Phase II Clinical Trial of Eribulin–Gemcitabine Combination Therapy in Previously Treated Patients With Advanced Liposarcoma or Leiomyosarcoma

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ABSTRACT

Purpose: Monotherapy with eribulin or gemcitabine has been found to be moderately effective in treating soft-tissue sarcomas (STS). In this study, we evaluated the efficacy and safety of eribulin–gemcitabine combination therapy for the two most common histologic types of STS, liposarcoma and leiomyosarcoma.

Patients and Methods: In this nonrandomized, multicenter, phase II study, we included patients with progressive disease who had received one or two courses of chemotherapy that included doxorubicin. Patients were administered 1.4 mg/m^2 eribulin and 1,000 mg/m^2 gemcitabine on days 1 and 8 every 3 weeks. The primary endpoint was progression-free survival rate at 12 weeks ($PFSR_{12wks}$), with null and alternative hypotheses of $PFSR_{12wks}$ ≤20.0% and ≥40.0%, respectively. Exploratory biomarker analyses with next-generation sequencing (NGS) were performed on pretreatment tumor samples.

Results: Among the 37 patients included, the overall $PFSR_{12wks}$ was 73.0%, achieving the primary endpoint. The objective response rate, disease control rate, median progression-free survival, and median overall survival were 16.2%, 78.4%, 5.6 months, and 31.9 months, respectively, without differences according to histologic type. New safety signals and treatment-related deaths were not documented. NGS-based transcriptome analysis revealed that functional enrichment in the TGFβ pathway was mostly associated with a poor outcome, whereas single genetic alterations largely failed to predict treatment outcome.

Conclusions: Eribulin–gemcitabine combination therapy showed promising activity and an acceptable safety profile in patients with liposarcoma or leiomyosarcoma. Gene expression profiling with pathway enrichment analysis would have possibilities to have predictive value for survival outcome, necessitating further investigation to confirm.

Introduction

Soft-tissue sarcomas (STS) are rare solid tumors that account for approximately 1% of malignancies in adults. They have over 100 histologic subtypes and predominately occur in the trunk, extremities, and retroperitoneum (1). Radical surgical resection is the cornerstone of curative treatment for localized disease (2, 3). Despite adequate surgical resection, most patients with STS remain at high risk for local and systemic recurrence (2, 3). Initial treatment for unresectable metastatic STS includes standard cytotoxic chemotherapy comprising anthracyclines with or without ifosfamide (4). Second-line therapies administered after the failure of first-line chemotherapy include ifosfamide, dacarbazine, gemcitabine, and taxanes (5). Trabectedin and pazopanib also demonstrated meaningful activity for advanced STS based on randomized phase III studies (6, 7).

In the last decade, there has been an effort to improve the quality of care for patients with STS based on the anatomic site and histologic type, and multiple ongoing clinical trials are focusing on tailoring therapy based on histologic type (8, 9). The most common histologic types of STS in adults include liposarcoma and leiomyosarcoma (collectively referred to as L-sarcomas), both of which are associated with distinct clinical and biologic features (10). Eribulin inhibits microtubule dynamics via a mechanism distinct from that of other tubulin-targeting drugs, thereby inducing cell-cycle arrest and vascular remodeling and reversing epithelial–mesenchymal transition (11). It has been approved for use in patients with metastatic breast cancer

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Translational Relevance

Effective systemic treatment options are largely limited for previously treated liposarcoma and leiomyosarcoma. In this phase II study, the eribulin–gemcitabine combination therapy demonstrated appreciable efficacy compared with monotherapy from the historic arm. Functional enrichment in the TGFβ pathway would hold predictive value for the survival outcomes of the combination therapy, although further confirmatory investigation may be required. Eribulin–gemcitabine combination therapy could present as a future treatment option for refractory soft-tissue sarcomas with liposarcoma or leiomyosarcoma histology.

previously treated with chemotherapy (12, 13). The safety and efficacy of eribulin in patients with STS have been also explored. In a single-arm phase II study, 31.6% (12/38) and 46.9% (15/32) of patients with leiomyosarcoma and liposarcoma, respectively, were progression-free at 12 weeks after the start of therapy, and eribulin was associated with a manageable tolerability profile (14). Based on these encouraging results, a phase III study comparing eribulin with dacarbazine was conducted, demonstrating a significantly longer median overall survival (OS) for eribulin than for dacarbazine (15). This survival benefit was particularly evident in patients with liposarcoma (16), and eribulin is currently considered a standard treatment option for pretreated patients with advanced liposarcoma (2, 5). However, monotherapy is generally associated with low response rates, which are associated with low progression-free survival (PFS) and OS. Phase II and III studies have reported objective response rates of eribulin of 2.9% (3/70) and 4.0% (9/228), respectively (14, 15).

Gemcitabine, a pyrimidine analogue, has shown limited efficacy in the treatment of metastatic STS (17). Therefore, it is often administered in combination with chemotherapeutic agents of other classes, including docetaxel (18), dacarbazine (19), and vinorelbine (20). Preclinical studies demonstrated the potential synergistic effect of eribulin–gemcitabine combination therapy (21). Notably, a phase I study showed favorable safety and efficacy of eribulin–gemcitabine combination therapy in 21 pretreated patients with advanced solid tumors, providing evidence for its use (22). However, the clinical effects of eribulin–gemcitabine combination therapy have not been investigated in patients with STS.

In this study, we aimed to investigate the efficacy and safety of eribulin–gemcitabine combination therapy in patients with pretreated liposarcoma and leiomyosarcoma, which are the two most common histologic types of adult STS. The primary endpoint was the PFS rate at 12 weeks after starting therapy ($PFSR_{12wks}$), which has commonly been used in prospective studies of patients with STS. In addition, we performed biomarker analysis based on next-generation sequencing (NGS) for DNA and RNA to assess whether these tests have predictive value for the outcomes of combination treatment. We ultimately intended to provide viable and valuable options for patients with advanced liposarcoma and leiomyosarcoma with genomic correlates.

Patients and Methods

Patients

In this nonrandomized, multicenter, phase II study, we included adult patients (age ≥18 years) with histologically proven advanced or metastatic liposarcoma or leiomyosarcoma; an Eastern Cooperative Oncology Group performance status of 0 to 2; adequate renal, liver, and bone marrow function [hemoglobin ≥ 9.0 g/dL; absolute neutrophil count ≥1,000 cells/μL; platelet count ≥ 75,000 cells/μL; bilirubin concentration ≤1.5 × upper limit of normal (ULN) or ≤2 × ULN in patients with liver metastasis; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations ≤3 × ULN or ≤5 × ULN, respectively, in patients with liver metastasis; alkaline phosphatase concentrations ≤3 × ULN or ≤5 × ULN in patients with liver or bone metastasis; and calculated creatinine clearance > 60 mL/minute]; and a history of one or two courses of chemotherapy including doxorubicin. Patients who had received more than three courses of cytotoxic chemotherapy and those with uncontrolled active central nervous system metastasis and/or carcinomatous meningitis, known hypersensitivity to administered drugs, or preexisting neuropathy of grade 2 or higher (as assessed using the NCI Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.03] were not enrolled. This study was approved by ethics committees and institutional review boards of all participating institutions (Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, Republic of Korea and Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea). All patients provided written informed consent. This study was conducted in accordance of Declaration of Helsinki.

Treatment

On days 1 and 8 of a 21-day cycle, 1.4 mg/m^2 of eribulin was administered as an intravenous bolus for 5 minutes, following which 1,000 mg/m^2 of gemcitabine was intravenously administered for 30 minutes. Doses were reduced or delayed in subsequent cycles for certain patients depending on toxicity. Intrapatient dose escalation was not allowed. Treatment was continued until any of the following occurred: disease progression, an intercurrent illness that prevented additional treatment, treatment delay of greater than 3 weeks for any reason, unacceptable adverse event(s), general or specific changes in the patient's condition that rendered additional treatment unacceptable (as judged by the investigator), or withdrawal of patient consent for treatment.

Response and safety evaluation

At the time of enrollment, patients were required to have measurable disease according to the RECIST version 1.1 (23), as in other studies (24–27). All patients underwent radiographic evaluation and tumor measurements at baseline and after every two treatment cycles (every 6 weeks). Confirmatory scans were performed at ≥4 weeks after initial documentation of an objective response. After 12 weeks, radiologic evaluation and tumor measurements can be extended from 6 weeks to 9 weeks at the discretion of the investigators. Treatment response was classified based on the RECIST version 1.1 as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). The objective response rate (ORR) was defined as the summation of CR and PR. Disease control rate (DCR) was defined as the summation of CR, PR, and SD. Toxicity was graded using the NCI-CTCAE version 4.03. Physical examinations, laboratory tests, and toxicity assessments were performed at baseline and before each treatment.

Tumor sample collection and NGS

Tumor samples were collected before initiation of eribulin–gemcitabine combination treatment. If the tumor content of the sample was estimated to be ≥40% on pathologic examination, targeted DNA and RNA sequencing (RNA-seq) were performed using TruSight Tumor 170 or 500 (Illumina). We extracted 40 ng DNA and RNA from

formalin-fixed, paraffin-embedded (FFPE) tissue using a Qiagen All Prep DNA/RNA FFPE kit (Qiagen). Library preparation was performed according to the manufacturer's instructions. After hybridization capture-based target enrichment, pair-ended sequencing (2 × 150 bp) was performed using a NextSeq sequencer (Illumina) according to the manufacturer's instructions.

Genetic alteration analysis

The raw DNA sequencing file was aligned to the human genome reference build Genome Research Consortium human build 38 (GRCh38) using Genome Analysis Toolkit (28). The tag containing the unique molecular identifiers information was marked using an in-house script. Mark duplicate process was performed using UmiAwareMarkDuplicatesWithMateCigar (Picard). All variants from Mutect2 were filtered using the following in-house filtering criteria: (i) total depth <100, (ii) variant allelic frequency <10%, (iii) combined annotation dependent depletion phred score <25, and (iv) minor allele frequency >0.1% in the gnomAD database for the global and East Asian populations (29). The pathogenicity of the filtered variants using haplotype caller was determined as per the recommendations of the American College of Medical Genetics and Genomics and Association for Molecular Pathology (30). We used CNVkit to analyze copy-number variations (CNV; 31). We only selected CNVs that had been previously reported in patients with liposarcoma and leiomyosarcoma. Gene-fusion candidates were analyzed using Star-fusion (32). A fusion expression filter was applied to the initial calls (fusion fragments per million total RNA-seq fragments < 0.5). An oncoplot was constructed using MAFtools (33).

Gene expression and transcriptome analysis

For RNA-seq, we applied the new Tuxedo protocol (34). Raw sequencing files were analyzed using HISAT2 and Stringtie. Raw counts were assigned based on Ensembl human gene annotations using Stringtie (35). The principal components and differentially expressed genes were analyzed using the DEseq2 R package (36). Raw fragments per kilobase of exon per million were calculated using the Ballgown R package (34). The enrichment scores for each pathway were calculated using the gene-set variation analysis (GSVA) R package (37).

Statistical analysis

The primary endpoint of the trial was $PFSR_{12wks}$, which was measured as a binary variable. This endpoint was assessed in all eligible patients who received treatment. Secondary endpoints included OS, PFS, response to therapy according to the RECIST version 1.1, onset time, response duration, and incidence and severity of adverse events according to the NCI-CTCAE version 4.03. Treatment was regarded as a success if a radiologic assessment performed at least 12 weeks after the start of therapy indicated SD, PR, or CR; all other outcomes were considered failures.

Based on the results of a previous retrospective analysis of the European Organization for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group database, we used a minimax two-stage study design (38). The null hypothesis (P0) was that the $PFSR_{12wks}$ would not warrant further investigation (P0 ≤ 20%), and the alternative hypothesis (P1) was that the $PFSR_{12wks}$ would warrant further investigation (P1 ≥ 40%) after treatment completion. Applying these hypotheses with $\alpha = 0.05$ (to account for type I error) and $\beta = 0.2$ (to account for type II error), we assumed that further testing would be warranted if there were ≥5 cases of successful treatment in the first 18 patients enrolled in the first stage. If ≥11 of 33 patients were progression-free or alive after 12 weeks of treatment, an additional investigation was considered to be warranted.

Categorical and continuous variables were compared using Fisher exact test and the Wilcoxon rank–sum test, respectively. PFS and OS were defined based on the time from initiation of treatment until evidence of disease progression or death from any cause. Survival was plotted using Kaplan–Meier curves and compared using the log–rank test. The censoring date was July 31, 2021. Statistical analyses were performed with SPSS version 26 (IBM Corp.) and GraphPad Prism version 6 (GraphPad Software).

Data availability

Targeted sequencing data have been deposited into the NCBI Sequence Read Archive with accession number PRJNA833874 (https://www.ncbi.nlm.nih.gov/sra). All other data generated in this study are available from the corresponding authors (H.S. Kim and J.-H. Ahn) upon reasonable request.

Results

Patient characteristics

Between March 2018 and September 2020, 37 patients were enrolled at two tertiary centers in Korea, all of whom were assessable for efficacy and safety. Baseline patient characteristics are listed in **Table 1**. The number of patients with leiomyosarcoma and liposarcoma was 20 (54.1%) and 17 (45.9%), respectively. The histopathologic subtypes of liposarcoma were as follows: dedifferentiated liposarcoma ($n = 11$, 64.7%), myxoid liposarcoma ($n = 5$, 29.4%), and pleomorphic liposarcoma ($n = 1$, 5.9%). The sites of origin of leiomyosarcoma were as follows: gynecologic system ($n = 10$, 50.0%), retroperitoneal area ($n = 6$, 30.0%), thorax ($n = 2$, 10.0%), gastrointestinal system ($n = 1$, 5.0%), and lower extremities ($n = 1$, 5.0%). Most patients had distant metastasis, with the lung being the most frequent site ($n = 28$, 75.7%), followed by the intraperitoneal area/pelvic cavity ($n = 24$, 64.9%), liver and lymph nodes ($n = 10$, 27.0%), and bone and muscle ($n = 9$, 24.3%). Most of the patients had previously undergone curative or palliative surgery ($n = 33$, 89.2%), and approximately half of them had received radiotherapy. Eribulin and gemcitabine had been administered as a second- or third-line palliative treatment in 25 (67.6%) and 12 (32.4%) patients, respectively. All patients had previously received doxorubicin, and more than half of them ($n = 20$, 54.1%) had received ifosfamide.

Clinical outcome

After a median follow-up of 20.1 months [95% confidence interval (CI), 16.9–24.4 months), 5 patients (13.5%) did not experience progression including 3 patients (8.1%) on active treatment, while 16 (43.2%) patients died (Supplementary Fig. S1). $PFSR_{12wks}$ in the overall patients were 73.0% (27/37; 95% CI, 58.0–88.0) with 75.0% (15/20) and 70.6% (12/17) for leiomyosarcoma and liposarcoma, respectively (**Fig. 1A**). Among patients with leiomyosarcoma, those with tumors arising from the female reproductive tract had a $PFSR_{12wks}$ of 80.0% (8/10), whereas the others had a $PFSR_{12wks}$ of 70.0% (7/10). Among patients with liposarcoma, patients with dedifferentiated liposarcoma had a $PFSR_{12wks}$ of 63.6% (7/11), whereas the others had a $PFSR_{12wks}$ of 83.3% (5/6). Median PFS was 5.6 months (95% CI, 2.5–8.6 months) in the overall patients, with 4.6 months and 5.7 months for leiomyosarcoma and liposarcoma, respectively (**Fig. 1B**). Median OS was 31.9 months (**Fig. 1C**; 95% CI, 16.0–47.9), without significant differences according to histologic subtypes as observed in the case of PFS.

Table 1. Baseline characteristics of patients according to histology.

	Total ($N = 37$)	Leiomyosarcoma ($N = 20$)	Liposarcoma ($N = 17$)	*P*
Age	56 (36–74)	52 (36–74)	60 (40–74)	0.635
Sex				0.021
Male	14 (37.8%)	4 (20.0%)	10 (58.8%)	
Female	23 (62.2%)	16 (80.0%)	7 (41.2%)	
Primary site				0.124
Thorax	2 (5.4%)	2 (10.0%)	0 (0.0%)	
Retroperitoneum	16 (43.2%)	6 (30.0%)	10 (58.8%)	
Gastrointestinal tract	3 (8.1%)	1 (5.0%)	2 (11.8%)	
Gynecological organ	13 (35.1%)	10 (50.0%)	3 (17.6%)	
Lower extremity	3 (8.1%)	1 (5.0%)	2 (11.8%)	
Histologic subtype of liposarcoma				—
Dedifferentiated	11 (29.7%)	—	11 (64.7%)	
Myxoid	5 (13.5%)	—	5 (29.4%)	
Pleomorphic	1 (2.7%)	—	1 (5.9%)	
Previous curative surgery				0.048
Yes	29 (78.4%)	13 (65.0%)	16 (94.1%)	
No	8 (21.6%)	7 (35.0%)	1 (5.9%)	
Previous perioperative radiotherapy				1.000
Yes	10 (27.0%)	5 (25.0%)	5 (29.4%)	
No	27 (73.0%)	15 (75.0%)	12 (70.6%)	
Previous palliative surgery				0.022
Yes	16 (43.2%)	5 (25.0%)	11 (64.7%)	
No	21 (56.8%)	15 (75.0%)	6 (35.3%)	
Previous palliative radiotherapy				1.000
Yes	13 (35.1%)	7 (35.0%)	6 (35.3%)	
No	24 (64.9%)	13 (65.0%)	11 (64.7%)	
Total line of previous treatment				1.000
1	25 (67.6%)	13 (65.0%)	12 (70.6%)	
2	12 (32.4%)	7 (35.0%)	5 (29.4%)	

Measurable tumor shrinkage and RECIST responses were seen regardless of histologic type (**Fig. 2A–B**). The overall ORR was 16.2% (6/37; 95% CI, 3.8–28.7) with 20.0% (4/20) and 11.8% (2/17) for leiomyosarcoma and liposarcoma, respectively. The overall DCR was 78.4% (29/37; 95% CI, 64.5–92.3) with 80.0% (16/20) and 76.5% (13/17) for leiomyosarcoma and liposarcoma, respectively.

Safety and tolerability

The most commonly observed adverse events were neutropenia ($n = 34$, 91.9%), nausea ($n = 14$, 37.8%), thrombocytopenia ($n = 12$, 32.4%), anemia ($n = 9$, 24.3%), myalgia ($n = 9$, 24.3%), and anorexia ($n = 8$, 21.6%; **Table 2**). 26 (70.3%) patients experienced at least one event of grade 3 or 4 toxicity, including neutropenia ($n = 26$, 70.3%), thrombocytopenia ($n = 6$, 16.2%), and anemia ($n = 4$, 10.8%). Grades 3 and 4 febrile neutropenia occurred in 2 (5.4%) and 0 (0.0%) patients, respectively. There were no treatment-related deaths.

At the time of analysis, 334 cycles of eribulin–gemcitabine combination therapy had been administered, with a median of seven cycles per patient (range 1–24). 15 patients (40.5%) received eribulin–gemcitabine combination therapy at full dose without delay. Dose reduction was performed in 22 patients (59.5%). 10 patients (27.0%) experienced both dose delays and reductions, and most dose modification events among them were due to hematologic adverse events ($n = 28$; 87.5%). The median treatment duration was 4.6 months (range: 0.3–21.9 months). The average relative dose intensities of eribulin and gemcitabine were 85.1% (95% CI, 80.2–90.1) and 90.2% (95% CI, 86.5%–93.9), respectively. No patient discontinued treatment because of treatment-related adverse events; most patients discontinued treatment due to disease progression (31/32; 96.9%), and 1 patient (1/31; 3.1%) withdrew consent.

NGS-based exploratory biomarker analysis

24 (64.9%) patients underwent targeted DNA sequencing and RNA-seq using the archival tissue, and their baseline characteristics did not differ from those who did not undergo NGS (Supplementary Table S1). *CDKN2A* and *PTEN* deletion were the most frequently observed genetic alterations [10 patients (41.7%); **Fig. 3A–E**]. Alterations in *TP53* and *ATRX* were frequently observed (37.5% and 33.3% of patients, respectively), similar to previous study (10). However, no genetic alteration could predict PFS at 12 weeks (Supplementary Table S2). Transcriptome analysis revealed differential transcriptional profiles according to histologic subtypes based on unsupervised principal component analysis (Supplementary Fig. S2). Correspondingly, analysis of differentially expressed genes based on histologic types revealed distinct expression profiles of lineage markers for leiomyosarcoma and liposarcoma (Supplementary Fig. S2). When the expression profiles were compared between patients with and without progression within 12 weeks, we found that transcriptome enrichment of 22 pathways from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database was significantly associated with PFS at 12 weeks (**Fig. 4A**; Supplementary Fig. S3). In contrast, only one gene was a significant predictor of $PFSR_{12wks}$ (Supplementary Fig. S4). The TGFβ pathway was the most significantly associated with PFS and OS (**Fig. 4B–D**). However, transcriptome enrichment in the TGFβ

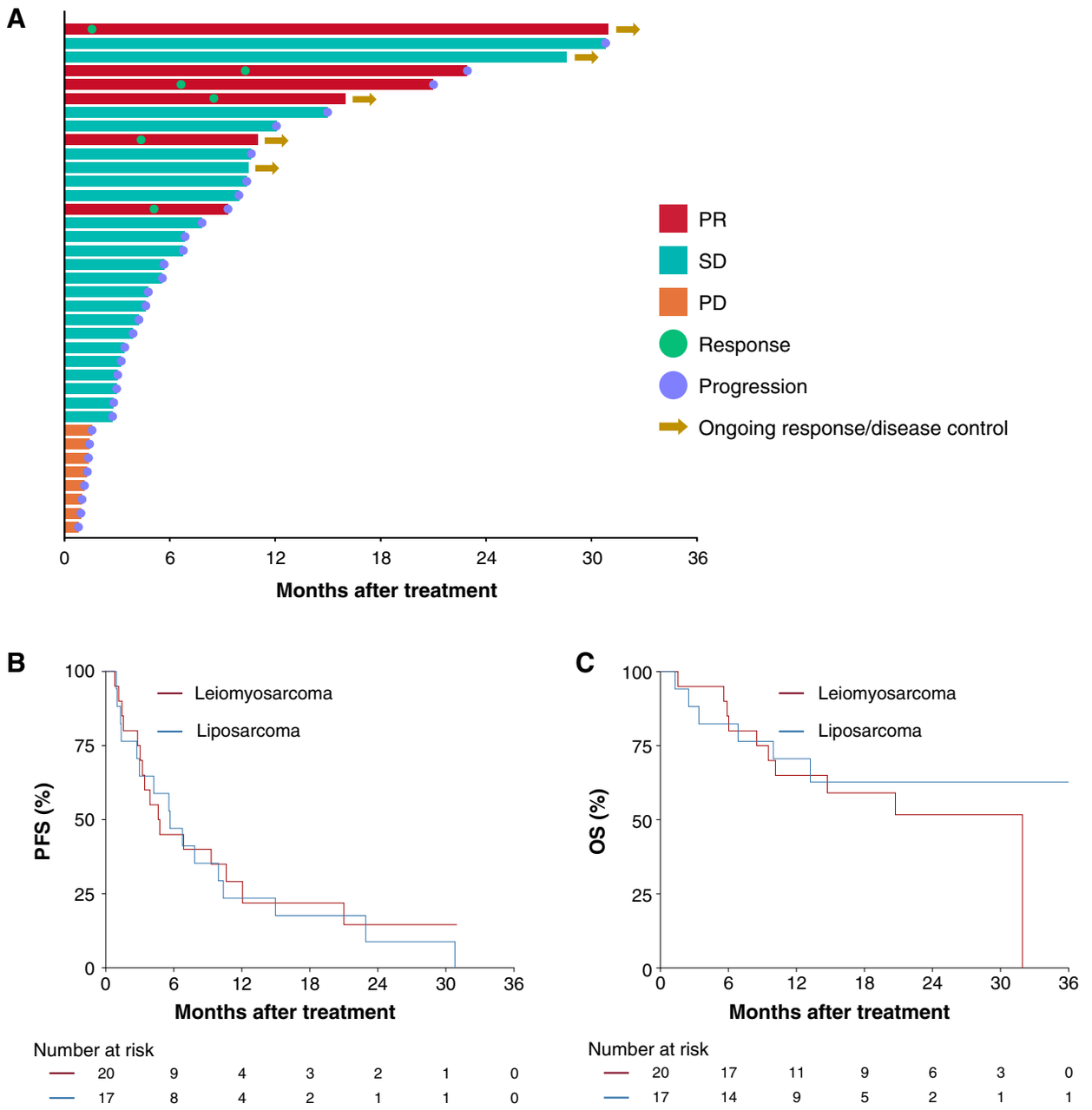

Figure 1.
Survival outcome. **A,** Swimmer plot of patients. **B,** Kaplan–Meier plot of PFS of patients. **C,** Kaplan–Meier plot of OS of patients.

pathway was not associated with OS in patients with liposarcoma or leiomyosarcoma from The Cancer Genome Atlas (TCGA) cohort (**Fig. 4E**), indicating that enrichment of the TGFβ pathway has predictive rather than prognostic value.

Discussion

This phase II study indicates that eribulin–gemcitabine combination therapy meets prespecified criteria for efficacy in previously treated patients with liposarcoma or leiomyosarcoma. The efficacy of eribulin–gemcitabine combination therapy is promising; for both liposarcoma and leiomyosarcoma, more than 70% of patients showed clinical benefit at 12 weeks, with 6 partial responders among 37 patients. The safety profiles of eribulin–gemcitabine combination treatment were consistent with those reported in previous studies and those of each agent; there were no unexpected safety signals or treatment-related deaths. To the best of our knowledge, this is the first study to demonstrate the activity and safety of eribulin–gemcitabine combination therapy for the two most common histologic types of adult STS.

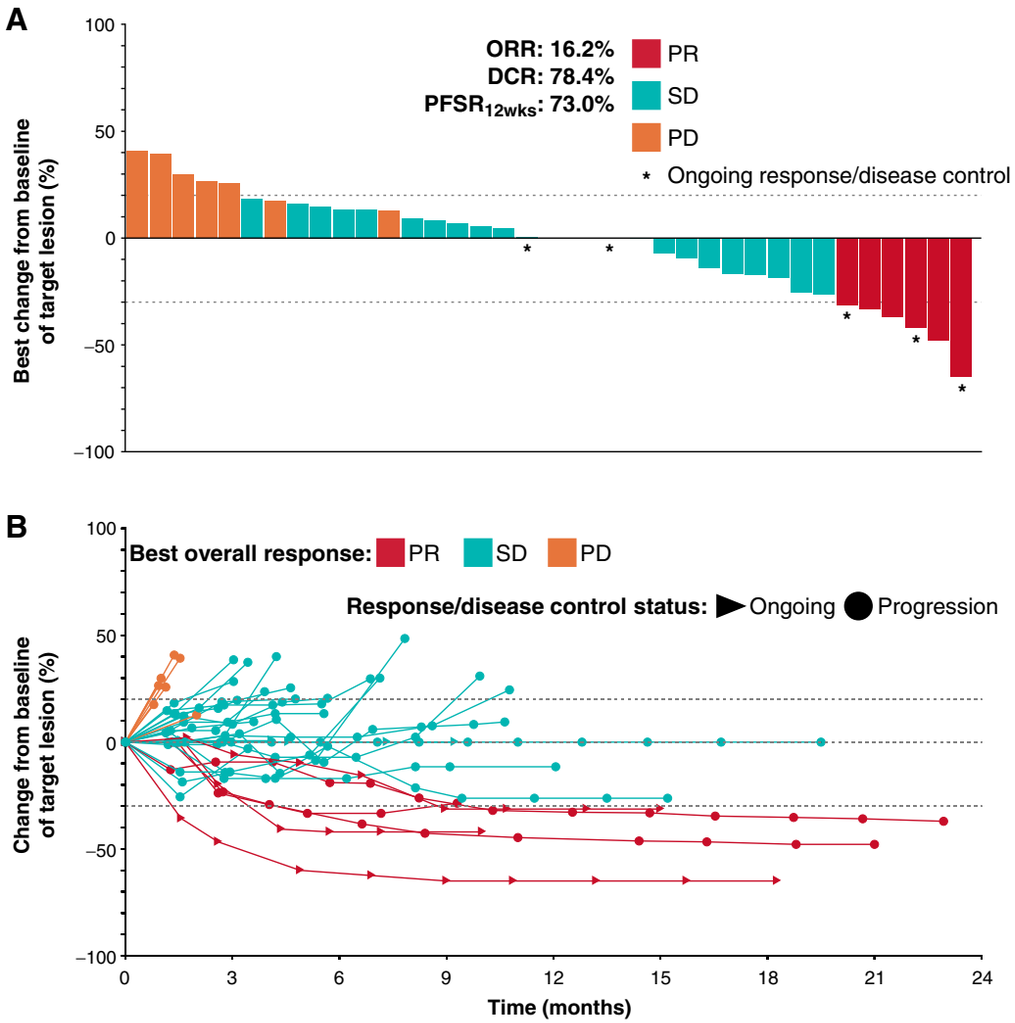

Figure 2.
Tumor reduction and response over time. **A,** Waterfall plot displaying the best percentage change from baseline in sum of target lesion diameters. **B,** Spider plot displaying the percentage change from baseline in sum of target lesion diameters over time.

Table 2. Summary of treatment-related adverse events occurring more than 5%.

Adverse event	Grade 1	Grade 2	Grade 3	Grade 4	Any grade (%)	Grade 3–4 (%)
Neutrophil decreased	0	8	16	10	34 (91.9)	26 (70.3)
Febrile neutropenia	—	—	2	0	— (—)	2 (5.4)
Nausea	11	3	0	0	14 (37.8)	0 (0.0)
Platelet decreased	3	3	3	3	12 (32.4)	6 (16.2)
Anemia	0	5	3	1	9 (24.3)	4 (10.8)
Myalgia	6	3	0	0	9 (24.3)	0 (0.0)
Anorexia	4	4	0	0	8 (21.6)	0 (0.0)
Constipation	5	2	0	0	7 (18.9)	0 (0.0)
Fatigue	4	0	0	0	4 (10.8)	0 (0.0)
Fever	4	0	0	0	4 (10.8)	0 (0.0)
Skin rash	4	0	0	0	4 (10.8)	0 (0.0)
Alopecia	2	1	0	0	3 (8.1)	0 (0.0)
ALT increased	1	1	1	0	3 (8.1)	0 (0.0)
AST increased	1	1	1	0	3 (8.1)	0 (0.0)
Cough	2	0	0	0	2 (5.4)	0 (0.0)
General weakness	1	1	0	0	2 (5.4)	0 (0.0)
Urticaria	1	1	0	0	2 (5.4)	0 (0.0)

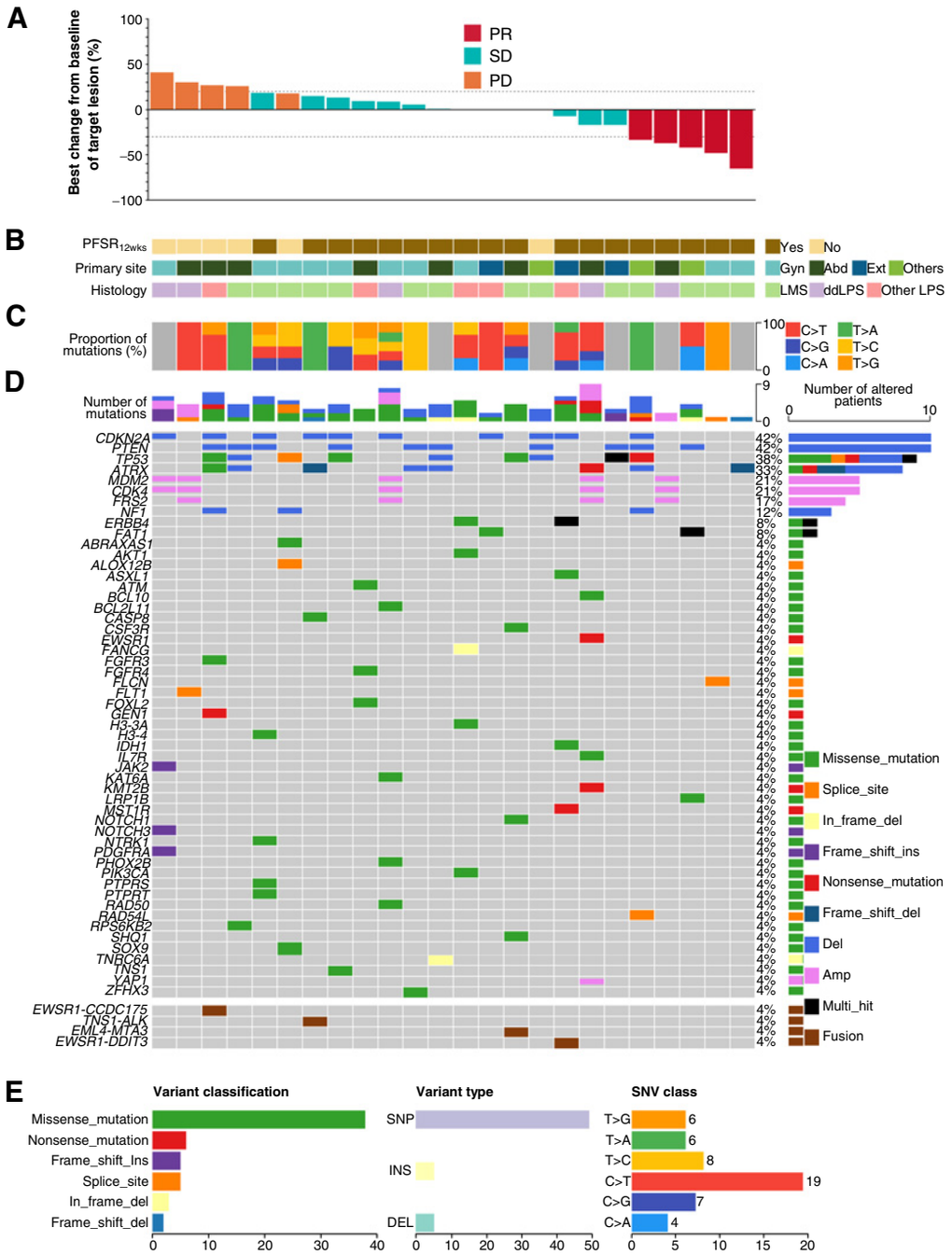

Figure 3.

Correlation of genetic alteration with response to treatment. **A,** Waterfall plot displaying the best percentage change from baseline in sum of target lesion diameters in patients with available NGS data. **B,** Clinicopathologic factors and PFS at 12 weeks after treatment. **C,** Relative proportion of base mutations. **D,** Plot showing pathogenic alterations. **E,** Distribution of variants. Abd, abdominal organ; ddLPS, dedifferentiated liposarcoma; Ext, extremitiy; Gyn, gynecologic organ; LMS, leiomyosarcoma; LPS, liposarcoma.

Traditional treatment options for STS after failure with anthracycline include ifosfamide (39), dacarbazine (40), gemcitabine (17), trabectedin (6), pazopanib (7), and eribulin (15). Gemcitabine-based combinations, especially those with docetaxel, have been used as salvage treatment (18). Furthermore, eribulin and gemcitabine combination has been shown to be effective for treating advanced breast (41) and urothelial cancer (42) with acceptable safety profiles. Based on the distinct action mechanism of eribulin and gemcitabine to arrest cell-cycle progression in the G_2–M phase (43) and G1/S phase (44), eribulin–gemcitabine combination therapy may increase

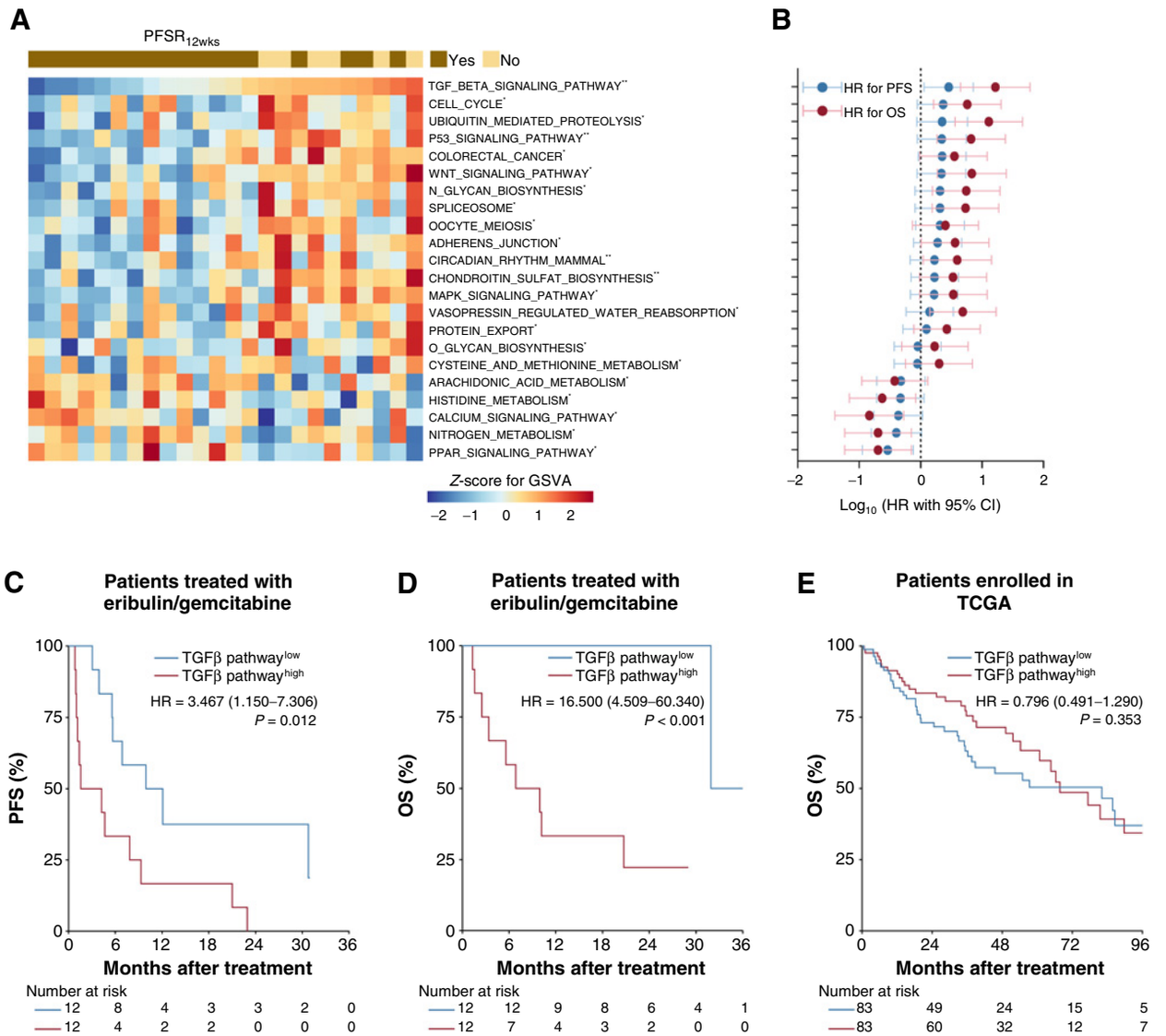

Figure 4.

Analysis for transcriptional enrichment of pathways associated with outcomes. **A,** Pathways significantly associated with disease progression within 12 weeks ($n = 22$) among pathways from the KEGG database ($n = 186$) based on GSVA. Cases were shown according to the GSVA score of the TGFβ signaling pathway, and the case with the highest GSVA score of TGFβ signaling pathways was placed on the right side. **B,** The corresponding statistics for survival among patients receiving eribulin–gemcitabine combination therapy. **C,** PFS and **D,** OS according to the GSVA score of the TGFβ signaling pathway in patients receiving eribulin–gemcitabine combination therapy (cut-off = median). **E,** OS according to the GSVA score of the TGFβ signaling pathway in patients with leiomyosarcoma and liposarcoma from TCGA cohort (cut-off = median). $^{*}P < 0.05$, $^{**}P < 0.01$.

apoptosis of cancer cells by modulating two different cell-cycle checkpoints, exerting synergistic antitumor activity. Based on this, we designed a single-arm phase II study incorporating eribulin and gemcitabine.

Although a randomized phase III study reported an OS benefit of 2 months with eribulin compared with dacarbazine (16), a lack of significant effect on PFS and low response rate (3.9%) indicated room for improvement. Although cross-trial comparisons should be interpreted with caution, our data indicate that eribulin and gemcitabine showed notable efficacy in terms of PFS (1.5 months for gemcitabine monotherapy, 2.6 months for eribulin monotherapy, and 5.6 months for combination therapy; refs. 14, 15). Specifically, pooled analysis of previous phase II and III studies on eribulin monotherapy for pretreated leiomyosarcoma and liposarcoma yielded an ORR of 4.0% (12/298) and a DCR of 56.7% (169/298), which were numerically lower than those in this study (ORR: 16.2% and DCR: 78.4%), suggesting that the antitumor activity of eribulin–gemcitabine combination therapy could be superior to that of eribulin monotherapy, resulting in a longer OS with combination therapy than with monotherapy (8.9 months for gemcitabine monotherapy, 13.5 months for eribulin monotherapy, and 31.9 months for eribulin–gemcitabine combination therapy; refs. 15, 45). However, it should be noted that several factors can be

attributed to longer median OS other than the combined effect of eribulin and gemcitabine. First, this study did not enroll patients who received more than three lines of treatment. Second, the subsequent treatment can affect OS within the context of evolving treatment landscape of STS. Third, this study was conducted only at tertiary representative centers where multidisciplinary sarcoma clinics are actively operated. Considering that this study is a small single arm phase II study with heterogenous sarcoma patient profiles, further exploration to investigate the clinical implication of eribulin–gemcitabine combination therapy is warranted.

The safety profile in this study was favorable and consistent with those of previous reports. In this study, grade 3 to 4 neutropenia occurred in 70.4% of patients, although only 2 (5.4%) patients experienced clinically significant neutropenic fever. This relatively higher incidence of grade 3 to 4 neutropenia can be attributed to frequent monitoring of blood counts per the treating physicians' discretion and a lower probability of prescription of prophylactic hematopoietic growth factor than in previous studies (18, 46). The nonhematologic toxicity of this regimen was also manageable, without any unexpected safety signals. Therefore, eribulin–gemcitabine combination therapy was safer than other commonly used combinatory regimens for metastatic STS as salvage treatments, including docetaxel and gemcitabine or ifosfamide, with a potential to maintain a high dose for a long duration.

Alterations in transcriptional networks can shape response and resistance to treatment, including chemotherapy (47). Here, activation of the TGFβ pathway was associated with poor outcomes in terms of $PFSR_{12wks}$, PFS, and OS. To further delineate predictive and prognostic value, we analyzed the survival outcomes of the TCGA liposarcoma or leiomyosarcoma cohort and found that their OS did not vary according to the magnitude of activation of this pathway. Although limited by its small sample size and exploratory nature, this experimental analysis indicated that transcriptional profiling of the TGFβ pathway holds promise as a stratification biomarker, rather than a prognostic biomarker, for treatment with eribulin and gemcitabine. In contrast, single genetic alterations were not associated with treatment outcomes, confirming the complexity of genomic characteristics of STS (10). Although the platforms for NGS used in the TCGA study differed from those used in our study, future studies with genomic profiling and treatment response assessment will encourage precision medicine and improve the chances of discovering novel targets.

In conclusion, eribulin–gemcitabine combination therapy had encouraging efficacy and an acceptable safety profile in patients with previously treated leiomyosarcoma and liposarcoma. Based on our findings, the clinical utility of eribulin and gemcitabine for treating STS should be explored in controlled trials with larger samples. Therapeutic implications of NGS should be determined to conceive strategies to identify patients who will benefit from these therapies. Further research is warranted regarding the optimal timing of eribulin and gemcitabine administration in relation to other currently available treatment options and the validity of treatment approaches based on histologic types or exploratory biomarkers.

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Authors' Contributions

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