Nivolumab and sunitinib combination in advanced soft tissue sarcomas: a multicenter, single-arm, phase Ib/II trial

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ABSTRACT

Background Sarcomas exhibit low expression of factors related to immune response, which could explain the modest activity of PD-1 inhibitors. A potential strategy to convert a cold into an inflamed microenvironment lies on a combination therapy. As tumor angiogenesis promotes immunosuppression, we designed a phase Ib/II trial to test the double inhibition of angiogenesis (sunitinib) and PD-1/PD-L1 axis (nivolumab).

Methods This single-arm, phase Ib/II trial enrolled adult patients with selected subtypes of sarcoma. Phase Ib established two dose levels: level 0 with sunitinib 37.5 mg daily from day 1, plus nivolumab 3 mg/kg intravenously on day 15, and then every 2 weeks; and level −1 with sunitinib 37.5 mg on the first 14 days of induction and then 25 mg per day plus nivolumab on the same schedule. The primary endpoint was to determine the recommended dose for phase II (phase I) and the 6-month progression-free survival rate, according to Response Evaluation Criteria in Solid Tumors 1.1 (partition II).

Results From May 2017 to April 2019, 68 patients were enrolled: 16 in phase Ib and 52 in phase II. The recommended dose of sunitinib for phase II was 37.5 mg as induction and then 25 mg in combination with nivolumab. After a median follow-up of 17 months (4–26), the 6-month progression-free survival rate was 48% (95% CI 41% to 55%). The most common grade 3–4 adverse events included transaminitis (17.3%) and neutropenia (11.5%).

Conclusions Sunitinib plus nivolumab is an active scheme with manageable toxicity in the treatment of selected patients with advanced soft tissue sarcoma, with almost half of patients free of progression at 6 months.

INTRODUCTION

Systemic treatment for advanced soft tissue sarcomas (STS) is an unmet need since no new drugs or combination schemes have offered significant overall survival (OS) benefit over doxorubicin alone in the last 40 years. Immunomodulation has become a topical area of interest in many tumors, being sarcoma not an exception. Nevertheless, while in paradigmatic immune-sensitive tumors such as melanoma the expression levels of tumor mutational burden (TMB), CD8+ lymphocytes and Programmed death-ligand 1 (PD-L1) were high enough, with values of 14 mutations per DNA megabase (Mb), 42%±23% and 35%, respectively, sarcomas appear at the low range of these expression values. Indeed, the median TMB reported was 2 mutations per Mb, the percentage of CD8+ lymphocytes was 23%±13% and the tumor PD-L1 expression was 6.6% in the largest series. Specific immune-checkpoint protein expression, such as PD-1/PD-L1 axis, has not demonstrated convincing prognostic or predictive value in sarcomas. Apart from the inherent heterogeneity of STS, other factors that introduce bias across these studies were different staging, diverse antibody clones used for PD-L1, and intra-study variability between tissue microarray or complete tumor block section. A genomic approach that can circumvent some constraints of PD-L1 immunohistochemistry has confirmed an independent negative prognostic value for PD-L1 overexpression in a large study of patients with STS. The elapsed time between tumor sampling and the time frame considered for the analysis is a common limitation of immunostaining and genomics, which is only eluded if biopsies are mandatory at baseline. Using transcriptomic analysis of the micro-environment cell population, which measures the expression of eight immune and two stromal cell populations, it was possible to...
classify sarcoma into five different sarcoma immune classes (SIC). Patients grouped as SIC E were considered the most immune-responsive subset, but only represented 17.8% of cases. Therefore, the immune cold microenvironment found in most sarcomas could explain the low probability of response or tumor control with anti-PD-1 compounds in monotherapy. In fact, two reported experiences with nivolumab showed a median progression-free survival (mPFS) of 1.7 months in 43 patients with STS and 1.8 months in 12 patients with STS, respectively. A pioneer study trialing pembrolizumab in the most frequent STS and bone sarcomas reported better outcomes with an mPFS of 4.2 months, 18% of responses and a 6-month progression-free survival rate (PFSR) of 32%. Immune barrier promoted by tumor angiogenesis is well established and there is an ever growing list of immune cells exhibiting the dual capacity of promoting immunosuppression and angiogenesis. This impelled us to design a double inhibition study with antiangiogenic and anti-PD-1 compounds, which could potentially synergize for the treatment of some advanced soft tissue and bone sarcomas. We present herein the phase Ib, common to both cohorts, and phase II part of the STS cohort of the IMMUNOSARC trial, which combined sunitinib and nivolumab. The bone sarcoma cohort will be reported separately.

METHODS

Study design and subjects

In this phase Ib/II, single-arm trial, STS cohort, patients aged over 18 years with advanced STS were considered for eligibility and enrolled in eight centers in Spain and Italy with expertise in sarcoma care. Central pathology review was mandatory before accrual. Patients had to be in progression in the previous 6 months according to Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. Eligible subtypes of sarcoma were chosen based on several reasons: histologies reported in transplant recipients (undifferentiated pleomorphic sarcoma (UPS), angiosarcoma, and synovial sarcoma), higher tumor infiltrating lymphocytes (UPS and epithelioid sarcoma), or sensitivity to antiangiogenic compounds (solitary fibrous sarcoma, extraskelatal myxoid chondrosarcoma, clear cell sarcoma, alveolar soft part sarcoma (ASPS), epithelioid hemangioendothelioma and angiosarcoma). Other relevant inclusion criteria are described in online supplemental methods.

Patients provided written informed consent before participating in the study.

Procedures

In phase I, two dose levels were defined: the starting dose (dose level 0) used sunitinib 37.5 mg orally on a daily basis from day 1. The de-escalation level (dose level −1) used sunitinib 37.5 mg on the first 14 days (induction) and from then on 25 mg per day. Nivolumab was given at 3 mg/kg intravenously on day 15 and every 2 weeks from then on at both dose levels. Phase I was completed when 10 dose-limiting toxicity (DLT) evaluable patients had been treated, associated with a DLT rate of less than 0.33. There was a de-escalation level if three or more DLTs occurred at the initial dose level.

Radiological assessment was performed at the local sites as well as by a mandatory independent central review. Tumor assessment was done every 8 weeks by CT scan or by MRI in accordance with RECIST 1.1. Other relevant procedures are described in online supplemental methods.

For translational purposes, tumor biopsies were required at baseline and at week 13, and blood samples were collected at baseline, day 1 of week 3, day 1 of week 13, and after each radiological evaluation.

The HTG EdgeSeq, ran in the VERIP service lab of HTG in Tucson (HTG Molecular Diagnostics, Tucson, Arizona, USA), and ProcartaPlex immunoassay (Invitrogen, Carlsbad, California, USA) protocols were applied in the exploratory translational study, and complete bioinformatic analyses are described in online supplemental methods.

Outcomes

The main endpoint for phase Ib was to determine the recommended dose for phase II. The main endpoint for phase II was 6-month PFSR according to RECIST. The secondary endpoints were median OS (mOS), overall response rate according to RECIST 1.1 and Choi criteria, toxicity profile according to Common Terminology Criteria for Adverse Events (CTCAE) V.4.0, and contribution from translational studies.

Sample size has been obtained for a one-arm, one-stage survival design based on Brookmeyer-Crowley-like test. The statistical test for survival probability was based on non-parametric estimation of survival distribution. For STS second-line cohort sample size has been obtained for the primary endpoint: 6-month PFSR. The estimated accrual time was 24 months. In this population, a 5% PFSR was considered not promising, whereas a 15% PFSR was considered promising. With a 0.05 type I error α and a power of 0.80, 48 patients were needed in this cohort.

Statistical analysis

The intention-to-treat population includes patients who had provided written informed consent, with central pathology confirmation, and fulfilling all the inclusion criteria and none of the exclusion criteria. The per-protocol population (efficacy) includes patients fulfilling the intention-to-treat population criteria, and additionally patients who had received sunitinib in the induction phase and at least one dose of nivolumab. The safety population includes all patients of the intention-to-treat population who had received at least one dose of sunitinib. The radiological evaluable population includes all patients of the intention-to-treat population who underwent at least one radiological assessment.
The initial dose level (sunitinib 37.5 mg/day) in phase Ib was ceased after observing three DLTs in six accrued patients: grade 3 fatigue (n=2) and grade 4 septic shock (n=1). In the subsequent de-escalation (−1) level, only one DLT (febrile neutropenia) was reported in 10 accrued patients. Thus, the recommended dose of sunitinib for phase II was 37.5 mg/day as induction on the first 14 days, followed by sunitinib 25 mg/day plus nivolumab 3 mg/kg every 2 weeks from day 15 on. One hundred and sixty-eight 2-week cycles were administered in phase Ib. Phase Ib toxicities by patients are depicted in table 2.

According to RECIST there were seven partial responses among 14 evaluable patients enrolled in this part (figure 2): two patients with clear cell sarcoma, two with ASPS, and one occurring in each of the following histologies: synovial sarcoma, chondrosarcoma and angiosarcoma. In the remaining non-responder patients, three had stable disease and four had progressive disease.

The clinical cut-off for the final data analyses for phase II was February 27, 2020. At that time, 5 (10%) of the 52 patients were still under treatment and 47 (90%) have discontinued the scheme. Of the 52 patients, 40 (77%) had disease stable and four had progressive disease.

Time-to-event variables were measured from the date of enrollment and were estimated according to the Kaplan-Meier method. Comparisons between the variables of interest were performed by the log-rank test. For variables not available at baseline (ie, RECIST or Choi response), a landmark analysis was performed. False discovery rate was applied to regulate multiple comparisons. Other statistical methods are described in online supplemental methods.

**RESULTS**

From May 31, 2017 to April 18, 2019, 91 patients with advanced and progressing STS were assessed for eligibility in phase Ib and in the STS cohort of phase II. Twenty-three patients were excluded (considering both phases) after screening. Sixty-eight patients were enrolled and treated with the experimental compounds: 16 in phase Ib and 52 in the STS cohort of phase II. The intention-to-treat population and safety population for phase II consisted of 52 eligible patients, while the per-protocol population consisted of 49 patients, since 3 patients had not ever received nivolumab. Radiological evaluable patients in phase II according to RECIST and Choi criteria were 46 and 40, respectively (figure 1). Patients’ demographics are depicted in table 1.

The clinical cut-off for the final data analyses for phase II was February 27, 2020. At that time, 5 (10%) of the 52 patients were still under treatment and 47 (90%) have discontinued the scheme. Of the 52 patients, 40 (77%) had disease stable and four had progressive disease. The clinical cut-off for the final data analyses for phase II was February 27, 2020. At that time, 5 (10%) of the 52 patients were still under treatment and 47 (90%) have discontinued the scheme. Of the 52 patients, 40 (77%) had disease stable and four had progressive disease.
partial responses (11%), 33 stabilizations (72%) and 7 progressions (15%). Complete response was observed in one patient with angiosarcoma and partial response in patients diagnosed with ASPS (n=2), angiosarcoma (n=1), extraskeletal myxoid chondrosarcoma (n=1) and synovial sarcoma (n=1). Central assessment according to Choi criteria showed 25 patients with partial response (63%), 10 with stable disease (25%) and 5 with progressive

| Table 1 Baseline characteristics | Phase Ib (n=16) | Phase II (n=52) |
|----------------------------------|----------------|-----------------|
| Median age (range) (months)      | 38 (25–78)     | 43 (19–77)      |
| Sex (male/female), n (%)         | 10 (63)/6 (37) | 30 (58)/22 (42) |
| Primary tumor localization, n (%)|                |                 |
| Somatic                          | 15 (94)        | 44 (85)         |
| Visceral                         | 1 (6)          | 8 (15)          |
| Primary tumor depth, n (%)       |                |                 |
| Deep                             | 16 (100)       | 40 (77)         |
| Superficial                      | 0              | 9 (17)          |
| UK                               | 0              | 3 (6)           |
| Extension at diagnosis, n (%)    |                |                 |
| Localized                        | 9 (56)         | 25 (48)         |
| Locally advanced                 | 2 (13)         | 15 (29)         |
| Metastatic                       | 5 (31)         | 12 (23)         |
| Resectable at diagnosis, n (%)   |                |                 |
| Resectable                       | 10 (63)        | 38 (73)         |
| Unresectable                     | 6 (37)         | 14 (27)         |
| Median metastatic-free interval (range) (months) | 8 (0–80) | 11 (0–141)     |
| Extension at baseline, n (%)     |                |                 |
| Locally advanced                 | 0              | 4 (8)           |
| Metastatic                       | 16 (100)       | 48 (92)         |
| Diagnosis (central), n (%)       |                |                 |
| Synovial sarcoma                 | 2 (13)         | 9 (17)          |
| UPS                              | 2 (13)         | 8 (15)          |
| Clear cell sarcoma               | 4 (25)         | 7 (14)          |
| Solitary fibrous tumor           | 0              | 7 (14)          |
| Epithelioid sarcoma              | 0              | 7 (14)          |
| Angiosarcoma                     | 2 (13)         | 5 (10)          |
| Extraskeletal myxoid chondrosarcoma | 0              | 4 (7)           |
| Alveolar soft part sarcoma       | 3 (19)         | 4 (7)           |
| Epithelioid hemangioendothelioma  | 0              | 1 (2)           |
| Osteosarcoma*                    | 2 (13)         | 0               |
| Chondrosarcoma*                  | 1 (6)          | 0               |
| ECOG PS at baseline, n (%)       |                |                 |
| 0                                | 6 (37)         | 25 (48)         |
| 1                                | 10 (63)        | 27 (52)         |
| Median previous lines (range)    | 1.5 (0–5)      | 1 (0–4)         |
| Previous antiangiogenic lines, n (%)|              |                 |
| 0                                | 14 (88)        | 41 (79)         |
| 1                                | 2 (12)         | 10 (19)         |
| 2                                | 0              | 1 (2)           |

*Included in phase Ib.

ECOG PS, Eastern Cooperative Oncology Group performance status; UK, unknown; UPS, undifferentiated pleomorphic sarcoma.
### Table 2  Toxicity profile in phase Ib

**Phase Ib (n=16)**

| Type of adverse event (n=16) | Any grade, n (%) | Grades 1–2, n (%) | Grade 3, n (%) | Grade 4, n (%) | Grade 5, n (%) |
|-----------------------------|------------------|------------------|---------------|---------------|---------------|
| **Hematological toxicity**  |                  |                  |               |               |               |
| Leukopenia                  | 12 (75.0)        | 11 (68.8)        | 1 (6.3)       | 0             | 0             |
| Neutropenia                 | 10 (62.5)        | 7 (43.8)         | 3 (18.8)      | 0             | 0             |
| Thrombocytopenia            | 9 (56.3)         | 7 (43.8)         | 2 (12.5)      | 0             | 0             |
| Anemia                      | 8 (50.0)         | 6 (37.5)         | 2 (12.5)      | 0             | 0             |
| Lymphopenia                 | 2 (12.5)         | 2 (12.5)         | 0             | 0             | 0             |
| Febrile neutropenia         | 1 (6.3)          | 0                | 0             | 1 (6.3)       | 0             |
| **Non-hematological toxicity** |                |                  |               |               |               |
| Fatigue                     | 14 (87.5)        | 11 (68.8)        | 3 (18.8)      | 0             | 0             |
| Hypertension                | 11 (68.8)        | 10 (62.5)        | 1 (6.3)       | 0             | 0             |
| Diarrhea                    | 8 (50.0)         | 8 (50.0)         | 0             | 0             | 0             |
| Oral mucositis              | 8 (50.0)         | 7 (43.8)         | 1 (6.3)       | 0             | 0             |
| Increased AST               | 7 (43.8)         | 6 (37.5)         | 1 (6.3)       | 0             | 0             |
| Nausea                      | 6 (37.5)         | 6 (37.5)         | 0             | 0             | 0             |
| Increased ALT               | 5 (31.3)         | 4 (25.0)         | 1 (6.3)       | 0             | 0             |
| Anorexia                    | 4 (25.0)         | 4 (25.0)         | 0             | 0             | 0             |
| Constipation                | 3 (18.8)         | 3 (18.8)         | 0             | 0             | 0             |
| Hyponatremia                | 3 (18.8)         | 3 (18.8)         | 0             | 0             | 0             |
| Hypothyroidism              | 3 (18.8)         | 2 (12.5)         | 1 (6.3)       | 0             | 0             |
| Palmar-plantar erythrodysesthesia syndrome | 3 (18.8) | 3 (18.8) | 0 | 0 | 0 |
| Skin/subcutaneous tissue disorders | 3 (18.8) | 3 (18.8) | 0 | 0 | 0 |
| Stomach pain                | 3 (18.8)         | 3 (18.8)         | 0             | 0             | 0             |
| Vomiting                    | 3 (18.8)         | 3 (18.8)         | 0             | 0             | 0             |
| Hypokalemia                 | 3 (18.8)         | 2 (12.5)         | 1 (6.3)       | 0             | 0             |
| Increased ALP               | 2 (12.5)         | 2 (12.5)         | 0             | 0             | 0             |
| Infection                   | 2 (12.5)         | 0                | 1 (6.3)       | 1 (6.3)       | 0             |
| Dysgeusia                   | 2 (12.5)         | 2 (12.5)         | 0             | 0             | 0             |
| Epistaxis                   | 2 (12.5)         | 2 (12.5)         | 0             | 0             | 0             |
| Fever                       | 2 (12.5)         | 2 (12.5)         | 0             | 0             | 0             |
| Hypermagnesemia             | 2 (12.5)         | 2 (12.5)         | 0             | 0             | 0             |
| Periodontal disease         | 2 (12.5)         | 2 (12.5)         | 0             | 0             | 0             |
| Arthralgia                  | 1 (6.3)          | 1 (6.3)          | 0             | 0             | 0             |
| Body pain                   | 1 (6.3)          | 1 (6.3)          | 0             | 0             | 0             |
| Broncho pulmonary hemorrhage| 1 (6.3)          | 1 (6.3)          | 0             | 0             | 0             |
| Renal function deterioration | 1 (6.3)          | 1 (6.3)          | 0             | 0             | 0             |
| Dry mouth                   | 1 (6.3)          | 0                | 1 (6.3)       | 0             | 0             |
| Flatulence                  | 1 (6.3)          | 1 (6.3)          | 0             | 0             | 0             |
| Headache                    | 1 (6.3)          | 1 (6.3)          | 0             | 0             | 0             |
| Hiccups                     | 1 (6.3)          | 1 (6.3)          | 0             | 0             | 0             |
| Hypercalcemia               | 1 (6.3)          | 1 (6.3)          | 0             | 0             | 0             |
| Hypocalcemia                | 1 (6.3)          | 1 (6.3)          | 0             | 0             | 0             |

Continued
disease (12%). Response assessment according to RECIST showed significant prognostic difference for PFS and OS; by contrast, Choi assessment only had prognostic relevance for PFS. Adding the 12 evaluable STS cases of phase I to the 46 evaluable patients with STS in phase II, the RECIST Overall Response Rate (ORR) was 21% (12 out of 58). The 18-month OS proportion was 100%, 75% and 44% for those with response, stable disease and progressive disease, according to RECIST, respectively (p=0.01) (table 3).

Ad hoc analyses showed a growth modulation index (GMI) in phase II of 0.7 (0.1–11.6), while 31% of patients exhibited GMI >1.33. On the other hand, the GMI in phase II in subtypes with known sensitivity to antiangiogenic compounds was 1.01 (0.04–26.82).

In the translational study, among 732 genes related to tumor immune response, 50 and 102 genes showed to have potential prognostic value for PFS and OS, respectively. Hierarchical clustering, considering the 102 genes with impact on OS, distributed samples into two groups with a distinct molecular pattern (online supplemental figure 1). The different outcome of these two groups was confirmed (table 3). Functional enrichment analysis showed that the genes upregulated in group 2 (molecular group with better survival) were highly associated with Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (online supplemental figure 2) cytokine–cytokine receptor interaction (hsa04060) (adjusted p=3.69×10^{-53}) or chemokine signaling pathway (hsa04062) (adjusted p=2.13×10^{-22}). On the other hand, genes upregulated in group 1 (molecular group with worse outcome) were

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**Table 2** Continued

| Type of adverse event (n=16) | Any grade, n (%) | Grades 1–2, n (%) | Grade 3, n (%) | Grade 4, n (%) | Grade 5, n (%) |
|-----------------------------|-----------------|-----------------|---------------|---------------|---------------|
| Hypernatremia 1 (6.3)       | 1 (6.3)         | 0               | 0             | 0             | 0             |
| Hypomagnesemia 1 (6.3)      | 1 (6.3)         | 0               | 0             | 0             | 0             |
| Hypophosphatemia 1 (6.3)    | 1 (6.3)         | 0               | 0             | 0             | 0             |
| Myalgia 1 (6.3)              | 1 (6.3)         | 0               | 0             | 0             | 0             |
| Odynophagia 1 (6.3)         | 1 (6.3)         | 0               | 0             | 0             | 0             |
| Tumor lesions bleeding 1 (6.3) | 1 (6.3) | 0             | 0             | 0             | 0             |
| Periorbital edema 1 (6.3)   | 1 (6.3)         | 0               | 0             | 0             | 0             |
| Proteinuria 1 (6.3)         | 1 (6.3)         | 0               | 0             | 0             | 0             |
| Thromboembolic event 1 (6.3) | 0               | 0             | 1 (6.3)       | 0             | 0             |
| Weight loss 1 (6.3)         | 1 (6.3)         | 0               | 0             | 0             | 0             |

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

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![Figure 2](image2.png) **Figure 2** Response to treatment by patient according to RECIST. All evaluable patients (N=60) from phase Ib (n=14) and phase II (n=46) are shown. Tumor diameter was measured in millimeters. The dashed lines represent 20% increase in diameter and 30% decrease in diameter (RECIST progression and response cut-offs, respectively). *No size variation. RECIST, Response Evaluation Criteria in Solid Tumors.

![Figure 3](image3.png) **Figure 3** Progression-free survival to treatment by patient, based on RECIST central radiological assessment. Each patient in the efficacy population in phase II is represented as bars (n=49). The vertical dashed line represents the median progression-free survival (5.6 months). The stars represent patients achieving RECIST objective responses. The arrows represent patients non-progression in the last central radiological assessment. RECIST, Response Evaluation Criteria in Solid Tumors.
### Table 3  Univariate analysis

Demographics and clinical characteristics in phase II (n=49)

| Analysis                        | Median PFS (central) (95% CI) | PFS at 6 months, % (95% CI) | P value | Median OS (95% CI) | OS at 18 months, % (95% CI) | P value |
|---------------------------------|-------------------------------|-----------------------------|---------|--------------------|----------------------------|---------|
| **Age**                         |                               |                             |         |                    |                            |         |
| 0–44                            | 4.5 (1.8 to 7.1)              | 45 (36 to 55)               | 0.4     | NR                 | 75 (65 to 84)              | 0.55    |
| >44                             | 6.5 (2.2 to 10.8)             | 52 (41 to 63)               |         | 24.1 (18.6 to 29.7) | 61 (51 to 71)              |         |
| **MFI (years)**                 |                               |                             | 0.59    |                    |                            | 0.97    |
| 0–1                             | 4.5 (2.6 to 6.3)              | 43 (33 to 52)               |         | 24.1 (NA)          | 66 (56 to 76)              |         |
| >1                              | 6.1 (0 to 14.6)               | 54 (44 to 65)               |         | NR                 | 68 (58 to 78)              |         |
| **Baseline ECOG**               |                               |                             | 0.2     |                    |                            | 0.098   |
| 0                               | 7.3 (4.2 to 10.4)             | 56 (46 to 66)               |         | NR                 | 80 (72 to 88)              |         |
| 1                               | 4.1 (3 to 5.2)                | 40 (29 to 50)               |         | 22.5 (8.8 to 36.2) | 51 (39 to 63)              |         |
| **Baseline extension**          |                               |                             | 0.9     |                    |                            | 0.22    |
| Metastatic                      | 6.1 (2.6 to 9.6)              | 50 (43 to 58)               |         | 24.1 (19.5 to 28.8)| 63 (56 to 71)              |         |
| Locally advanced                | 3.8 (1.3 to 6.2)              | 25 (2 to 48)                |         | NR                 | 100 (NA)                   |         |
| **Previous lines**              |                               |                             | 0.28    |                    |                            | 0.096   |
| 0–1                             | 5.6 (0.8 to 10.3)             | 49 (39 to 59)               |         | NR                 | 76 (67 to 86)              |         |
| >1                              | 4.1 (0.6 to 7.7)              | 47 (37 to 58)               |         | 21 (6.5 to 35.6)   | 55 (44 to 66)              |         |
| **Previous antiangiogenic**     |                               |                             | 0.27    |                    |                            | 0.71    |
| No                              | 4.4 (2.6 to 6.2)              | 41 (33 to 49)               |         | 24.1 (19.7 to 28.6)| 65 (57 to 73)              |         |
| Yes                             | 15.5 (4.3 to 26.7)            | 73 (59 to 86)               |         | NR                 | 72 (58 to 86)              |         |
| **Central RECIST response**     |                               |                             | 0.005   | 0.01               |                            |         |
| CR/PR                           | 11.7 (10.9 to 12.4)           | 100                         |         | NR                 | 100                        |         |
| SD                              | 3.7 (1.6 to 5.9)              | 39                          |         | NR                 | 75                         |         |
| PD                              | 0                             | 0                           |         | 3.9 (0 to 11.6)    | 44                         |         |
| **Central Choi response**       |                               |                             | 0.005   | 0.97               |                            |         |
| PR                              | 5.6 (0.6 to 10.6)             | 48                          |         | 22.3 (19.3 to 25.3)| 68                         |         |
| SD                              | 4.4 (0 to 17.8)               | 50                          |         | NR                 | 80                         |         |
| PD                              | 0                             | 0                           |         | NR                 | 67                         |         |
| **RNA expression genes from phase Ib and II (n=28)** |

| Gene   | Median PFS (95% CI) | PFS at 6 months, % (95% CI) | OS at 18 months, % (95% CI) | P value |
|--------|--------------------|-----------------------------|-----------------------------|---------|
| **PDGFD†** | 3.5 (1.7 to 5.2) | 17 (6 to 27) | 25 (12 to 37) | 0.005 |
| 0–4.9  |                   | 9.8 (7.2 to 12.5) | 93 (87 to 100) |         |
| >4.9   | 11.5 (3.9 to 19.1) | 74 (63 to 85) | 47 (35 to 59) | 0.022 |
| **IL16†** | 4 (2.5 to 5.5) | 24 (14 to 35) | 90 (68 to 100) | 0.005 |
| 0–6.1  |                   | 13.9 (0.6 to 27.3) | 90 (80 to 99) |         |
| >6.1   | 13.5 (8.2 to 18.8) | 90 (80 to 99) | 79 (70 to 88) | 0.007 |
| **TAL1†** | 3 (0.2 to 5.7) | 12 (1 to 24) | 25 (10 to 40) | 0.016 |
| 0–3.5  |                   | 8.4 (0 to 18.7) | 79 (70 to 88) |         |
| >3.5   | 7.5 (3.8 to 11.1) | 64 (53 to 75) | 90 (80 to 99) | 0.007 |
| **IL23A†** | 5.2 (2.1 to 8.2) | 38 (25 to 52) | 38 (25 to 52) | 0.27 |
| 0–4.1  |                   | 12.7 (6.2 to 19.2) | 86 (76 to 95) |         |
| >4.1   | 7.5 (2 to 12.9) | 58 (45 to 71) | 82 (73 to 92) | 0.017 |
| **TNFSF13B†** | 3 (0.1 to 5.9) | 10 (0 to 19) | 30 (15 to 44) | 0.007 |
| 0–5.3  |                   | 9.8 (0 to 21.4) | 82 (73 to 92) |         |
| >5.3   | 7.5 (4.2 to 10.7) | 71 (60 to 82) | 83 (74 to 92) | 0.005 |
| **CD276†** | 9.6 (4.8 to 14.4) | 67 (56 to 78) | 83 (74 to 92) | 0.005 |
| 0–8.4  |                   | 8.4 (0 to 17.5) | 22 (8 to 36) |         |
| >8.4   | 3 (0.5 to 5.4) | 11 (1 to 22) | 22 (8 to 36) |         |
greatly associated with metabolic and energetic processes (online supplemental figure 3).

Other clinical and translational results are described in online supplemental results.

**DISCUSSION**

In this phase Ib/II trial, we found that the 6-month PFSR was 48% according to RECIST and independent central review. This outcome widely exceeds the 15% considered promising in the statistical assumption of this trial. This threshold was based on the European Organisation for Research and Treatment of Cancer (EORTC) recommendation cut-off for activity, in terms of 6-month PFSR, in second line drugs of advanced STS. This indicator was chosen considering that a valuable immunotherapy should induce durable disease control; accordingly, 3-month PFSR would not be useful enough in detecting the potential added value of immunomodulation in sarcoma. The scheme was in general manageable even when it required transient dose interruptions in up to 59% of patients. Neutropenia, which could be related to FLT3 inhibition by sunitinib, was found at a higher proportion than sarcoma trials with anti-PD-1 alone or sunitinib alone. The difference with the latter could be explained by the shorter use of sunitinib due to earlier progression in the monotherapy trial.

The mOS of 24 months is worth mentioning; phase III trials showing significant benefits of some active drugs in STS second line reported mOS ranging from 12.5 to 13.5 months. It will be important to analyze postprotocol therapies and microenvironment transformation after the immunomodulatory treatment since the clinical impact of the sunitinib and nivolumab scheme could also have an influence on subsequent postprotocol therapies. We have analyzed the potential bias of selecting some more indolent histologies or those histologies more sensitive to antiangiogenics as the reason for this prolonged survival, but we did not find significant differences in survival between groups.

The 6-month PFSR of 48% and the mOS of 24 months showed in our trial favorably compare with the activity reported with anti-PD-1 or sunitinib in monotherapy in previous STS trials. The SARC028 study reported 32% and 11.4 months for 6-month PFSR and mOS, respectively, in 42 patients with STS treated with pembrolizumab. Nevertheles, the SARC028 study selected the four more frequent histotypes for the STS cohort, making comparison with our study difficult since only synovial sarcoma and UPS were common histologies in both trials. The Alliance-091401 trial reported 15% and 10.7 months, respectively, for the same indicators in 43 patients with STS treated in the monotherapy arm with nivolumab. Another study was prematurely closed, with 12 patients with uterine leiomyosarcoma treated with nivolumab, after observing an mPFS of 1.8 months at the interim analysis and with no patient free of progression at 6 months. Sunitinib was also trialed as monotherapy in a phase II study of 50 patients with STS, reporting a 6-month PFSR of 22% with an mPFS of 1.8 months, while OS data were not provided. Of note, the GMI in our study is comparable with those reported with active chemotherapy in second line, and the median elapsed time of 1.17 months between previous progression and enrollment reinforces the outcome in terms of PFS. Furthermore, the GMI in the subset of subtypes with higher potential sensitivity to antiangiogenics was even better. A limitation of this study is inherent to design assumption: it is not possible to distinguish separately the efficacy of antiangiogenic or anti-PD-1. The differences detected between immunomodulation with combination approach and monotherapy underline the feature that STS exhibits, in general, a cold immune microenvironment. In line with this, a combined immunotherapy approach tried to convert...
a cold into inflamed microenvironment in sarcomas. Thus, the combination arm of Alliance-091401 addressed a double immune-checkpoint inhibition with nivolumab plus ipilimumab. Patients in the combination arm had a 6-month PFSR of 28% and a mOS of 14.3 months, all the efficacy indicators being superior to monotherapy arm. The lower 6-month PFSR and the shorter OS in the combination arm in comparison with our trial could be explained, at least partially, by the restriction in the number of ipilimumab cycles (four at maximum). Conceptually similar to our study, the administration of antiangiogenic plus anti-PD-1 agents was explored in a single-center phase II trial with axitinib plus pembrolizumab. The 6-month PFSR was 46.9% and the mOS was 18.7 months, which were both closely similar to our study. A substantially different aspect was based on the proportion of ASPS; while in the axitinib-pembrolizumab 36% of the patients had this subtype, in our trial only 7% had this diagnosis. Thus, for the non-ASPS cases of that trial, the mPFS was 3 months, the 6-month PFSR was 38.1%, the mOS was 13.1 months, while no patient survived beyond 2 years. All these parameters seemed to be worse than ours, although limited by indirect comparisons. The indolence and special sensitivity of this subtype to both antiangiogenic and immunomodulation could have influenced the survival results. In our series, four of seven accrued patients with ASPS (considering both phases) had partial response (57%), whereas in a randomized phase II trial exploring cediranib versus placebo in ASPS the authors reported responses in 6 of 31 (19%) patients.29 In fact, excluding ASPS from the analysis of PFS due to its inherent indolence, we were not able to find statistical differences between potential antiangiogenic sensitive subtypes and others. In other words, ASPS cases could represent bias for the main endpoint of 6-month PFSR. Intriguingly, ASPS does not exhibit some features of the classic immune sensitive microenvironment; thus, the proportion of tumor infiltrating lymphocytes, or specifically CD8+ or FOXP3+ subpopulations, per cubic millimeter is lower in ASPS than in non-translocation-related sarcomas. Additionally, TMB is lower in ASPS compared with other sarcomas, such as synovial sarcoma or Ewing sarcoma. The activity of anti-PD-1 in ASPS has been related with molecular mismatch-repair deficiency signature, even in the absence of high TMB, but a broader number of cases to confirm this point will be required.31

The proportion of responding patients, 13% in phase II or 21% joining STS evaluable patients from phase Ib and II of our trial, is similar to the 18% reported with pembrolizumab in the SARCO28 study. This could be explained by STS heterogeneity, even within specific subtype; for instance, from seven evaluable patients with UPS in our phase II, only two had stabilization and five had progressive disease, with an mPFS of 1.8 months. By contrast, in the SARCO28 study, from nine patients with UPS, four responded, three were stable and two progressed, with an mPFS of 7 months. This emphasizes the concept of the relevance of the microenvironment in the immunomodulation results in sarcoma. A potential molecular prognostic signature with impact on survival has been proposed in our exploratory translational research. Of note, inflammatory processes were associated with better survival, whereas metabolic processes were linked to worse outcome. The role of metabolism in tumor immune evasion has been previously described.32 33 Also, the significant prognostic value of PDGFD or IL16 overexpression in longer survival, shown in online supplemental results, is a finding that deserves to be further explored.

Based on the findings of this study, six subtypes of sarcoma were selected for a multicohort phase II trial, which has been recently activated, with the same regimen. In the translational research, only 28 samples were used for transcriptomics, which is a limitation of this study. However, a broad comprehensive analysis of the immune populations within the tumor and their association with gene signatures and clinical outcomes is currently ongoing and expected to be published as a separate correlative study. Sunitinib inhibits several tyrosine kinase receptors (TKR), including angiogenic (eg, Vascular Endothelial Growth Factor Receptor (VEGFR) types 1 and 2, and PDGFR-α and PDGFR-β), pro-oncogenic (eg, fms like tyrosine kinase 3 (FLT3), rearranged during transfection (RET), and KIT) or immune-related pathways (eg, Colony stimulating factor 1 receptor (CSF1R)). Although the main hypothesis of this study is to convert the sarcoma cold microenvironment into an inflamed one, by inhibiting immunosuppression driven by angiogenesis, we cannot discard the potential added value of targeting other TKRs. In fact, it seems that CSF1 expression has been described as an adaptive mechanism of resistance to anti-PD-1 inhibition in melanoma, while the combination of anti-CSF1R and anti-PD-1 inhibitions was demonstrated to be active in BRAFV600E-driven, transplant mouse melanomas. The efficacy of this combination was dependent on the elimination of tumor-associated macrophages (TAMs).34 Likewise, it has been reported that the combination of CSF1R inhibitor with anti-PD-L1/PD-1 axis blockade is also highly active in hepatozellcular carcinoma mouse models, increasing intratumoral CD8+ T cells infiltrate and reducing TAMs.35 Translational and preclinical studies will also address the potential role of targeting these TKRs for anti-PD-1/PD-L1 axis inhibition efficacy in STS.

In conclusion, our study supports the idea that the PD-1/PD-L1 axis inhibition per se is not enough in the context of STS. Combination strategies promoting inflamed microenvironment resulted in a higher efficacy in this setting. Targeting new immune-checkpoint inhibitors and selecting histological subtypes could also be advantageous in future trials. Correlative studies searching predictive signatures will allow a better selection of patients for immunotherapy.

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Patient consent for publication

Not required.

Ethics approval

Study procedures were in accordance with the guidelines established by the ethics committees of each hospital and with the Declaration of Helsinki. Approval was obtained from the ethics committees.

Provenance and peer review

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Data availability statement

Raw data from HTG Molecular OBP panel experiments are available in the Gene Expression Omnibus (GEO) from NCBI.

Supplemental material

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