde, Cousin, Grellety, Italiano); Department

of Medical Oncology, Centre Oscar Lambret, Bordeaux, France (Penel); Department of Pathology, Gustave Roussy, Villejuif, France (Adam); INSERM U981, Villejuif, France (Adam); Department of Medical Oncology, Oncopole Toulouse, Toulouse France (Chevreau); Department of Medical Oncology, Centre Leon Berard, Lyon, France (Blay); Department of Medical Oncology, Institut Gustave Roussy, Villejuif, France (Le Cesne); Department of Medical Oncology, Institut de Cancérologie de l'Ouest, Nantes, France (Bompas); Department of Medical Oncology, Institut Curie, Paris, France (Piperno-Neumann); Department of Medical Oncology, Centre Oscar Lambret, Lille, France (Ryckewaert); Immusmol, Bordeaux, France (Bessede); INSERM, Dijon, France (Ghiringhelli); Unité de Recherche et d'Epidémiologie Cliniques, Institut Bergonié, Bordeaux, France (Pulido); INSERM CIC 1401, Bordeaux, France (Pulido).

Author Contributions: Dr Italiano had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Toulmonde, Adam, Blay, Bompas, Grellety, Italiano.
Acquisition, analysis, or interpretation of data: Toulmonde, Penel, Adam, Chevreau, Blay, Le Cesne, Piperno-Neumann, Cousin, Ryckewaert, Bessede, Ghiringhelli, Pulido, Italiano.
Drafting of the manuscript: Toulmonde, Adam, Blay, Italiano.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Pulido, Italiano.
Obtained funding: Blay, Grellety, Italiano.
Administrative, technical, or material support: Toulmonde, Adam, Chevreau, Blay, Bessede.
Study supervision: Toulmonde, Blay, Ryckewaert, Ghiringhelli, Italiano.

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