

Pharmacotherapeutics for bone and soft tissue tumours

Edited by

Hong Duan, Wei Xu and Wenli Zhang

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Pharmacotherapeutics for bone and soft tissue tumours

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Oral vinorelbine and continuous low doses of cyclophosphamide in pediatric rhabdomyosarcoma: a real-world study

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Introduction: Metronomic maintenance therapy (MMT) has significantly improved the survival of patients with high-risk rhabdomyosarcoma in clinical trials. However, there remains a lack of relevant data on its effectiveness in real-world situations.

Methods: We retrospectively retrieved data of 459 patients < 18 years of age diagnosed with rhabdomyosarcoma at Sun Yat-sen University Cancer Center from January 2011 to July 2020 from our database. The MMT regimen was oral vinorelbine 25–40 mg/m² for twelve 4-week cycles on days 1, 8, and 15, and oral cyclophosphamide 25–50 mg/m² daily for 48 consecutive weeks.

Results: A total of 57 patients who underwent MMT were included in the analysis. The median follow-up time was 27.8 (range: 2.9–117.5) months. From MMT to the end of follow-up, the 3-year PFS and OS rates were 40.6% ± 6.8% and 58.3% ± 7.2%, respectively. The 3-year PFS was 43.6% ± 11.3% in patients who were initially diagnosed as low- and intermediate-risk but relapsed after comprehensive treatment (20/57), compared with 27.8% ± 10.4% in high-risk patients (20/57) and 52.8% ± 13.3% in intermediate-risk patients who did not relapse (17/57). The corresponding 3-year OS for these three groups was 65.8% ± 11.4%, 50.1% ± 12.9%, and 55.6% ± 13.6%, respectively.

Conclusion: We present a novel study of MMT with oral vinorelbine and continuous low doses of cyclophosphamide in real-world pediatric patients with RMS. Our findings showed that the MMT strategy significantly improved patient outcomes and may be an effective treatment for high-risk and relapsed patients.

KEYWORDS

pediatric, rhabdomyosarcoma, metronomic maintenance therapy, soft tissue sarcoma, chemotherapy

1 Introduction

Rhabdomyosarcoma (RMS), which represents a high-grade neoplasm in which cancer cells have a propensity for myogenic differentiation, is the most common soft tissue sarcoma in children and adolescents (Skapek et al., 2019). Over the last 3 decades, despite many advances with comprehensive treatment strategies involving multiple disciplines, such as chemotherapy, surgery, and radiotherapy, the chance of cure for children with recurrent and widely metastatic disease remains very low (Yohe et al., 2019). According to the risk stratification of Children's Oncology Group (COG) (Crane et al., 2022), the overall survival rate can exceed 90% among low-risk patients, approximately 70% among intermediate-risk patients, but only less than 30% among high-risk patients. Moreover, multidrug combinations or the addition of targeted therapy did not significantly improve the survival of patients with high risk and patients who were low or intermediate risk at diagnosis but had refractory or relapsed disease (Haduong et al., 2022).

Vinorelbine (VNR) has been confirmed as an effective treatment in previously treated advanced childhood sarcomas (Casanova et al., 2022). VNR and continuous low doses cyclophosphamide (CTX) showed a good response rate in relapsed, refractory, or metastatic RMS (Casanova et al., 2004; Klingebiel et al., 2008; Minard-Colin et al., 2012). Interestingly, a randomized trial revealed that the addition of metronomic maintenance therapy (MMT) with VNR plus CTX for children with high-risk RMS resulted in a significant increase in overall and event-free survival in the European paediatric Soft tissue sarcoma Study Group (EpSSG) (Bisogno et al., 2019). The introduction of maintenance chemotherapy included six cycles of intravenous VNR 25 mg/m² on days 1, 8, and 15, and daily oral CTX 25 mg/m² on days 1–28 (Bisogno et al., 2019). Patients at a high risk of relapse had a 5-year overall survival (OS) rate of 86.5% in the MMT group with manageable toxicity compared with 73.3% in the non-MMT group.

In fact, the detailed clinical application of MMT in RMS still needs to be discussed. First, more convenient drug preparations should be selected. Intravenous VNR has demonstrated encouraging results in RMS. Oral VNR appears to be a more convenient and more economical attractive candidate for the management of RMS. Previous studies have shown that oral VNR has the same pharmacokinetic-pharmacodynamic relationship as intravenous VNR (Gebbia and Puozzo, 2005). In our study, one major improvement in maintenance therapy would be the use of oral VNR instead of intravenous VNR. Second, the duration of MMT is another crucial issue. Although the duration of maintenance therapy for high-risk RMS patients was 6 months (Bisogno et al., 2019), the follow-up EpSSG PaR-RMS trial (EudraCT Number: 2018-000515-24) is investigating the role of a longer duration of MMT with CTX and VNR (randomization 6 vs. 12 months). Randomization of between 1 and 2 years of maintenance with this MMT has also been proposed for stage IV RMS. Third, which group of patients can benefit more from MMT needs to be further studied. Until now, the role of MMT has not been studied in patients who were at low and intermediate-risk at diagnosis but relapsed or had small

residual lesions at the end of treatment. Apart from high-risk patients, these patients also have a high risk of recurrence. Therefore, these patients may also benefit from maintenance treatment. Additionally, finding the right dose remains important for the successful use of MMT.

In the present study, we used oral VNR instead of intravenous VNR for a longer duration of maintenance with 1 year. We evaluated the efficacy of MMT not only in RMS patients with high risk but also in those with low and intermediate-risk at diagnosis but relapsing or with small residual lesions at the end of comprehensive treatment.

2 Patients and methods

2.1 Study population

Patients < 18 years of age diagnosed with RMS at Sun Yat-sen University Cancer Center from January 2011 to July 2020 were retrospectively identified from our database. Patients who were lost to follow-up after initial examination and treatment were excluded. The inclusion criteria of patients undergoing MMT were as follows: 1) First relapsed patients achieved clinical complete remission (cCR) or complete remission (CR) after comprehensive treatment. cCR was defined as patients who had residual lesions but with no fluorodeoxyglucose metabolism detected by PET/CT; 2) intermediate-risk patients who achieved cCR or patients with Intergroup Rhabdomyosarcoma Study (IRS) stage III who did not receive radiotherapy; and 3) high-risk patients who achieved CR or cCR after standard treatment were assigned to continue maintenance chemotherapy. Patients' data were followed up by telephone and access to outpatient and inpatient data. Patient follow-up was current through 31 December 2021. This study was approved by the Ethics Committee of Sun Yat-sen University Cancer Center (Approval Number: B2022-489-01) and conducted according to the latest version of the Declaration of Helsinki. The requirement for informed consent was waived by the institutional review committee.

2.2 Risk stratification

Risk stratification for RMS is based on a pretreatment Tumor Node Metastasis (TNM) staging system and surgical/pathologic clinical grouping system. Patients were divided into low-risk, intermediate-risk, and high-risk groups according to COG risk stratifications (Rudzinski et al., 2015). In our study, patients in low-risk Subset A and Subset B were included in the low-risk group.

2.3 Treatment protocol

All patients received chemotherapy, surgery, and/or local radiotherapy, followed by MMT (Table 1). Chemotherapy regimens were administered alternately at 3-week intervals. Patients who relapsed were given multiple cycles of chemotherapy with different drug combinations proven to be effective at present in combination with surgery (if surgical

TABLE 1 Chemotherapy regimens for RMS.

Chemotherapy regimens	Drugs dosage and administration
Low-risk group	VCR1.5 mg/m ² /d (≥ 2 mg), iv, d1
VAC 8 cycles	ACT-D 45 ug/kg/d (≥ 2,500 ug), iv drip, d1 CTX 1.2 g/m ² /d, iv drip, d1
Intermediate-risk group	CTX 1.0 g/m ² /d, iv drip, d1
CAV	THP 50 mg/m ² /d, iv, d1
(Cycle 1, 3, 5, 7, 9)	VCR1.5 mg/m ² /d (≥ 2 mg), iv, d1
I.E.,	IFO 1.5 g/m ² /d, iv drip, d1-5
(Cycle 2, 4, 6, 8, 10)	Etoposide 100 mg/m ² /d, iv drip, d1-5
High-risk group	CTX 1.0 g/m ² /d, iv drip, d1-2
CAV	THP 50 mg/m ² /d, iv, d1
(Cycle 1, 3, 5, 7, 9, 11 and 13)	VCR1.5 mg/m ² /d (≥ 2 mg), iv, d1
I.E.,	IFO 1.8 g/m ² /d, iv drip, d1-5
(Cycle 2, 4, 6, 8, 10, 12 and 14)	Etoposide 100 mg/m ² /d, iv drip, d1-5
During radiotherapy ^a	VCR1.5 mg/m ² /d (≥ 2 mg), iv, d1
VI 2 cycles	Irinotecan 50 mg/m ² /d, iv drip, d1-5

^aDuring radiotherapy, the VI, regimen was administered concurrently for sensitization, and 2 cycles of VI, were not included in the total cycles of treatment.

Mesna will be used with cyclophosphamide and ifosfamide.

VAC: vincristine (VCR), actinomycin-D (Act-D), and cyclophosphamide (CTX); CAV: cyclophosphamide, pirarubicin (THP), and vincristine; I.E.: ifosfamide (IFO), and etoposide; VI: vincristine, irinotecan.

resection was possible after assessment by the surgeon) and radiotherapy (if radiotherapy was possible after assessment by the radiologist), followed by MMT after cCR or CR was achieved.

Despite the reliance on low doses, right dosing remains important for successful use of MMT. A minimum level of exposure to anticancer agents is essential to obtain a meaningful clinical effect. Considering the convenience of drug use for children, we have given a dosage selection range to facilitate the cutting and rounding of tablet or capsule drugs. The MMT regimen was oral VNR 25–40 mg/m² for twelve 4-week cycles on days 1, 8, and 15, and oral CTX 25–50 mg/m² daily for 48 consecutive weeks. Patients in the intermediate-risk and high-risk groups generally underwent surgery in the 10th week and radiotherapy in the 16th week; however, radiotherapy within the 12th week was considered for high-risk patients with parameningeal lesions or central system involvement. The starting time of MMT was the time when peripheral blood leukocytes reached $3 \times 10^9/L$ or neutrophils reached $1 \times 10^9/L$ after the end of treatment. The duration of MMT was 48 weeks (if tolerated) or until disease progression, relapse, or metastasis. Disease assessment was performed by computed tomography or magnetic resonance imaging every 3 months during MMT.

2.4 Statistical analysis

Progression-free survival (PFS) was defined as the time from the start of MMT to the occurrence of disease progression or all-cause death or time of last follow-up if no event had occurred. OS was defined as the time from the start of MMT to all-cause death or last

follow-up. PFS and OS were censored at the date of the last follow-up visit. Survival analysis was performed using the Kaplan-Meier method, and data were compared using the log-rank test. Statistical analyses were performed by SPSS version 26.0 and GraphPad Prism 9.0. A *p*-value of <0.05 was considered statistically significant.

3 Results

3.1 Patient characteristics

Between January 2011 and July 2020, a total of 459 patients with RMS were treated in our center, and 57 patients (12.4%) undergoing MMT were eventually included in the analysis. The patients' characteristics are summarized in Table 2. The median age of the patients was 6.6 years (range: 0.2–17.9 years). The male-to-female ratio was 1.38:1.0. ERMS was the main pathological type (70.2%). The most common primary sites were retroperitoneal (26.3%), parameningeal (24.6%), trunk (19.3%), and extremities (14.0%). Of the 459 patients, 163 were at low risk, 170 were at intermediate risk, and 124 were at high risk. Among 57 patients undergoing MMT, 14 cases were low risk at initial diagnosis but relapsed, 23 cases were intermediate risk, and 20 cases were high risk at diagnosis. Among the 20 high-risk patients receiving MMT, the most common sites of initial metastasis were lymph nodes (10/20), multiple bones (8/20), lung and bone marrow (4/20), liver (3/20), abdominal pelvis cavity (2/20), pancreas (2/20), bladder (1/20), kidney (1/20), testis (1/20), and adrenal gland (1/20).

Among the 14 patients who were low risk at initial diagnosis receiving MMT, six cases relapsed after comprehensive

TABLE 2 Patient characteristics.

Characteristic	n (%)
Sex	
Female	24 (42.1)
Male	33 (57.9)
Age at diagnosis, year	
≤1	3 (5.3)
1–9	39 (68.4)
≥10	15 (26.3)
Histology	
Alveolar	14 (24.6)
Embryonal	40 (70.2)
Spindle cell/sclerosing	0 (0)
Pleomorphic	0 (0)
NOS/unknown	3 (5.3)
FOXO1 fusion status	
Fusion-positive	3 (21.4)
Unknown	11 (78.6)
Primary size	
≤5 cm	24 (42.1)
>5 cm	25 (43.9)
Unknown	8 (14.0)
Tumor site	
Extremity	8 (14.0)
Parameningeal	14 (24.6)
Bladder/prostate	1 (1.8)
Testicle	2 (3.5)
Head and neck	4 (7.0)
Retroperitoneal	15 (26.3)
Trunk	11 (19.3)
Orbit	2 (3.5)
TNM staging	
1	7 (12.3)
2	9 (15.8)
3	25 (43.9)
4	16 (28.1)
IRS staging	
I	5 (8.8)
II	9 (15.8)
III	23 (40.4)
IV	20 (35.1)
Number of metastatic sites	
0	37 (64.9)
1	10 (17.5)
2	4 (7.0)
3	5 (8.8)
4	1 (1.8)
Radiation therapy techniques	
3DCRT	4 (7.0)
IMRT	24 (42.1)
VMAT	6 (10.5)
TOMO	7 (12.3)

NOS, not otherwise specified; TNM, tumor node metastasis; IRS, intergroup rhabdomyosarcoma study; 3DCRT, three-dimensional conformal radiation therapy; IMRT, intensity modulated radiation therapy; VMAT, volumetric modulated arc therapy.

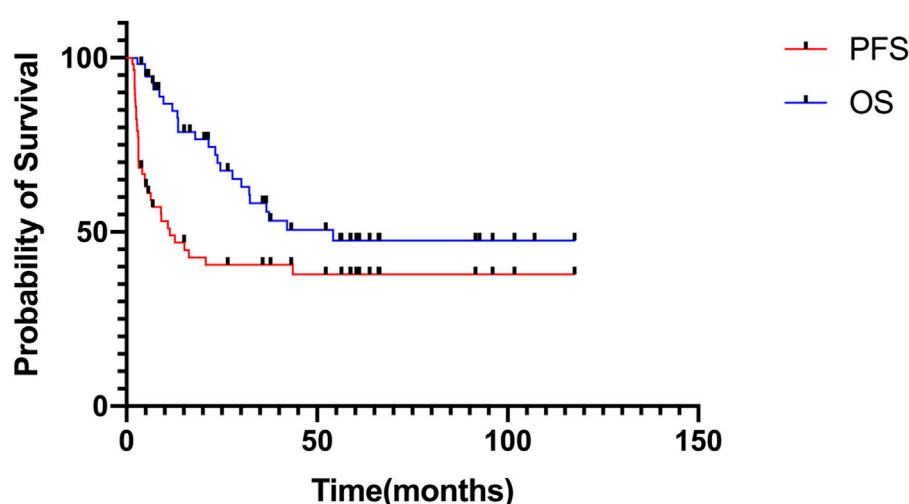


FIGURE 1

Progression-free survival and overall survival of patients receiving MMT in the entire cohort.

treatment, and eight cases relapsed without treatment after initial surgical resection. Most of the intermediate-risk patients receiving MMT were those with a poor response to standard treatment, among whom 12 patients achieved cCR at the end of comprehensive treatment, one patient relapsed without treatment after surgery, five patients relapsed after comprehensive treatment, and five patients with IRS stage III did not receive radiotherapy. In high-risk patients, MMT was generally considered. However, MMT was not administered to all high-risk patients, especially those with poor chemotherapy tolerance. Additionally, MMT is not currently recognized as part of standard care, so the preferences of physicians and patients greatly influence the selection of high-risk patients. All patients achieved cCR or CR after comprehensive therapies at the time of enrollment.

3.2 Treatment outcome

The median follow-up time was 27.8 months (range: 2.9–117.5 months). In the entire cohort, the 3-year PFS and OS rates were $40.6\% \pm 6.8\%$, and $58.3\% \pm 7.2\%$, respectively; the 5-year PFS and OS rates were $37.9\% \pm 6.9\%$, and $47.6\% \pm 7.7\%$, respectively (Figure 1). The median duration of MMT was 4 months (range: 1–36 months). There was an extension of treatment duration in four patients following their wishes, and the total treatment duration in the patients was 14, 17, 22, and 36 months.

The disease status of patients at the time of MMT administration was CR ($n = 24$), cCR ($n = 33$). At the end of MMT, 11 of the 24 patients with CR had progressive disease (PD), and 13 patients maintained CR. Of the remaining 33 patients with cCR, four patients had CR, seven patients had cCR, and 22 patients had PD, including one patient who developed a second tumor.

After MMT, 4 of the 33 patients with PD died without additional treatment. Among the 29 treated patients, 20 received chemotherapy alone, two received chemotherapy and surgery, four received

chemotherapy, surgery, and radiotherapy, and three received chemotherapy and radiotherapy. After MMT, 12 patients developed metastasis, and the most common site of metastasis was the lungs (3/12).

Among the 57 patients receiving MMT, we compared the survival of high-risk patients with that of non-high-risk patients at diagnosis. Non-high-risk patients were defined as low- and intermediate-risk patients at diagnosis who relapsed after comprehensive treatment, intermediate-risk patients who achieved cCR or patients with IRS stage III who did not receive radiotherapy. The 3-year PFS was $27.8\% \pm 10.4\%$ in high-risk patients versus $48.1\% \pm 8.6\%$ in non-high-risk patients [hazard ratio (HR) 1.73 (95% CI 0.82–3.63); $p = 0.11$], and the 3-year OS was $50.1\% \pm 12.9\%$ in high-risk patients versus $61.6\% \pm 8.8\%$ in non-high-risk patients [HR 1.52 (95% CI 0.64–3.58); $p = 0.30$]. The 5-year PFS was $27.8\% \pm 10.4\%$ in high-risk patients versus $44.4\% \pm 8.7\%$ in non-high-risk patients, and the 5-year OS was $20.0\% \pm 15.7\%$ in high-risk patients versus $54.6\% \pm 9.1\%$ in non-high-risk patients (Figure 2).

We further analyzed the survival of low- and intermediate-risk patients at diagnosis who relapsed and found that the 3-year PFS was $43.6\% \pm 11.3\%$ in these patients (20/57), compared with $27.8\% \pm 10.4\%$ in high-risk patients (20/57) and $52.8\% \pm 13.3\%$ in intermediate-risk patients who did not relapse (17/57). The corresponding 3-year OS for these three groups was $65.8\% \pm 11.4\%$, $50.1\% \pm 12.9\%$, and $55.6\% \pm 13.6\%$, respectively. The corresponding 5-year PFS for these three groups was $37.4\% \pm 11.3\%$, $27.8\% \pm 10.4\%$, and $52.8\% \pm 13.3\%$ ($p = 0.15$). The 5-year OS for these three groups was $53.2\% \pm 12.3\%$, $20.0\% \pm 15.7\%$, and $55.6\% \pm 13.6\%$ ($p = 0.58$). However, there was no significant difference between the relapsed group and the other two groups (Figure 3).

4 Discussion

Current studies on MMT are limited to a few clinical trials showing that administration of MMT after standard therapy

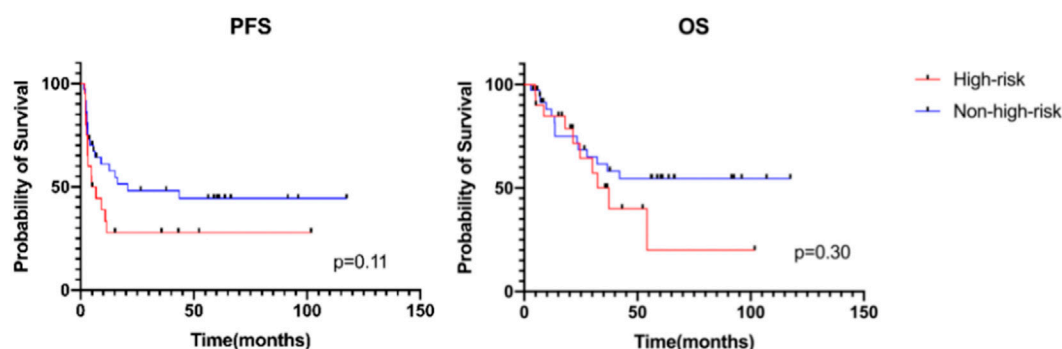


FIGURE 2

Progression-free survival and overall survival of patients receiving MMT between different risk groups.

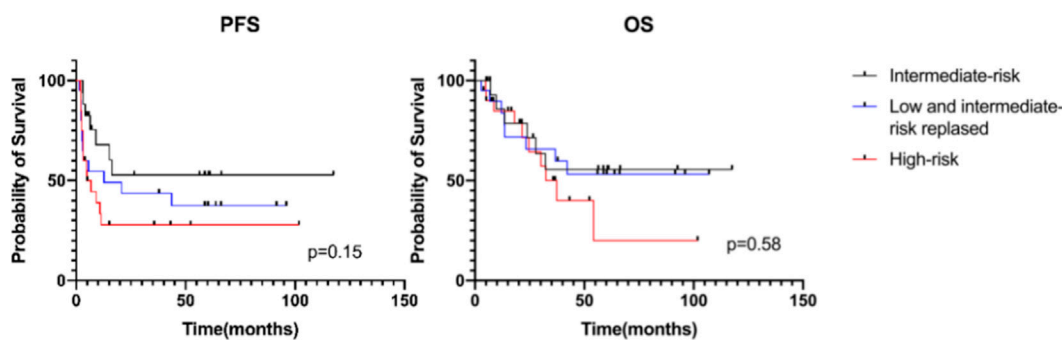


FIGURE 3

Progression-free survival and overall survival of patients receiving MMT by risk group stratum.

improves the overall survival of RMS patients (Casanova et al., 2004; Klingebiel et al., 2008; Minard-Colin et al., 2012; Bisogno et al., 2019; Koscielniak et al., 2022). In the present study, we included nearly 10 years of pediatric RMS patients receiving MMT and found that their OS in the real world was slightly lower than what has been observed in the largest MMT clinical trials (Bisogno et al., 2019) but better than the current COG study in high-risk and relapse/refractory patients without MMT (Haduong et al., 2022). That may be because the risk stratification in the RMS 2005 study (Bisogno et al., 2019) was different from that of the COG study (Crane et al., 2022), and its definition of high-risk patients was similar to that of the intermediate-risk patients in our study, resulting in a higher 5-year OS of 86.5% after standard treatment and maintenance therapy. Based on a similar risk stratification study of patients with metastatic RMS receiving maintenance therapy (Schoot et al., 2022), the PFS and OS was similar to our patients, with improved outcomes compared to historical cohorts. Therefore, our study is similar to previous MMT studies (Casanova et al., 2004; Klingebiel et al., 2008; Minard-Colin et al., 2012; Koscielniak et al., 2022; Schoot et al., 2022), showing the effectiveness of MMT in relapsed and high-risk RMS patients. In the absence of a clear application method and indication of MMT in RMS at present, our study has the following highlights. Most importantly, VNR was

administered orally rather than intravenously, which was more convenient for children than previous studies (Casanova et al., 2004; Klingebiel et al., 2008; Minard-Colin et al., 2012; Bisogno et al., 2019) and did not affect the final survival. Furthermore, we extended the use population of MMT in RMS. We included not only high-risk patients but also relapsed patients after standard treatment or those who had a high risk of recurrence. Another crucial issue relies on the duration of treatment. The duration of maintenance therapy for high-risk patients with RMS was 6 months (Bisogno et al., 2019). However, in the follow-up European EpSSG protocol, a 1-year duration design has already been proposed for patients with high-risk RMS. Randomization of the duration of maintenance of 1 or 2 years has also been proposed for stage IV RMS. Moreover, in EpSSG's MTS 2008 study (Schoot et al., 2022), metastatic patients treated with MMT for up to 1 year delayed the median time from random assignment to relapse from 6.9 months to 10.1 months, with the majority of events taking place after the 24-week window for maintenance treatment. Therefore, we adopted 48 weeks of MMT in the expectation for better disease control, which provides available data for a longer duration of maintenance treatment. Therefore, our data provide a new perspective basis for further clinical application of MMT in the future to improve the survival of RMS patients, especially those with poor prognosis. It may be possible to expand

the use of MMT in patients who relapsed or achieved cCR after comprehensive therapy.

Given the availability of oral drugs and convenience of medication for patients, VNR was changed from intravenous administration to oral administration in this study. Compared with the intravenous route, oral anticancer drugs have many advantages, especially the reduction of local intravenous toxicity at the injection site and the overall improvement of patient convenience and quality of life. It is also a favorable choice in long-term medication management of disease. For VNR, oral administration had the same pharmacokinetic-pharmacodynamic relationship as intravenous administration (Gebbia and Puozzo, 2005). Oral dosing in children is challenged by VNR liquid capsule formulation and CTX tablet limitations. Therefore, in our study, the doses of VNR and CTX fluctuated and were rounded on the basis of VNR 40 mg/m² and CTX 25–50 mg/m². Repeated administration of VNR at the same dose and frequency in pediatric patients has been shown to produce similar systemic exposures (Hamimed et al., 2022). Therefore, it is believed that oral VNR can be used to improve outcomes and quality of life in patients with RMS.

The oral dose of VNR is usually 60 mg/m²; however, too-high doses of VNR can cause protumoral host responses and prevent desired effects (Shaked et al., 2019). Therefore, we tried a lower dose of VNR of 25–40 mg/m². It seems it does not lower the efficacy which would be consistent with a metronomic-based mechanisms of action. The dosage of CTX in metronomic therapy varies in different studies. CTX (30 mg/m² PO daily) was continually given in pediatric recurrent solid tumors (Stempak et al., 2006). Another study showed that CTX was given at 40 mg/m²/day (PO) combined with vinblastine in patients with desmoplastic small round cell tumors, which was correlated with prolonged time to relapse (Scheer et al., 2019). A higher dose of CTX (2.5 mg/kg/day PO) (Kieran et al., 2005), etoposide, temozolomide, in combination with alternating cytostatic biologic therapy, celecoxib and isotretinoin were studied in patients with malignant central nervous system tumors (Choi et al., 2008). In fact, the right dose to ensure efficient and low toxicity is a critical issue that needs to be further studied.

The standard treatment regimen at our center was modified based on current COG studies. We adopted the VAC (vincristine/actinomycin-D/cyclophosphamide) regimen every 3 weeks in the low-risk group, which was similar to the Intergroup Rhabdomyosarcoma Study-IV (IRS-IV) (Crist et al., 2001), D9602 (Raney et al., 2011), and ARST0331 (Walterhouse et al., 2014). ARST0331 achieved good clinical results, reducing toxicity without affecting OS and simplifying the treatment of low-risk patients. The regimen in the present study was similar to those in the above studies, although the total dose of VCR was reduced and the cumulative CTX dose (9.6 g/m²) was doubled compared with that of ARST0331 (4.8 g/m²). VDC/IE, has been proven to be as effective for intermediate-risk RMS as IRS-IV (Arndt et al., 2008). Considering the large cumulative dose of CTX in D9803 (Arndt et al., 2009), ifosfamide was used to replace CTX to prevent losing the therapeutic effect, and the CAV/IE regimen was used alternately for 10 cycles in the intermediate-risk group in this study. Considering the effectiveness recorded in the ARST0531 study (Hawkins et al., 2018), it was changed to the VI regimen for 2 cycles during radiotherapy. In this study, the chemotherapy

regimen in high-risk patients was similar to COG's intensive multiagent therapy (Weigel et al., 2016). A CAV/IE regimen was used every 3 weeks for 14 cycles and 2 cycles of the VI regimen during radiotherapy, in which doxorubicin was replaced with pirarubicin owing to its cardiotoxic effects (Dantchev et al., 1979).

We found that the PFS and OS of high-risk patients at diagnosis were worse than those of non-high-risk patients at diagnosis; however, there was no significant difference between the two groups, which may be explained by the fact that most of the non-high-risk patients at diagnosis included in this study were relapsed patients or those who had a high risk of recurrence. Additionally, we further grouped metastatic patients receiving MMT according to Oberlin prognostic factors (Oberlin et al., 2008) and found that the 5-year PFS was 15.4% ± 10% in patients with two or more Oberlin risk factors versus 57.1% ± 18.7% in patients with one or no risk factors [HR 2.12 (95% CI 0.71–6.28); *p* = 0.23], and the 5-year OS was 26.9% ± 15.7% in patients with two or more Oberlin risk factors versus 66.7% ± 19.2% in patients with one or no risk factors [HR 3.58 (95% CI 1.04–12.4); *p* = 0.08]. Both PFS and OS in patients with two or more Oberlin risk factors were lower than those with one or no risk factor (Supplementary Figure S1), suggesting the feasibility of Oberlin prognostic factors in metastatic patients receiving MMT in the real world.

There is currently no universal standard regimen for relapsed patients with RMS. Some studies have found that the prognosis of non-metastatic relapsed patients depends on several related factors, such as radiotherapy, tumor size, and intensity of treatment, and the survival rate varies from 2% to 60% (Chisholm et al., 2011; Affinita et al., 2020; Heske et al., 2021). However, we found that the 3-year OS of non-metastatic relapsed patients was approximately 70%, higher than that of previous studies, indicating that MMT may be a new and effective standard of care in patients with non-metastatic relapsed RMS. MMT is mostly used in high-risk patients in clinical trials and has not been used in low- and intermediate-risk patients; therefore, the present study provides a new perspective that the use of MMT after salvage therapy in non-metastatic relapsed patients can significantly improve the outcome.

Previous studies showed that in group III participants for IRS-IV, the response at the end of treatment was not associated with disease recurrence or death, resection of the residual mass was not associated with improved prognosis, and aggressive alternative therapy may not be warranted (Rodeberg et al., 2009). However, in this study, when patients received MMT after the end of treatment, 11 of 24 patients with CR progressed, compared with 22 of the remaining 33 patients with cCR. Given the benefit of MMT, nearly 70% of patients with cCR progressed even when patients received MMT. Thus, for patients who failed to achieve a CR at the end of treatment, MMT was one of the recommended therapies (André et al., 2020), a strategy that needs to be further confirmed in future clinical trials.

In terms of toxicity, MMT with oral VNR and continuous low doses of CTX was generally safe, with no treatment-related deaths. No grade 3 or 4 toxic events were observed. In addition, bone marrow suppression rarely occurs during MMT because of regular monitoring of routine blood tests and adjustment of the dose of oral drugs according to the results of routine blood tests. Although some patients occasionally had mild gastrointestinal symptoms,

there was no need to go to the hospital for treatment of adverse reactions.

This study had some limitations. On the one hand, this was a retrospective study with patient selection bias, and the sample size was relatively small. On the other hand, as not all patients were examined for FOXO1 fusion genes, the latest risk grouping based on positive/negative fusion genes was limited.

In conclusion, our study showed that MMT with oral VNR and continuous low doses of CTX are effective and feasible for pediatric patients with RMS in the real world. This treatment could be further studied in patients with high-risk and relapsed RMS in prospective clinical trials.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#), further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Sun Yat-sen University Cancer Center (Approval Number: B2022-489-01). Written informed consent to participate in this study was provided by the participants and legal guardian/next of kin.

Author contributions

YL, LW, and YH conceived the idea for the present study. YL wrote the manuscript. XS, JW, JH, FS, JZ, ZZ, and YZ carried out data collection and performed data analysis, statistical analysis, and literature search. LW and YH made the figures and tables. MS and SL reviewed the manuscript and contributed to the intellectual scientific content of the manuscript domain specific expertise; all authors agree to be accountable for the content of the work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphar.2023.1132219/full#supplementary-material>

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Case Report: Should Regorafenib be prescribed as a continuous schedule in gastrointestinal stromal tumors? Three case reports on Regorafenib personalized schedule

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Introduction: Regorafenib is a tyrosine kinase inhibitor (TKI) approved in metastatic gastrointestinal stromal tumor (GIST), colorectal cancer, and hepatocarcinoma. Anyway, the toxicity profile of Regorafenib standard schedule is associated with poor compliance and a high rate of discontinuation. For this reason, there is a growing need for a Regorafenib personalized schedule emerging from the scientific community.

Objective: The aim of this case series was to describe the experience of our sarcoma referral center with the continuous administration of Regorafenib as an alternative regimen to treat metastatic GIST patients.

Methods: We retrospectively collected clinical, pathological, and radiological data of patients with metastatic GIST treated with daily personalized Regorafenib at a single tertiary referral center from May 2021 to December 2022.

Results: We identified three patients fulfilling the inclusion criteria. The average follow-up since the start of Regorafenib was 19.1 months (12–25 months). All three patients had started a standard third-line Regorafenib schedule according to guidelines. The reasons for switching to a continuous schedule were as follows: exacerbation of symptoms during week-off treatment in the first patient, a serious adverse event (AE) in the second patient, and a combination of both conditions in the third. After switching, none of the patients reported severe AEs, and they improved control of tumor-related symptoms. Two of the

patients experienced disease progression after 16 months (9 months of which is continuous schedule) and 12 months (8.1 months of which is continuous schedule) of Regorafenib, respectively; the third patient is still receiving continuous Regorafenib at the time of writing, with a progression-free survival of 25 months (14 months after the modified schedule start).

Conclusion: With a similar efficacy and lower toxicities, a daily, personalized Regorafenib schedule seems to be a promising alternative to the standard regimen for metastatic GIST patients, including the frail ones. Further prospective analyses are needed to confirm the safety and efficacy of such regimen.

KEYWORDS

Regorafenib, sarcoma, GIST, case report, personalized therapy

Introduction

Despite overall rarity, gastrointestinal stromal tumors (GISTs) represent the most common subtype of mesenchymal tumors, with an incidence of 1.5/100,000 people/year worldwide. The median age at diagnosis is mid-60 years of age, with an equal distribution between men and women (1).

In the majority of cases, GISTs are diagnosed as a localized tumor, and only radical surgery is intended as a curative treatment. Perioperative treatment with a TKI (Imatinib mesylate, an inhibitor of KIT, PDGFRA, and ABL) is recommended in high-risk patients according to the risk assessment classifications and to the mutational pattern (2–6). In moderate-risk patients, perioperative treatment should be discussed with the patient (5, 6).

However, approximately 20% of patients present with metastases at diagnosis (7, 8) and up to 40% of patients who receive surgery tend to recur (9). In metastatic GIST, TKIs are the standard of care according to mutational status (6). Imatinib represents the first-line treatment for patients harboring an Imatinib-sensitive mutation (10), while Sunitinib constitutes the second-line treatment according to the results of the pivotal phase 3 trial (11).

Finally, Regorafenib represents the standard third-line treatment, based on the results of a phase 3 trial (GRID) (12), in which Regorafenib, at the dose of 160 mg daily orally for the first 3 weeks of each 4-week cycle (160 mg/day d1–21 q28), allowed a significant improvement of PFS versus placebo.

The efficacy of Regorafenib, an oral multi-TKI able to inhibit several kinases, including VEGFR1 to 3, TEK, KIT, RET, RAF1, BRAF, PDGFR and FGFR, is unfortunately afflicted by a high incidence of drug-related adverse events (AEs) and often requires personalized dose adaptations (13). Moreover, owing to the nature of GIST, some patients report an exacerbation of cancer-related symptoms during the week-off treatment (14). As an alternative schedule, Regorafenib continuous administration with a lower daily dose (120 mg/day continuously) but the same dose intensity in a 4-week cycle has been evaluated as feasible in a phase I study (15) and in a retrospective study (16).

Unfortunately, resistance to Regorafenib eventually occurs. Resistance to anti-angiogenetic agents can be a consequence of

genetic/epigenetic modifications in cancer cells and/or in tumor endothelial cells (17). In order to avoid/delay resistance, several combinations of TKIs with other multiple angiogenetic drugs or immunotherapeutic agents are being studied.

Based on these biological and clinical data, we present our experience with a continuous schedule of Regorafenib.

Methods

We retrospectively collected clinical, pathological, and radiological data of patients with metastatic GIST treated with continuous Regorafenib 120 mg/day after failure or intolerance to Imatinib and Sunitinib at Humanitas Research Hospital from May 2021 to December 2022. We anonymously collected data through the clinical records in an electronic database. All the patients signed an informed consent to the clinical research according to the institutional requirements.

Results

We included three patients who fulfilled the inclusion criteria. In the same period of time, no other GIST patient received Regorafenib standard schedule in our institute. Patients' characteristics are summarized in Table 1. The average follow-up since the start of Regorafenib was 19.1 months (12–25 months). All three patients had started a standard Regorafenib schedule. Below, we will briefly describe the clinical history of each patient. In Figure 1, we reported the timeline of each patient.

Patient 1

In April 2018 a 50-year-old man without relevant comorbidities accessed the Emergency Room due to abdominal increased volume, pain, and worsening of performance status (PS) according to the

TABLE 1 Features of patients.

	Patient 1	Patient 2	Patient 3
Age (years)	50	53	62
Sex	M	M	M
Site	Small bowel	Stomach	Small bowel
Mutational status	KIT (exon 11)	KIT (exon 11)	Wild type
Metastasis at diagnosis	Yes	No	No
Previous lines	2	2	2
PS ECOG at Regorafenib start	3	1	3
AEs $\geq$ G2—standard schedule	No	Yes ¹	Yes ²
AEs $\geq$ G2—modified schedule	No	No	No
Duration of modified schedule (months)	9	14	8.1
PFS with Regorafenib (months)	17	25	12
Subsequent lines	Imatinib	No	No
FU since start of Regorafenib (months)	20.3	25	12
FU since diagnosis (months)	58	152	35
Status	Dead	Alive	Dead

M, male; PS, performance status; ECOG, Eastern Cooperative Oncology Group; Rego, Regorafenib; AEs, adverse events; PFS, progression-free survival; FU, follow-up.

¹ Hypothyroidism G2, anemia G3, nausea G2, anorexia G2, sialorrhea G2.

² Anemia G3, hypothyroidism G2.

Eastern Cooperative Oncology Group (ECOG). An abdominal ultrasound showed a large mass of 17 × 14 cm and a CT scan confirmed the lesion associated with peritoneal localizations. A fine needle biopsy allowed the diagnosis of GIST presumably from the small bowel, with a mitotic index of 10/35 HPF (high power field). The tumor was stained positive for CD34, DOG1, and Caldesmon. The molecular pattern showed a mutation in KIT exon 11 [c.1657_1668del12; p.Y553_Q556del].

He received first-line therapy with Imatinib since May 2018, with an almost immediate symptoms relief and improvement of PS. After a month, a CT scan showed a trend to a reduction of the known lesions and the best overall response (BOR) as partial response (PR) occurred after 14 months of treatment, with subsequent disease progression (PD) after 21 months. Therefore, second line-therapy with Sunitinib was started. The BOR occurred after 4 months as stable disease (SD) according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.1 (18), alongside a metabolic response. After 9 months, clinical and radiological PD occurred and PS of the patient was dramatically compromised (PS 3) because of complete bowel occlusion and intense pain. Despite the clinical situation and the need for total parenteral nutrition, we proposed a further treatment with Regorafenib, and the patient accepted.

Therefore, Regorafenib was gradually administered through the nasogastric tube with an initial reduced dose, obtaining a slow clinical improvement and a partial resolution of the bowel occlusion. After 1 month, the patient was receiving the standard dose of Regorafenib of 160 mg/day with the classic schedule; parenteral nutrition was progressively withdrawn in favor of oral nutrition.

The patient obtained as BOR a PR after 2 months of treatment, followed by SD. Nevertheless, the patient reported a significant

worsening of abdominal symptoms during the week-off treatment, with an almost complete resolution at the restart of a new cycle of treatment. For this reason, according to few published data, the Regorafenib schedule was switched to a personalized regimen, administering 120 mg/day continuously. This schedule was well tolerated and provided an SD for a further 9 months. The patient reported no more symptoms, worsening periods, or AEs, indeed denoting subjective wellbeing. The overall PFS with Regorafenib was 17 months. At the time of PD, we proposed a rechallenge with Imatinib, but unfortunately, the patient's clinical conditions dramatically worsened because of abdominal pain, bowel occlusion, and ascites, leading to death after less than 2 months from the initiation of Imatinib.

Patient 2

A 53-year-old man without comorbidities except a low body mass index (BMI 17.15) came to our institute in 2018 with a diagnosis of gastric GIST with liver metastases. According to his medical history, he had undergone a total gastrectomy for a gastric GIST in another hospital in 2010. The histopathologic report had confirmed the diagnosis with a positivity for CD117, CD34, and DOG1 and a mitotic index of 18/25 HPF. Molecular analysis had shown a mutation in KIT exon 11 [W557-v559>Y]. The first CT scan performed after surgery had revealed a single liver metastasis, so a systemic treatment with Imatinib had been started, achieving a complete response (CR). After a treatment discontinuation of a few months, multifocal liver progression had occurred, so the patient had restarted Imatinib, obtaining disease control.

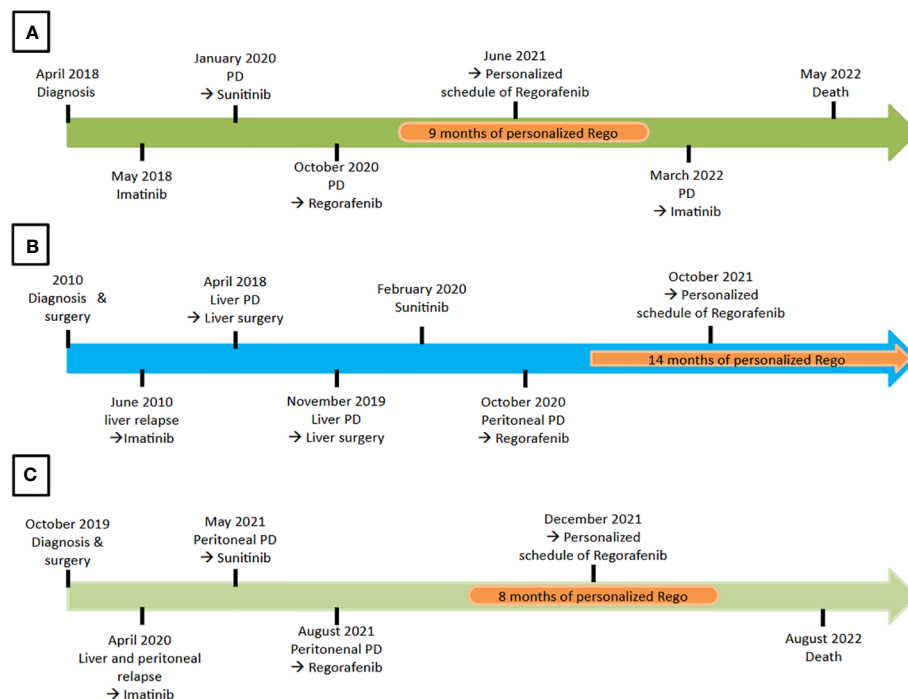


FIGURE 1

Timeline of treatments for each patient. (A) The patient 1 received 9 months of Regorafenib personalized treatment. (B) The patient 2 was still receiving personalized schedule of Regorafenib at the moment of the analysis. (C) The patient 3 received 8 months of Regorafenib personalized schedule.

In April 2018, the patient, motivated by the long-lasting disease stability, accessed our institute to be evaluated for surgery and underwent multiple liver resections. The pathologic report detected four metastases of GIST, and the molecular pattern showed a mutation in KIT exon 11 [W557_V559>Y] and a new mutation in c-KIT exon 17 [D816G].

A new liver relapse occurred 2 months after surgery and Imatinib was gradually restarted, obtaining a metabolic CR after 1 month of treatment and a morphological SD after 6 months. After a further liver oligoprogression, the patient underwent a new liver resection, with histological confirmation of two metastases with the same mutational pattern of the previously resected ones. Nearly immediately after surgery, a liver relapse was detected and a new line of treatment with Sunitinib was started, with a PFS of 9 months and SD as BOR obtained after 2 months. At the time of PD, the CT scan detected pulmonary, liver, and new peritoneal lesions. Third-line treatment with Regorafenib was started 160 mg/day with the classic schedule. The patient interrupted the therapy after only 1 month because of a symptomatic (G2) hypothyroidism due to an autoimmune thyroiditis that warranted steroids and hormone replacement. Over the Regorafenib interruption, a stereotactic body radiotherapy was performed on all the known metastases. After 2 months, Regorafenib was cautiously restarted at 80 mg/day and then gradually increased until reaching a 160 mg/day standard schedule. Due to gastrointestinal toxicities (G2 nausea, G2 anorexia, and G2 sialorrhea), the dose was reduced to 120 mg/day d1–21 q28, with a morphologic SD and a metabolic CR after 3 months. As a

consequence of an intestinal bleeding and G3 anemia that required blood transfusions, Regorafenib was interrupted. At the complete recovery, considering the medical history of the patient, the previous toxicities, and the low BMI, we proposed the resumption of Regorafenib with a personalized continuous schedule of 80 mg/day. This schedule has been well tolerated, without requiring new interruptions and with no more AEs other than G1 (hand skin reaction), obtaining SD. The patient is still progression-free after 25 months of treatment (14 months personalized schedule) and in subjective and objective good clinical conditions.

Patient 3

A 63-year-old man came to our institute in 2021 for a second opinion for an ileal GIST with metachronous metastases. As significant comorbidities, he had had a myocardial infarction in 1977 and a stroke without neurological sequelae in 2007. In 2019, an abdominal large mass of 13 × 4.4 × 15 cm was detected due to abdominal pain, and in October 2019, surgery of the lesion was performed in another center. The pathology report diagnosed a high-risk [Mettinen et al. (2, 3)] ileal GIST with a positive staining for CD117, CD34, and DOG1 and a mitotic index greater than 5/25 HPF. Because of patient comorbidities, no adjuvant therapy was proposed and a liver and peritoneal relapse was detected after 6 months from surgery. First-line therapy with Imatinib 400 mg/day was proposed with initial SD, then PD after 9 months. Thus,

Imatinib was increased to 800 mg/day, but a worsening of the clinical conditions occurred, leading the patient to the Emergency Room of our Institute with G3 acute heart failure that required hospitalization. A CT scan showed a further dimensional PD. In May 2021, after complete clinical recovery, a second-line treatment with Sunitinib was gradually started until the dose of 37.5 mg/day. The pathologic review of the histologic specimen confirmed the diagnosis, and the molecular analysis showed no mutations in the c-KIT gene. It was not possible to determine PDGFR α gene status due to poor sample quality and quantity.

After 2 months of treatment, a severe gastrointestinal bleeding conditioning G3 anemia and acute kidney failure led to another hospitalization of the patient and discontinuation of the treatment. The CT scan showed an abdominal PD conditioning a severe bilateral hydronephrosis that required a right ureteral stenting and a left nephrostomy. Although the patient was suffering from abdominal pain and poor clinical conditions (PS ECOG 3), having achieved initial hematologic recovery and a clinical stabilization, in agreement with the patient in August 2021, standard schedule Regorafenib was started and progressively increased up to 120 mg/day d1–21 q28. The patient experienced a mild clinical improvement and radiologic SD, but reported a worsening of the symptoms (abdominal pain) over the week-off treatment. Moreover, owing to G3 anemia that required multiple blood transfusions, the treatment had to be interrupted and restarted at the recovery with a personalized schedule. Thus, we proposed a Regorafenib continuous schedule (120 mg/day), obtaining a good tolerance and no more AEs $\geq$ G2, with the only AEs being G1 anemia and G1 hand-foot syndrome. Global clinical conditions significantly improved with subjective wellbeing, radiologic SD as BOR after 1 month, and metabolic PR after 5 months. A radiologic and clinical PD occurred after 12 months of therapy with Regorafenib (8 months personalized schedule) Regorafenib, and the patient was hospitalized for best supportive care. Unfortunately, his conditions, compromised by abdominal pain and bowel obstruction, did not allow any further treatment and he passed away 2 months after the discharge.

Discussion

Regorafenib is approved as a third-line therapy in metastatic GIST, but it presents a challenging toxicity profile often requiring a personalization of therapeutic schedule. In the pivotal phase III GRID trial (12), 98% of patients receiving Regorafenib experienced at least one drug-related AE and 60% of the study population reported a G3 or higher AE [according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0]. Dose modifications were required in 72% of patients in the experimental arm, and 6% discontinued treatment due to AEs. This incidence of $\geq$ G3 AEs was definitely more elevated than the one documented for Imatinib and Sunitinib, respectively, of 20.5% (10) and 20% (11).

Similar results were also reported in the phase III CORRECT trial (19) of Regorafenib in pretreated metastatic colorectal cancer,

where the $\geq$ G3 AEs occurred in 54% of patients, leading to a dose reduction or treatment discontinuation in 67% of cases.

The severe toxicity profile of Regorafenib across different cancers has also been highlighted by a systematic meta-analysis (13) that included seven studies and 2,099 patients: the authors registered 47% dose reductions, 57.2% dose interruptions, and 9.7% permanent discontinuations.

The published data were also confirmed in clinical practice as recently collected by Nannini et al. in a retrospective study conducted across several Italian sarcoma centers (20, 21), evaluating the real-life treatment strategies in 152 metastatic GIST patients on Regorafenib. Among them, only 32.2% received treatment at the standard dose, while the vast majority (67.8%) received a personalized dose/schedule, upfront or during the course of treatment. The most frequent dose modification was daily dose reduction to 120 mg or 80 mg maintaining the regular schedule, a scheme adopted in 54% and 21% of cases, respectively. Other dose adjustments affected only the schedule or both dose and schedule. The authors reported not only a complete or partial resolution of AEs in all patients receiving personalized treatment, but also a significant positive impact of this approach on PFS (mPFS 9.7 versus 5.6 months), observing also a trend towards OS improvement. Thus, the customization of a personalized regimen in the daily clinical practice allowed the achievement of a better disease control, probably due to the continuity of treatment.

A continuous schedule has been rarely adopted in Nannini et al. (20, 21), although it would be the best approach to maintain the dose intensity and to meet the unique GIST biology. Indeed, the kinase-addicted nature of this tumor requires a continuous suppression, as clinically described in a prospective study enrolling 57 GIST patients treated with Regorafenib (14), in which 26% of the patients treated with standard schedule suffered from an exacerbation of cancer-related symptoms during the week-off treatment, with a quick improvement at the new cycle start.

Imatinib and Sunitinib treatment schedule is based on this rationale. The discontinuation of Imatinib results in early disease progression (22), so that prosecution of treatment is continuously recommended. Despite the pivotal study of Sunitinib (11) with an intermittent schedule of 50 mg/day 4-weeks-on, 2-weeks-off, the equivalent dose-intensity regimen with 37.5 mg/day continuously was investigated and finally recognized as standard regimen in sarcoma referral centers (23). Indeed, even short suspensions of anti-VEGF agents lead to tumor regrowth, with full revascularization after 7 days of drug withdrawal (24).

Regarding Regorafenib, a continuous schedule was explored in a phase I study showing a favorable clinical activity and safety profile (15). In a subsequent retrospective study in GIST patients (16), 79% received a continuous dose of Regorafenib 120 mg/day. Overall, $\geq$ G3 AEs were reported in 43% of patients, while treatment discontinuation due to AEs were registered remarkably in 21% of patients on classic schedule versus 14% of patients on continuous schedule.

Perhaps, to identify the correct dose and schedule of oral TKIs for every patient, a monitoring of drug plasma concentrations

should be determined, as investigated in GIST patients on Imatinib (25) and recently re-proposed with Pazopanib (26). This personalized model might be appropriate but hardly feasible in clinical practice due to costs and complex techniques.

Resistance to anti-angiogenic therapies is a significant challenge in oncology, as it leads to a lack of response and disease progression. It can develop due to genetic/epigenetic changes in cancer cells or endothelial cells and it can imply different mechanisms of tumor angiogenesis, as intussusceptive microvascular growth, vasculogenic mimicry, and vascular co-option. To overcome resistance, alternative therapeutic strategies have been explored, such as combining multiple anti-angiogenic drugs or anti-angiogenic drugs with immune checkpoint inhibitors, as it has been successfully done in other angiogenesis-dependent tumors such as renal cancer (17). In GIST, this combination is being studied in different clinical trials (27). Potentially, in the future, pan-omics profiling could allow physicians to identify the most suitable treatment for each patient (17).

As for personalization of the cure, our cases support the previously reported data on continuous schedule Regorafenib, showing a comparable efficacy through a steady suppression of the oncogenic pathways and guaranteeing a better tolerance even in frail patients who had experienced serious AEs with standard schedule Regorafenib.

Certainly, our analysis presents several limitations. Being a retrospective analysis, data can be affected by bias or missing. Secondly, the study was conducted at a single tertiary referral center, which may limit the generalizability of the findings to other populations or healthcare settings. Thirdly, the sample size is small, preventing us from applying statistical analysis and drawing robust conclusions. Lastly, the short follow-up may limit the assessability of long-term safety and efficacy of continuous schedule Regorafenib in this setting.

Also, a personalized approach itself presents some limitations, such as the physician team's expertise in identifying patients that could benefit the most from the schedule adjustment and the difficult generalizability of personalized schedules.

On the other hand, a personalized schedule allows to take into account the patient's needs and perspective, leading to an increased awareness of his cure plan and a better compliance.

Our study provides initial evidence for the potential benefits of a continuous, personalized Regorafenib schedule, and these findings suggest that such a regimen may be a promising alternative to the standard, with similar efficacy and lower toxicities. However, further prospective studies with larger sample sizes and longer follow-up periods are needed to confirm the safety and efficacy of this treatment approach.

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Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Internal Humanitas Research Hospital IRCCS committee. The ethics committee waived the requirement of written informed consent for participation.

Author contributions

Conceptualization and first draft: AFB, MSG, RF. Data collection: MSG, RF, AL. Review: AFB, MSG, NG, AL, RF, LS, LVS, FD, FS, SLR, AS. Supervision: AFB, AS, AL. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Chemotherapeutic drugs for soft tissue sarcomas: a review

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Despite the low incidence of soft tissue sarcomas (STSs), hundreds of thousands of new STS cases are diagnosed annually worldwide, and approximately half of them eventually progress to advanced stages. Currently, chemotherapy is the first-line treatment for advanced STSs. There are difficulties in selecting appropriate drugs for multiline chemotherapy, or for combination treatment of different STS histological subtypes. In this study, we first comprehensively reviewed the efficacy of various chemotherapeutic drugs in the treatment of STSs, and then described the current status of sensitive drugs for different STS subtypes. anthracyclines are the most important systemic treatment for advanced STSs. Ifosfamide, trabectedin, gemcitabine, taxanes, dacarbazine, and eribulin exhibit certain activities in STSs. Vinca alkaloid agents (vindesine, vinblastine, vinorelbine, vincristine) have important therapeutic effects in specific STS subtypes, such as rhabdomyosarcoma and Ewing sarcoma family tumors, whereas their activity in other subtypes is weak. Other chemotherapeutic drugs (methotrexate, cisplatin, etoposide, pemetrexed) have weak efficacy in STSs and are rarely used. It is necessary to select specific second- or above-line chemotherapeutic drugs depending on the histological subtype. This review aims to provide a reference for the selection of chemotherapeutic drugs for multi-line therapy for patients with advanced STSs who have an increasingly long survival.

KEYWORDS

chemotherapeutic drugs, sarcoma, review, chemotherapy, efficacy

1 Introduction

Soft tissue sarcomas (STSs) are rare malignancies, accounting for only approximately 1% of all malignancies (Bhatt et al., 2016; Yang et al., 2019). There are more than 70 histological subtypes, and the clinical characteristics and prognoses of these subtypes greatly vary (Amadeo et al., 2020; Parikh et al., 2018; von Mehren et al., 2022; Tos et al., 2023). Despite the low incidence of STSs, hundreds of thousands of new STS cases are diagnosed annually worldwide, and approximately half of them eventually progress to advanced stages (Corey et al., 2014; Bhatt et al., 2016; Hung et al., 2019; Yang et al., 2019). Currently, chemotherapy is the first-line treatment for advanced STSs (Cojocaru et al., 2022; de Juan Ferre et al., 2021; von Mehren et al., 2020). Anthracyclines (mainly doxorubicin) were found to be effective against STSs in 1973 (Tan et al., 1973; Sritharan and Sivalingam, 2021). Since then, various clinical trials have been conducted to prolong survival or reduce adverse events in patients with STSs using intensive chemotherapy, non-anthracycline regimens, or alternative anthracycline drugs. These drugs include, but are not limited to, oxazaphosphorines, trabectedin, gemcitabine, taxanes, dacarbazine, eribulin, vinca alkaloid agents (vindesine, vinblastine, vinorelbine, vincristine), methotrexate, cisplatin, etoposide, and pemetrexed (Ratan and Patel, 2016; Hatcher et al., 2017; Smrke et al., 2020). The characteristics, efficacy, and safety of these drugs for STSs vary, and the responses of different STS subtypes to these

chemotherapeutic drugs greatly vary. Although some drugs have shown good efficacy in individual subtypes, none have exceeded the efficacy and safety achieved by doxorubicin for STSs. To date, doxorubicin remains the first-line chemotherapeutic drug for STSs (Cojocaru et al., 2022; de Juan Ferre et al., 2021; von Mehren et al., 2020; Smrke et al., 2020; Smolle et al., 2020; Meyer and Seetharam, 2019; Yang et al., 2022; Gronchi et al., 2017). Selecting second- or higher-line drugs for advanced STS remains a challenge (Frezza et al., 2017; Kim et al., 2019; Haddox and Riedel, 2020; Younger et al., 2021; Kojima et al., 2022).

In the past decade, anti-vascular endothelial factor receptor multi-target tyrosine kinase inhibitors (TKIs), such as pazopanib, have been widely used in STS, which is a major breakthrough in the treatment of this type of malignancy, leading to significantly prolonged survival in patients with STS (Tang et al., 2021; Kyriazoglou et al., 2022; Thirasastr et al., 2022). Immunotherapeutic agents, such as programmed cell death protein 1 inhibitors, have also shown some therapeutic effects in some STS histological subtypes (Tang et al., 2021; Banks and D'Angelo, 2022; Tawbi et al., 2017). Furthermore, the combination of conventional chemotherapeutic drugs with targeted agents (TKIs or immunotherapeutic agents) is considered the next breakthrough in STS treatment (Tang et al., 2021; Patel et al., 2022; Principe et al., 2022; Tian and Yao, 2022). There are significant differences in the efficacy of different chemotherapeutic drugs combined with different targeted agents (Kyriazoglou et al., 2022; Principe et al., 2022; Fuchs et al., 2023). Based on the different STS subtypes, selecting potential chemotherapeutic drugs to combine with targeted drugs is important to achieve better efficacy (Principe et al., 2022; Tian and Yao, 2022). In recent years, few studies have systematically summarized the differences between the chemotherapeutic drugs used to treat STSs and the differences in the efficacy of these drugs in different STS subtypes. This leads to difficulties in selecting appropriate chemotherapeutic drugs for combination treatment of different STS subtypes.

In this study, we first comprehensively reviewed the efficacy of various chemotherapeutic drugs in the treatment of STSs, and then described the current status of sensitive drugs for different STS subtypes. We aim to provide a reference for the selection of chemotherapeutic drugs for multi-line therapy for patients with advanced STSs who have an increasingly long-survival.

2 Efficacy of different drugs in STSs

As a traditional method of cancer treatment, chemotherapy has been used in STSs for more than 50 years since the introduction of doxorubicin in the 1970s. Currently, chemotherapeutic drugs, such as anthracyclines, ifosfamide, trabectedin, gemcitabine, paclitaxel, dacarbazine, and eribulin, have therapeutic effects in STSs and are widely used in clinical treatment (Seddon, 2016; Bleloch et al., 2017; Frezza et al., 2017; Hatcher et al., 2017; Smrke et al., 2020). Here, we comprehensively reviewed the clinical trial results of various drugs for STSs to accurately describe the efficacy of them in STSs. To improve the reliability of this study, we attempted to use data from multicenter, prospective, phase II–IV clinical trials as much as possible. In the case of the absence of prospective clinical trial

results, a multicenter retrospective study with large sample size conducted by multinational sarcoma organizations was included in the analysis. During our review, we found that the outcomes of different studies were presented using different measures, including the objective response rate (ORR), disease control rate, median progression-free survival (PFS), PFS rate, median overall survival (OS), and OS rate. We uniformly selected the most common measures, ORR, median PFS, and median OS, as comparative indicators.

2.1 Anthracyclines

Anthracyclines are among the most effective chemotherapeutics for cancer. They are glycoside drugs comprising the amino sugar daunosamine linked to a hydroxyanthraquinone aglycone, and they induce cell death through multiple intracellular targets: reactive oxygen species generation, DNA-adduct formation, topoisomerase II inhibition, histone eviction, Ca^{2+} and iron hemostasis regulation, and ceramide overproduction (Rabbani et al., 2005; Jasra and Anampa, 2018; Martins-Teixeira and Carvalho, 2020). Doxorubicin is the most effective and widely used anthracycline for the treatment of STSs (Table 1). Several other anthracyclines, such as idoxorubicin, epirubicin, and pegylated liposomal doxorubicin, have also been used for the clinical treatment of STSs (Table 1). However, none of the other anthracyclines exceed the efficacy of doxorubicin in STSs (Table 1) (Nielsen et al., 2000a; Judson et al., 2001; Chamberlain et al., 2019; Martins-Teixeira and Carvalho, 2020; Peter et al., 2022).

Doxorubicin (adriamycin) was isolated from *Streptomyces suis* and *S. peucetius* in the late 1960s (Tan et al., 1973; Peter et al., 2022). Since its Food and Drug Administration approval in 1974, doxorubicin alone or in combination with other drugs has been widely used as a first-line therapy for a myriad of cancers (Aubel-Sadron and Lodos-Gagliardi, 1984; Sun et al., 2017). Doxorubicin induces cell death through multiple intracellular targets, including reactive oxygen species generation, DNA adduct formation, topoisomerase II inhibition, histone eviction, Ca^{2+} and iron hemostasis regulation, and ceramide overproduction. Moreover, doxorubicin-treated dying cells undergo cellular modifications that enable neighboring dendritic cell activation and enhance the presentation of tumor antigens. In addition, doxorubicin aids in the immune-mediated clearance of tumor cells (Carvalho et al., 2009; Sritharan and Sivalingam, 2021).

To date, Numerous clinical trials have demonstrated the efficacy of doxorubicin alone or doxorubicin-based chemotherapy for the treatment of STSs (Sritharan and Sivalingam, 2021; Peter et al., 2022). The results of the representative multicenter prospective clinical trials over the past 30 years are listed in Table 1. Due to various reasons such as the long-time interval between different clinical trials and errors caused by small sample sizes, the reported efficacy of doxorubicin monotherapy for STS varies significantly. However, clinical trials with sample sizes exceeding 100 in the past decade have shown that the ORR of doxorubicin monotherapy with a conventional dose ($75 \text{ mg/m}^2/\text{d1/3w}$) for STSs was 14%–20%, with a median PFS of 4.6–6.8 months (Table 1).

To further improve the efficacy of chemotherapy, doxorubicin in combination with other drugs has also been widely used (Table 1).

TABLE 1 Outcomes of representative clinical trials of anthracyclines in nonspecific STSs.

Drugs and dosages	Years of report	Study types	Setting	Number of patients	Outcomes			References
					ORR (%)	M-PFS (months)	M-OS (months)	
Single agent								
Doxorubicin 75 mg/m ² /d1/3w	2020	Phase III trial	Anthracycline-naïve	251	18.3	6.8	19.7	Tap et al. (2020)
Doxorubicin 60 mg/m ² /d1/3w	2020	Phase II trial	First-line	40	7.7	4.3	9.8	Hartmann et al. (2020)
Doxorubicin 75 mg/m ² /d1/3w	2020	Phase II trial	First-line	39	15.4	5.3	14.3	Grünwald et al. (2020)
Doxorubicin 75 mg/m ² /d1/3w	2017	Phase III trial	First-line	323	18	6.0	19.0	Tap et al. (2017)
Doxorubicin 75 mg/m ² /d1/3w	2017	Phase III trial	First-line	129	20	5.4	17.9	Seddon et al. (2017)
Doxorubicin 75 mg/m ² /d1/3w	2016	Phase II trial	Anthracycline-naïve	65	11.9	4.1	14.7	Tap et al. (2016)
Doxorubicin 75 mg/m ² /d1/3w	2016	Phase III trial	First-line	221	19.9	5.2	16.9	Ryan et al. (2016)
Doxorubicin 75 mg/m ² /d1/3w	2016	Phase II trial	First-line	59	17	5.5	13.7	Martin-Broto et al. (2016)
Doxorubicin 75 mg/m ² /d1/3w	2015	Phase II trial	First-line	40	5	4.6	14.3	Chawla et al. (2015)
Doxorubicin 75 mg/m ² /d1/3w	2014	Phase III trial	First-line	228	14	4.6	12.8	Judson et al. (2014)
Doxorubicin 75 mg/m ² /d1/3w	2009	Phase II trial	First-line	64	23.4	6.5	-	Maurel et al. (2009)
Doxorubicin 75 mg/m ² /d1/3w	2007	Phase III trial	First-line	110	11.8	2.5	12.0	Lorigan et al. (2007)
Doxorubicin 75 mg/m ² /d1/3w	2001	Phase II trial	First-line	44	7	2.7	8.2	Judson et al. (2001)
Doxorubicin 75 mg/m ² /d1/3w	1998	Phase III trial	First-line	104	14	3.7	10.5	Nielsen et al. (1998)
Doxorubicin 75 mg/m ² /d1/3w	1995	Phase III trial	First-line	263	23.3	10.7	12.1	Santoro et al. (1995)
Doxorubicin 70 mg/m ² /d1/3w	1990	Phase III trial	First-line	151	17	3	9.4	Borden et al. (1990)
Doxorubicin 70 mg/m ² /d1/3w	1987	Phase III trial	First-line	83	25	3.5	9.6	Mouridsen et al. (1987)
Aldoxorubicin 350 mg/m ² /d1/3w	2015	Phase II trial	First-line	83	23	8.3	15.8	Chawla et al. (2015)
Pegylated liposomal doxorubicin 50 mg/m ² /d1/4w	2001	Phase II trial	First-line	50	10	2.2	10.7	Judson et al. (2001)
Epirubicin 150 mg/m ² /d1/3w	1998	Phase III trial	First-line	106	15	3.3	11.0	Nielsen et al. (1998)
Epirubicin 50 mg/m ² /d1–3/3w	1998	Phase III trial	First-line	106	14	2.8	10.5	Nielsen et al. (1998)
Epirubicin 75 mg/m ² /d1/3w	1987	Phase III trial	First-line	84	18	2.8	11.2	Mouridsen et al. (1987)

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TABLE 1 (Continued) Outcomes of representative clinical trials of anthracyclines in nonspecific STSs.

Drugs and dosages	Years of report	Study types	Setting	Number of patients	Outcomes			References
					ORR (%)	M-PFS (months)	M-OS (months)	
Combination regimens								
Doxorubicin 75 mg/m ² /d1/3w plus olaratumab 20 mg/kg/d1,8/3w in cycle 1, and 15 mg/kg in subsequent cycles	2020	Phase III trial	First-line	258	14	5.4	20.4	Tap et al. (2020)
Doxorubicin 75 mg/m ² /d1/3w plus evofosfamide 300 mg/m ² /d1, 8/3w	2017	Phase III trial	First-line	317	28	6.3	18.4	Tap et al. (2017)
Doxorubicin 75 mg/m ² /d1/3w plus olaratumab 20 mg/kg/d1, 8/3w	2016	Phase II trial	First-line	65	18.2	6.6	16.5	Tap et al. (2016)
Doxorubicin 75 mg/m ² /d1/3w plus palifosfamide 150 mg/m ² /d1–3/3w	2016	Phase III trial	First-line	226	28.3	6.0	15.9	Ryan et al. (2016)
Doxorubicin 60 mg/m ² /d1/3w plus trabectedin 1.1 mg/m ² /d1/3w	2016	Phase II trial	First-line	54	17	5.7	13.3	Martin-Broto et al. (2016)
Doxorubicin 25 mg/m ² /d1–3/3w plus ifosfamide 2.5 g/m ² /d1–4/3w	2014	Phase III trial	First-line	228	26	7.4	14.3	Judson et al. (2014)
Doxorubicin 30 mg/m ² /d1–3/2w for 3 cycles followed by ifosfamide 2.5 g/m ² /d1–5/3w for 3 cycles	2009	Phase II trial	First-line	62	24.1	6.0	-	Maurel et al. (2009)
Doxorubicin 20 mg/m ² /d1–3/3w plus ifosfamide 2.5 g/m ² /d1–3/3w plus dacarbazine 300 mg/m ² /d1–3/3w	2009	Phase III trial	First-line	74	35	9.8	17.7	Fayette et al. (2009)
Doxorubicin 25 mg/m ² /d1–3/3w plus ifosfamide 3 g/m ² /d1–3/3w plus dacarbazine 400 mg/m ² /d1–3/3w	2009	Phase III trial	First-line	71	38	9.1	17.3	Fayette et al. (2009)
Doxorubicin 30 mg/m ² /d1–3/2w for 3 cycles followed by ifosfamide 2.5 g/m ² /d1–5/3w for 3 cycles	2004	Phase II trial	First-line	57	38	5.6	13.5	Maurel et al. (2004)
Pegylated liposomal doxorubicin 45 mg/m ² /d1/3w plus paclitaxel 150 mg/m ² /d1/3w	2004	Phase II trial	First-line	42	16	5.7	13.2	Bafaloukos et al. (2004)
Doxorubicin 50 mg/m ² /d1/3w plus ifosfamide 5 g/m ² /d1/3w	2000	Phase III trial	First-line	149	21	11.0	13.1	Le Cesne et al. (2000)
Doxorubicin 75 mg/m ² /d1/3w plus ifosfamide 5 g/m ² /d1/3w	2000	Phase III trial	First-line	145	23.3	8.6	12.8	Le Cesne et al. (2000)
Doxorubicin 50 mg/m ² /d1/3w plus ifosfamide 5 g/m ² /d1/3w	1995	Phase III trial	First-line	258	28.1	10.3	12.8	Santoro et al. (1995)
Cyclophosphamide 500 mg/m ² /d1/3w plus vincristine 1.5 mg/m ² /d1/3w plus doxorubicin 50 mg/m ² /d1/3w plus dacarbazine 750 mg/m ² /d1/3w	1995	Phase III trial	First-line	142	28.4	11.2	11.9	Santoro et al. (1995)
Doxorubicin 60 mg/m ² /d1/3w plus dacarbazine 1,000 mg/m ² /d1/3w	1993	Phase III trial	First-line	170	17	4	12	Antman et al. (1993)
Doxorubicin 60 mg/m ² /d1/3w plus dacarbazine 1,000 mg/m ² /d1/3w plus ifosfamide 5–7.5 g/m ² /d1–3/3w	1993	Phase III trial	First-line	170	32	6	13	Antman et al. (1993)
Doxorubicin 70 mg/m ² /d1/3w plus vindesine 3 mg/m ² /d1/3w	1990	Phase III trial	First-line	147	18	4	9.9	Borden et al. (1990)
Doxorubicin 60 mg/m ² /d1/3w plus ifosfamide 5 g/m ² /d1/3w	1989	Phase II trial	First-line	42	36	7	8	Loehrer et al. (1989)

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TABLE 1 (Continued) Outcomes of representative clinical trials of anthracyclines in nonspecific STSs.

Drugs and dosages	Years of report	Study types	Setting	Number of patients	Outcomes			References
					ORR (%)	M-PFS (months)	M-OS (months)	
Doxorubicin 60 mg/m ² /d1/3w plus dacarbazine 900 mg/m ² /d1/3w plus ifosfamide 7.5 g/m ² /d1/3w	1989	Phase II trial	First-line	105	47	9.5	16	Elias et al. (1989)
Doxorubicin 60 mg/m ² /d1/3w plus dacarbazine 250 mg/m ² /d1/3w	1987	Phase III trial	First-line	104	33	7.2	8.6	Baker et al. (1987)
Doxorubicin 60 mg/m ² /d1/3w plus dacarbazine 250 mg/m ² /d1/3w plus cyclophosphamide 500 mg/m ² /d1/3w	1987	Phase III trial	First-line	112	34	6.0	9.8	Baker et al. (1987)
Doxorubicin 60 mg/m ² /d1/3w plus dacarbazine 250 mg/m ² /d1/3w plus actinomycin D 1.2 mg/m ² /d1/3w	1987	Phase III trial	First-line	119	24	5.4	11.7	Baker et al. (1987)

STSs, soft tissue sarcomas; ORR, objective response rate; M-PFS, median progression-free survival time; M-OS, median overall survival.

The drug most commonly used in combination with doxorubicin is ifosfamide. The ORR of doxorubicin plus ifosfamide in treating STSs is 21%–38%, with a median PFS of 5.6–11 months (Table 1). Although the combination of doxorubicin and ifosfamide improves the ORR and median PFS compared with doxorubicin alone, it does not improve the median OS (Table 1) and instead increases hematological toxicity such as leucopenia and anemia (Maurel et al., 2009; Judson et al., 2014; Wang et al., 2021). Therefore, recently, this combined regimen is not recommended as a first-line chemotherapy for advanced STSs but is only recommended for preoperative neoadjuvant chemotherapy of high-risk STSs (Judson et al., 2014; Weiss et al., 2020). Furthermore, no combination regimen has been shown to significantly extend the median OS over doxorubicin monotherapy in patients with advanced STSs (Table 1). Notably, the doxorubicin plus ifosfamide plus dacarbazine achieves the highest ORR (38%) and median PFS (9.8 months) in patients with advanced STSs (Table 1). This combined regimen should also be tested in the setting of neoadjuvant therapy.

In summary, as the most recognized chemotherapeutic drug, doxorubicin is the cornerstone of advanced STS chemotherapy. The testing of new drugs in the field of STSs is always guided by doxorubicin. With the invention and testing of an increasing number of targeted and immunotherapeutic drugs, various doxorubicin-based combination therapies will be widely tested and applied for the treatment of STSs.

2.2 Oxazaphosphorines

Oxazaphosphorines are a class of bifunctional alkylating agents that have been extensively investigated over the past 50 years and have a wide spectrum of anticancer and immune-regulating activities (Giraud et al., 2010). Most oxazaphosphorines are designed as prodrugs that require cytochrome P450 enzyme-mediated bioactivation to generate highly reactive alkylating nitrogen mustards, which exert their chemotherapeutic effects by attacking specific nucleophilic groups of DNA molecules in target cancer cells (Misiura, 2006; Liang et al., 2007; Wang and Wang,

2012). In STS chemotherapy, ifosfamide is the most widely used oxazaphosphorine with definite efficacy (Table 2). Other oxazaphosphorines, such as cyclophosphamide, trofosfamide, evofosfamide, and palifosfamide, have also been used for the treatment of STSs (Table 2). However, to date, none of the other oxazaphosphorines have exceeded the efficacy of ifosfamide in STSs (Table 2) (Giraud et al., 2010; Mulder et al., 2015; Tap et al., 2017; Hartmann et al., 2020).

Among the chemotherapeutic drugs for STSs, ifosfamide is the second most effective after doxorubicin (Tascilar et al., 2007). Ifosfamide was first synthesized in the 1960s. It was introduced as a chemical modification of cyclophosphamide with a different position of its two chloroethyl groups on the central ring, providing a structure with greater water solubility and antitumor activity and a better toxicity profile (Kerbusch et al., 2001; Misiura, 2006; Tascilar et al., 2007). Numerous clinical trials and retrospective studies have demonstrated the efficacy of ifosfamide alone or ifosfamide-based chemotherapy for the treatment of STSs. The results of representative multicenter prospective clinical trials are presented in Table 2. Because there are no cardiotoxicity concerns, ifosfamide can be administered at significantly higher doses than doxorubicin. Current evidence indicates that the ORR of using large doses of ifosfamide to treat STSs is significantly higher than that of using low doses (Table 1). The efficacy of ifosfamide in the treatment of STSs is slightly lower than that of doxorubicin (ORR, 5.5%–25% vs. 5%–25%; median PFS, 2.2–3.5 vs. 2.5–10.7 months; median OS, 7.2–12.8 vs. 8.2–19.7 months, respectively) (Table 1; Table 2). Therefore, doxorubicin is still considered the first choice of chemotherapy for advanced STSs (Lorigan et al., 2007). However, ifosfamide may be superior to doxorubicin in synovial sarcoma (Nielsen et al., 2000b; Carter et al., 2020).

In the real world, ifosfamide is most commonly used in combination with doxorubicin (Table 1; Table 2). The ORR of the doxorubicin plus ifosfamide in treating STS is 21%–38%, and the median PFS is 5.6–11 months (Table 1; Table 2). Compared with doxorubicin or ifosfamide alone, the combination of doxorubicin and ifosfamide increases the ORR and median PFS but does not improve the median OS in patients with advanced STSs (Table 1; Table 2) (Maurel et al., 2009; Judson et al., 2014; Wang et al., 2021).

TABLE 2 Outcomes of representative clinical trials of oxazaphosphorines in nonspecific STSs.

Drugs and dosages	Years of report	Study types	Setting	Number of patients	Outcomes			References
					ORR (%)	M-PFS (months)	M-OS (months)	
Single agent								
Ifosfamide 3 g/m ² /d1–3/3w	2007	Phase III trial	First-line	109	5.5	2.2	10.9	Lorigan et al. (2007)
Ifosfamide 9 g/m ² /3 days continuous infusion/3w	2007	Phase III trial	First-line	107	8.4	3.0	10.9	Lorigan et al. (2007)
Ifosfamide 5 g/m ² /d1/3w	2002	Phase II trial	1–2 line	49	10	2.6	12	van Oosterom et al. (2002)
Ifosfamide 3 g/m ² /d1–3/3w	2002	Phase II trial	1–2 line	49	25	3.3	10	van Oosterom et al. (2002)
Ifosfamide 12 g/m ² /3-day continuous infusion/4w	2000	Phase II trial	1–2 line	114	16	3.5	12.8	Nielsen et al. (2000b)
Ifosfamide 2 g/m ² /d1–4/3w	1989	Phase II trial	First-line	110	24	-	7.2	Antman et al. (1989)
Ifosfamide 5 g/m ² /d1/3w	1987	Phase II trial	First-line	68	18	-	-	Bramwell et al. (1987)
Trofosfamide 300 mg/d on days 1–7, then 150 mg/d continuously	2020	Phase II trial	First-line	80	6.6	2.8	12.3	Hartmann et al. (2020)
Cyclophosphamide 1.5 g/m ² /d1/3w	1987	Phase II trial	First-line	67	8	-	-	Bramwell et al. (1987)
Combination regimens								
Doxorubicin 75 mg/m ² /d1/3w plus evofosfamide 300 mg/m ² /d1,8/3w	2017	Phase III trial	First-line	317	28	6.3	18.4	Tap et al. (2017)
Doxorubicin 75 mg/m ² /d1/3w plus palifosfamide 150 mg/m ² /d1–3/3w	2016	Phase III trial	First-line	226	28.3	6.0	15.9	Ryan et al. (2016)
Doxorubicin 25 mg/m ² /d1–3/3w plus ifosfamide 2.5 g/m ² /d1–4/3w	2014	Phase III trial	First-line	228	26	7.4	14.3	Judson et al. (2014)
Doxorubicin 30 mg/m ² /d1–3/2w for 3 cycles followed by ifosfamide 2.5 g/m ² /d1–5/3w for 3 cycles	2009	Phase II trial	First-line	62	24.1	6.0	-	Maurel et al. (2009)
Doxorubicin 20 mg/m ² /d1–3/3w plus ifosfamide 2.5 g/m ² /d1–3/3w plus dacarbazine 300 mg/m ² /d1–3/3w	2009	Phase III trial	First-line	74	35	9.8	17.7	Fayette et al. (2009)
Doxorubicin 25 mg/m ² /d1–3/3w plus ifosfamide 3 g/m ² /d1–3/3w plus dacarbazine 400 mg/m ² /d1–3/3w	2009	Phase III trial	First-line	71	38	9.1	17.3	Fayette et al. (2009)
Doxorubicin 30 mg/m ² /d1–3/2w for 3 cycles followed by ifosfamide 2.5 g/m ² /d1–5/3w for 3 cycles	2004	Phase II trial	First-line	57	38	5.6	13.5	Maurel et al. (2004)
Ifosfamide 2 g/m ² /d1–2/3w plus etoposide 100 mg/m ² /d1–5/3w plus cisplatin 20 mg/m ² /d1–5/3w	2000	Phase II trial	First-line	104	46	4.6	8	Pápai et al. (2000)
Doxorubicin 50 mg/m ² /d1/3w plus ifosfamide 5 g/m ² /d1/3w	2000	Phase III trial	First-line	149	21	11.0	13.1	Le Cesne et al. (2000)
Doxorubicin 75 mg/m ² /d1/3w plus ifosfamide 5 g/m ² /d1/3w	2000	Phase III trial	First-line	145	23.3	8.6	12.8	Le Cesne et al. (2000)
Ifosfamide 1.5 g/m ² /d1–3/3w plus etoposide 600 mg/m ² /d1/3w	1997	Phase II trial	First-line	86	41	-	19	Saeter et al. (1997)

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TABLE 2 (Continued) Outcomes of representative clinical trials of oxazaphosphorines in nonspecific STSs.

Drugs and dosages	Years of report	Study types	Setting	Number of patients	Outcomes			References
					ORR (%)	M-PFS (months)	M-OS (months)	
Doxorubicin 50 mg/m ² /d1/3w plus ifosfamide 5 g/m ² /d1/3w	1995	Phase III trial	First-line	258	28.1	10.3	12.8	Santoro et al. (1995)
Cyclophosphamide 500 mg/m ² /d1/3w plus vincristine 1.5 mg/m ² /d1/3w plus doxorubicin 50 mg/m ² /d1/3w plus dacarbazine 750 mg/m ² /d1/3w	1995	Phase III trial	First-line	142	28.4	11.2	11.9	Santoro et al. (1995)
Doxorubicin 60 mg/m ² /d1/3w plus dacarbazine 1,000 mg/m ² /d1/3w plus ifosfamide 5–7.5 g/m ² /d1–3/3w	1993	Phase III trial	First-line	170	32	6	13	Antman et al. (1993)
Doxorubicin 60 mg/m ² /d1/3w plus ifosfamide 5 g/m ² /d1/3w	1989	Phase II trial	First-line	42	36	7	8	Loehrer et al. (1989)
Doxorubicin 60 mg/m ² /d1/3w plus dacarbazine 900 mg/m ² /d1/3w plus ifosfamide 7.5 g/m ² /d1/3w	1989	Phase II trial	First-line	105	47	9.5	16	Elias et al. (1989)
Cyclophosphamide 200 mg/d1–7,15–21/4w plus sirolimus 4 mg/d	2012	Phase II trial	≥2 line	48	2	3.4	9.9	Schuetze et al. (2012)

STSs, soft tissue sarcomas; ORR, objective response rate; M-PFS, median progression-free survival; M-OS, median overall survival.

TABLE 3 Outcomes of representative clinical trials of trabectedin in nonspecific STSs.

Drugs and dosages	Years of report	Study types	Setting	Number of patients	Outcomes			References
					ORR	M-PFS (months)	M-OS (months)	
Trabectedin 1.5 mg/m ² /24 h–infusion/3w	2022	Phase IV trial	≥2 line	128	11.7%	5.2	15.2	Grunwald et al. (2022)
Trabectedin 1.5 mg/m ² /24 h–infusion/3w	2021	Phase III trial	≥2 line	52	13.7%	3.1	13.6	Le Cesne et al. (2021)
Trabectedin 1.5 mg/m ² /24 h–infusion/3w	2020	Phase II trial	First-line	24	-	4	12	Grosso et al. (2020)
Trabectedin 1.5 mg/m ² /24 h–infusion/3w	2017	Phase IV trial	No line	218	26.6%	5.9	21.3	Buonadonna et al. (2017)
Trabectedin 1.2 mg/m ² /24 h–infusion/3w	2015	Phase II trial	≥2 line for translocation-related sarcomas	37	8%	5.6	-	Kawai et al. (2015)
Trabectedin 1.3 mg/m ² /3 h–infusion/3w	2015	Phase II trial	First-line	47	14.8%	2.8	-	Bui-Nguyen et al. (2015)
Trabectedin 1.5 mg/m ² /24 h–infusion/3w	2015	Phase II trial	First-line	43	4.7%	3.1	-	Bui-Nguyen et al. (2015)
Trabectedin 1.5 mg/m ² /24 h–infusion/3w	2014	Phase III trial	First-line for translocation-related sarcomas	61	5.9%	16.1	38.9	Blay et al. (2014)
Trabectedin 1.5 mg/m ² /24 h–infusion/3w	2005	Phase II trial	≥2 line	99	8.1%	3.5	9.2	Le Cesne et al. (2005)
Trabectedin 1.1 mg/m ² /d1/3w plus doxorubicin 60 mg/m ² /d1/3w	2016	Phase II trial	First-Line	54	17%	5.7	13.3	Martin-Broto et al. (2016)

STSs, soft tissue sarcomas; ORR, objective response rate; M-PFS, median progression-free survival; M-OS, median overall survival.

Currently, this combined regimen is recommended for preoperative neoadjuvant chemotherapy in high-risk STSs (Judson et al., 2014; Weiss et al., 2020). Other drugs that are commonly used in combination with ifosfamide include dacarbazine and etoposide (Table 2). Notably, the doxorubicin plus ifosfamide plus dacarbazine has the highest ORR (32%–47%) and median PFS (6–9.8 months) in patients with advanced STSs (Table 2). The combination of these three drugs has not received sufficient attention in the era of targeted therapy and immunotherapy for STSs.

In summary, as a chemotherapeutic agent that is as well-known as doxorubicin, ifosfamide has an important effect on the chemotherapy of STSs. Ifosfamide is also worthy of further testing for the treatment of synovial sarcoma. However, the other oxazaphosphorines have not exceeded the role of ifosfamide in STSs.

2.3 Trabectedin

Trabectedin is a natural compound initially isolated from the marine ascidian *Ecteinascidia turbinata* and can be obtained by high-purity chemical synthesis (Trabectedin, 2003; Cuevas and Francesch, 2009; Ganjoo and Patel, 2009). It has a unique structure with three-fused tetrahydroisoquinoline rings, which allow it to inhibit cancer cells by causing single- and double-strand DNA breaks, and several other key cellular biological processes and tumor microenvironments (Trabectedin, 2003; Cuevas and Francesch, 2009; Gordon et al., 2016; Larsen et al., 2016; Ratan and Patel, 2017; Wang et al., 2022). Trabectedin was approved in Europe in 2007 for the treatment of advanced STSs with previous anthracycline treatment failure and in the United States in 2015 for the treatment of patients with advanced leiomyosarcoma and liposarcoma with previous anthracycline treatment failure (Nakamura and Sudo, 2022). It is the most studied and widely used chemotherapeutic drug for STSs, in addition to doxorubicin and ifosfamide (Rastogi and Bakhshi, 2016; Dang et al., 2021; Le Cesne, 2022; Nakamura and Sudo, 2022; Wang et al., 2022). The ORR of trabectedin monotherapy for STS is 4.7%–14.8%, the median PFS is 2.8–5.9 months, and the median OS is 9.2–21.3 months (Table 3). Although these data are similar to those of doxorubicin or ifosfamide monotherapy, recent randomized controlled studies have demonstrated that trabectedin cannot replace doxorubicin as a first-line treatment for advanced STSs (Bui-Nguyen et al., 2015; Martin-Broto et al., 2016). In addition, several studies have demonstrated that the efficacy of trabectedin in the treatment of leiomyosarcoma and liposarcoma at the second- or above-line setting is significantly higher than in other STS subtypes (median PFS 5.1 versus 1.4 months, respectively) (Rastogi and Bakhshi, 2016; Schuetze, 2021; Vincenzi et al., 2023). Therefore, it is necessary to conduct randomized controlled clinical trials in a first-line setting to compare the activity of trabectedin and doxorubicin in these histological subtypes (Blay et al., 2014; Dang et al., 2021).

2.4 Taxanes

Taxanes are an important class of antitumor drugs that can interfere with the function of microtubules in cells, leading to

chromosomal non-aggregation in multipolar spindles, mitotic failure, and ultimately cell death induction (Yared and Tkaczuk, 2012; Weaver, 2014). They include paclitaxel and docetaxel and various analogs or processes thereof.

Paclitaxel was originally extracted from Pacific yew trees with a minimal yield. After its synthesis, paclitaxel has been widely used for the treatment of many cancers with significant therapeutic effects (Mekhail and Markman, 2002). However, the efficacy of paclitaxel monotherapy in the treatment of the majority of STSs is poor (Casper et al., 1998). Currently, paclitaxel alone is the recommended treatment for angiosarcoma (Skubitz and Haddad, 2005; Bui et al., 2018; Pink et al., 2021). A 2004 study demonstrated that a combination of paclitaxel and liposomal doxorubicin achieved appropriate efficacy in the treatment of STSs (Bafaloukos et al., 2004). However, because this chemotherapy regimen has no significant advantages over other regimens, it is rarely mentioned.

Docetaxel is a reprocessed taxol-like substance produced by the needles of *Taxus chinensis*. The chemical structures between docetaxel and paclitaxel differ in two ways (Ojima et al., 2016). These small changes make docetaxel different from paclitaxel in terms of water solubility, cellular effects, and pharmacology (Zhang et al., 2019). However, docetaxel monotherapy for STSs has also been proven ineffective (Santoro et al., 1999; Verweij et al., 2000). The efficacy of the combination of docetaxel and gemcitabine in the treatment of STSs is significantly higher than that of docetaxel alone or gemcitabine alone (Maki et al., 2007). Moreover, the efficacy of docetaxel plus gemcitabine is comparable to that of doxorubicin alone (Table 4). However, docetaxel plus gemcitabine is cumbersome, costly, and toxic than doxorubicin monotherapy; therefore, it is not recommended as a first-line treatment for advanced STSs (Seddon et al., 2017). Notably, docetaxel plus gemcitabine is deemed more effective in patients with leiomyosarcoma than in patients with other histological subtypes (Bay et al., 2006; Maki, 2007; Pautier et al., 2012; Choi et al., 2018).

In summary, single-drug taxane is not recommended for the treatment of STSs. However, the docetaxel plus gemcitabine is considered second only to doxorubicin-based chemotherapy for STSs.

2.5 Gemcitabine

Gemcitabine is a cytotoxic nucleoside analog widely used in the treatment of malignant tumors. The metabolites of gemcitabine in cells can inhibit DNA synthesis via the inhibition of ribonucleotide reductase and compete with the nucleoside deoxycytidine as a fraudulent base, thereby producing antitumor effects (Barton-Burke, 1999; Wong et al., 2009). Although gemcitabine is widely used in other cancers, its efficacy alone in STSs is poor, with an ORR of 3%–8% and a median PFS of 1.5–3 months (Table 5). However, gemcitabine monotherapy has better efficacy in leiomyosarcoma and angiosarcoma (Pautier et al., 2012; Stacchiotti et al., 2012; Ducoulombier et al., 2016; Watson et al., 2022). Fortunately, gemcitabine combined with other drugs (such as docetaxel plus gemcitabine described above) can achieve better efficiency in STSs (Table 4; Table 5). Moreover, docetaxel plus gemcitabine has an efficacy comparable to that of doxorubicin-based chemotherapy in leiomyosarcoma and epithelioid sarcoma (Ducoulombier et al.,

TABLE 4 Outcomes of representative clinical trials of taxanes in nonspecific STSs.

Drugs and dosages	Years of report	Study types	Setting	Number of patients	Outcomes			References
					ORR (%)	M-PFS (months)	M-OS (months)	
Single agent								
Docetaxel 100 mg/m ² /d1/3w	2001	Phase II trial	≥2 line	27	15	2.4	7.7	Köstler et al. (2001)
Docetaxel 100 mg/m ² /d1/3w	2000	Phase II trial	First-line	42	0	1.6	9.8	Verweij et al. (2000)
Docetaxel 100 mg/m ² /d1/3w	1999	Phase II trial	≥2 line	36	2.8	1.4	11.7	Santoro et al. (1999)
Docetaxel 100 mg/m ² /d1/3w	1998	Phase II trial	First-line	30	10.7	-	-	Bramwell et al. (1998)
Docetaxel 100 mg/m ² /d1/3w	1996	Phase II trial	First-line	18	5.9	-	-	Edmonson et al. (1996)
Paclitaxel 250 mg/m ² /d1/3w	1998	Phase II trial	Any-line	28	7	3.5	-	Casper et al. (1998)
Paclitaxel 200 mg/m ² /d1/3w	1997	Phase II trial	≥2 line	19	0	-	-	Patel et al. (1997)
Paclitaxel 250 mg/m ² /d1/3w	1995	Phase II trial	First-line	48	12.5	1.6	12	Balcerzak et al. (1995)
Combination regimens								
Gemcitabine 900 mg/m ² /d1,8/3w plus docetaxel 100 mg/m ² /d8/3w	2021	Phase II trial	≥2 line	45	18	4.1	15.9	Somaiah et al. (2021)
Gemcitabine 900 mg/m ² /d1,8/3w plus docetaxel 75 mg/m ² /d8/3w	2019	Phase II trial	Any-line	70	20	5.6	21.1	Jones et al. (2019)
Gemcitabine 675 mg/m ² /d1,8/3w plus docetaxel 75 mg/m ² /d8/3w	2017	Phase III trial	First-line	128	20	5.5	15.7	Seddon et al. (2017)
Gemcitabine 1,000 mg/m ² /d1,8/3w plus docetaxel 35 mg/m ² /d1,8/3w	2012	Phase II trial	≥2 line	30	16.7	2.5	8.4	Lee et al. (2012)
Gemcitabine 900 mg/m ² /d1,8/3w plus docetaxel 100 mg/m ² /d8/3w	2007	Phase II trial	Any-line	73	16	6.2	17.9	Maki et al. (2007)
Pegylated liposomal doxorubicin 45 mg/m ² /d1/4w plus paclitaxel 150 mg/m ² /d1/4w	2004	Phase II trial	First-line	42	16	5.7	13.2	Bafaloukos et al. (2004)

STSs, soft tissue sarcomas; ORR, objective response rate; M-PFS, median progression-free survival; M-OS, median overall survival.

2016; Choi et al., 2018; Frezza et al., 2018). The efficacy of gemcitabine in combination with other drugs for STSs is inferior to that of docetaxel plus gemcitabine (Table 5). In addition, gemcitabine in combination with emerging drugs, such as pazopanib and eribulin, has also been tested for the treatment of STSs (Somaiah et al., 2021; Lopez-Alvarez et al., 2022). In summary, gemcitabine and docetaxel have similar efficacy and status in the treatment of STSs. The efficacy of their single-drug treatment is relatively low, whereas the gemcitabine plus docetaxel has comparable efficacy to first-line chemotherapy for STSs.

2.6 Dacarbazine

Dacarbazine is an alkylating agent, similar to oxazaphosphorine, which binds to DNA through metabolites in the body and

establishes cross connections between the two strands, causing DNA replication to stop and ultimately leading to cell death (Huitema et al., 2000; Kantrowitz-Gordon et al., 2018; Karati et al., 2022). Additionally, dacarbazine exerts immunostimulatory effects (Ugurel et al., 2013). Dacarbazine has a long history of application in STSs, with only mild activity, an ORR of 3%–4%, and a median PFS of 2–2.7 months (Table 6). Therefore, it is often used as a control drug in clinical trials of new drugs for second- or above-line treatment of STS (Demetri et al., 2016; Schoffski et al., 2016). In terms of combined use, dacarbazine is most commonly used in combination with doxorubicin and ifosfamide, and the efficacy is significant (Tables 1, 2, and 6). The combined regimen of dacarbazine and gemcitabine also has some efficacy in STSs (Table 6), but it is rarely used. In summary, as a veteran drug for the treatment of STSs, it is worthwhile to use dacarbazine in patients with STSs who have failed multiline treatment. In addition, a

TABLE 5 Outcomes of representative clinical trials of gemcitabine in nonspecific STSs.

Drugs and dosages	Years of report	Study types	Setting	Number of patients	Outcomes			References
					ORR (%)	M-PFS (months)	M-OS (months)	
Single agent								
Gemcitabine 1,200 mg/m ² /d1,8/3w	2007	Phase II trial	Any-line	49	8	3	11.5	Maki et al. (2007)
Gemcitabine 1,000 mg/m ² /1w	2006	Phase II trial	First-line	46	7	2	6	Von Burton et al. (2006)
Gemcitabine 1,250 mg/m ² /d1,8,15/4w	2003	Phase II trial	Any-line	25	4	-	15	Okuno et al. (2003)
Gemcitabine 1,250 mg/m ² /d1,8/3w	2002	Phase II trial	≥2 line	31	3.23	1.5	8.9	Svancárová et al. (2002)
Combination regimens								
Gemcitabine 1,000 mg/m ² /d1,8/3w plus pazopanib 800 mg/d	2021	Phase II trial	≥2 line	45	11	4.1	12.4	Somaiah et al. (2021)
Gemcitabine 900 mg/m ² /d1,8/3w plus docetaxel 100 mg/m ² /d8/3w	2021	Phase II trial	≥2 line	45	18	4.1	15.9	Somaiah et al. (2021)
Gemcitabine 1,000 mg/m ² /d1,8/3w plus pazopanib 800 mg/d	2021	Phase II trial	≥2 line	43	11	5.6	13.1	Schmoll et al. (2021)
Gemcitabine 900 mg/m ² /d1,8/3w plus docetaxel 75 mg/m ² /d8/3w	2019	Phase II trial	Any-line	70	20	5.6	21.1	Jones et al. (2019)
Gemcitabine 675 mg/m ² /d1,8/3w plus docetaxel 75 mg/m ² /d8/3w	2017	Phase III trial	First-line	128	20	5.5	15.7	Seddon et al. (2017)
Gemcitabine 1,000 mg/m ² /d1,8/3w plus docetaxel 35 mg/m ² /d1,8/3w	2012	Phase II trial	≥2 line	30	16.7	2.5	8.4	Lee et al. (2012)
Gemcitabine 1800 mg/m ² /3w plus dacarbazine 500 mg/m ² /2w	2011	Phase II trial	≥2 line	57	12	4.2	16.8	Garcia-Del-Muro et al. (2011)
Gemcitabine 800 mg/m ² /d1,8/3w plus vinorelbine 25 mg/m ² /d1,8/3w	2007	Phase II trial	Any-line	40	10	3.4	-	Dileo et al. (2007)

STSs, soft tissue sarcomas; ORR, objective response rate; M-PFS, median progression-free survival; M-OS, median overall survival.

combined regimen of dacarbazine and other new drugs (such as trabectedin, eribulin, TKIs) is worth studying.

2.7 Eribulin

Similar to taxanes, eribulin inhibits microtubule polymerization. Similar to trabectedin, it is an anticancer drug found in marine organisms (Ratan and Patel, 2017). Eribulin is a synthetic analog of the naturally occurring anticancer agent halichondrin B in marine sponges (Shetty and Gupta, 2014). It exerts anticancer effects via multiple pathways. These pathways include the normalization of tumor blood vessels, inhibition of microtubule growth, isolation of microtubule proteins, reduction of microtubule supply, and reversal of the transition from mesenchymal to epithelial cells (Young and Woll, 2016; Phillips et al., 2022). In addition, eribulin has an important effect on the tumor immune microenvironment (Phillips et al., 2022). Although eribulin has various antitumor mechanisms, single-drug chemotherapy has limited efficacy in STSs (ORR, 0%–8%; median

PFS, 2–4 months) (Table 7). However, eribulin alone has better efficacy in leiomyosarcoma and liposarcoma, especially in liposarcoma (Kawai et al., 2022). Owing to the short time since eribulin was approved for the treatment of STSs, there have been no clinical trials on eribulin-based combined chemotherapy for STSs. Eribulin also has therapeutic effects on angiosarcoma, pleomorphic sarcoma, synovial sarcoma, rhabdomyosarcomas, and myxofibrosarcoma (Phillips et al., 2022). Therefore, it is necessary to continue studying the activity of various eribulin-based combination regimens in STSs.

2.8 Other chemotherapeutic drugs

In addition to the abovementioned drugs for treating STSs, many other drugs have been tested for the treatment of STSs. Vinca alkaloids (vindesine, vinblastine, vinorelbine, vincristine) have been widely tested in STSs, ultimately proving that they have important therapeutic effects in specific histological subtypes of STSs, such as

TABLE 6 Outcomes of representative clinical trials of dacarbazine in nonspecific STSs.

Drugs and dosages	Years of report	Study types	Setting	Number of patients	Outcomes			References
					ORR (%)	M-PFS (months)	M-OS (months)	
Single agent								
Dacarbazine 1,200 mg/m ² /d1/3w	2021	Phase II trial	≥2 line	79	3	2.7	8	Van Tine et al. (2021)
Dacarbazine 1,200 mg/m ² /d1/3w	2011	Phase II trial	≥2 line	52	4	2	8.2	Garcia-Del-Muro et al. (2011)
Combination regimens								
Gemcitabine 1800 mg/m ² /d1/3w plus dacarbazine 500 mg/m ² /d1/2w	2011	Phase II trial	≥2 line	57	12	4.2	16.8	Garcia-Del-Muro et al. (2011)
Doxorubicin 20 mg/m ² /d1–3/3w plus ifosfamide 2.5 g/m ² /d1–3/3w plus dacarbazine 300 mg/m ² /d1–3/3w	2009	Phase III trial	First-line	74	35	9.8	17.7	Fayette et al. (2009)
Doxorubicin 25 mg/m ² /d1–3/3w plus ifosfamide 3 g/m ² /d1–3/3w plus dacarbazine 400 mg/m ² /d1–3/3w	2009	Phase III trial	First-line	71	38	9.1	17.3	Fayette et al. (2009)
Dacarbazine 500 mg/m ² /d1/2w plus gemcitabine 1800 mg/m ² /d1/2w	2007	Phase II trial	≥2 line	23	4	3.6	8.6	Losa et al. (2007)
Cyclophosphamide 500 mg/m ² /d1/3w plus vincristine 1.5 mg/m ² /d1/3w plus doxorubicin 50 mg/m ² /d1/3w plus dacarbazine 750 mg/m ² /d1/3w	1995	Phase III trial	First-line	142	28.4	11.2	11.9	Santoro et al. (1995)
Doxorubicin 60 mg/m ² /d1/3w plus dacarbazine 1,000 mg/m ² /d1/3w	1993	Phase III trial	First-line	170	17	4	12	Antman et al. (1993)
Doxorubicin 60 mg/m ² /d1/3w plus dacarbazine 1,000 mg/m ² /d1/3w plus ifosfamide 5–7.5 g/m ² /d1–3/3w	1993	Phase III trial	First-line	170	32	6	13	Antman et al. (1993)
Doxorubicin 60 mg/m ² /d1/3w plus dacarbazine 900 mg/m ² plus ifosfamide 7.5 g/m ² /d1/3w	1989	Phase II trial	First-line	105	47	9.5	16	Elias et al. (1989)
Doxorubicin 60 mg/m ² /d1/3w plus dacarbazine 250 mg/m ² /d1/3w	1987	Phase III trial	First-line	104	33	7.2	8.6	Baker et al. (1987)
Doxorubicin 60 mg/m ² /d1/3w plus dacarbazine 250 mg/m ² /d1/3w plus cyclophosphamide 500 mg/m ² /d1/3w	1987	Phase III trial	First-line	112	34	6.0	9.8	Baker et al. (1987)
Doxorubicin 60 mg/m ² /d1/3w plus dacarbazine 250 mg/m ² /d1/3w plus actinomycin D 1.2 mg/m ² /d1/3w	1987	Phase III trial	First-line	119	24	5.4	11.7	Baker et al. (1987)

STSs, soft tissue sarcomas; ORR, objective response rate; M-PFS, median progression-free survival; M-OS, median overall survival.

rhabdomyosarcomas, whereas their activity in other subtypes is weak (Table 7). Methotrexate is one of the main drugs for the treatment of osteosarcomas (Belayneh et al., 2021). However, it is not involved in STS activity (Table 7) (Karakousis et al., 1980). Similarly, cisplatin is also one of the main drugs used for the treatment of osteosarcomas (Belayneh et al., 2021), with only slight activity in STSs (Table 7) (Brenner et al., 1982; Sordillo et al., 1987; Budd et al., 1990). The combined regimen of cisplatin with vinblastine or pemetrexed shows poor efficacy in STSs (Table 7) (Keohan et al., 1997; Kim et al., 2021). Cisplatin plus epirubicin has some activity in STSs (Table 7) (Jelić et al., 1990; Jelić et al., 1997), but this regimen is rarely used in the real world due to

its high toxicity (Leahy et al., 2012; Nagar et al., 2018; Kim et al., 2019). As a widely used anticancer drug, etoposide has been tested repeatedly in STSs (Belani et al., 1994). However, whether administered orally or intravenously, the activity of etoposide alone in the STS is weak (Table 7) (Licht et al., 1994; Crawley et al., 1997; Keizer et al., 1997; Kebudi et al., 2004). Although ifosfamide plus etoposide shows some activity in STSs (Table 7) (Saeter et al., 1997; Yalçın et al., 1998; Pápai et al., 2000), this combined regimen is rarely used in the real world (Leahy et al., 2012; Nagar et al., 2018; Kim et al., 2019). In addition, researchers tested the activity of pemetrexed in STSs, and the results were disappointing (Table 7) (Hartmann et al., 2013; Kim et al., 2021).

TABLE 7 Outcomes of representative clinical trials of eribulin and other drugs in nonspecific STSs.

Drugs and dosages	Years of report	Study types	Setting	Number of patients	Outcomes			References
					ORR (%)	M-PFS (months)	M-OS (months)	
Eribulin 1.4 mg/m ² /d1,8/3w	2022	Phase IV trial	Any-line	252	8.1	2.5	10.8	Kawai et al. (2022)
Eribulin 1.4 mg/m ² /d1,8/3w	2017	Phase II trial	≥2 line	51	0	4.1	13.2	Kawai et al. (2017)
Eribulin 1.4 mg/m ² /d1,8/3w	2011	Phase II trial	≥2 line	115	4.3	2.1/2.9 ^a	-	Schoffski et al. (2011)
Pemetrexed 500 mg/m ² /d1/3w plus cisplatin 75 mg/m ² /d1/3w	2021	Phase II trial	≥2 line	37	13.5	2.6	52	Kim et al. (2021)
Pemetrexed 500 mg/m ² /d1/3w	2013	Phase II trial	≥2 line	48	5	1.6	6	Hartmann et al. (2013)
Ifosfamide 2 g/m ² /d1–2/3w plus etoposide 100 mg/m ² /d1–5/3w plus cisplatin 20 mg/m ² /d1–5/3w	2000	Phase II trial	First-line	104	46	4.6	8	Pápai et al. (2000)
Ifosfamide 1.5 g/m ² /d1–3/3w plus etoposide 600 mg/m ² /d1/3w	1997	Phase II trial	First-line	86	41	-	19	Saeter et al. (1997)
Cisplatin 100 mg/m ² /d1/3w plus vinblastine 1.2 mg/m ² /d1/3w	1997	Phase II trial	≥2 line	20	0	-	-	Keohan et al. (1997)
Etoposide 50 mg/m ² /d1–21/4w	1997	Phase II trial	≥2 line	27	0	-	-	Keizer et al. (1997)
Epirubicin 60 mg/m ² /d1–3/3w plus cisplatin 30 mg/m ² /d2–5/3w	1997	Phase II trial	First-line	56	54	-	-	Jelić et al. (1997)
Etoposide 200 mg/m ² /72 h–infusion/3w	1997	Phase II trial	≥2 line	16	0	1.4	3.7	Crawley et al. (1997)
Cyclophosphamide 500 mg/m ² /d1/3w plus vincristine 1.5 mg/m ² /d1/3w plus doxorubicin 50 mg/m ² /d1/3w plus dacarbazine 750 mg/m ² /d1/3w	1995	Phase II trial	First-line	142	28.4	11.2	11.9	Santoro et al. (1995)
Cisplatin 400 mg/m ² /d1–5/4w	1990	Phase II trial	Any-line	40	15	-	-	Budd et al. (1990)
Epirubicin 60 mg/m ² /d1–3/3w plus cisplatin 30 mg/m ² /d2–5/3w	1990	Phase II trial	First-line	35	57	-	-	Jelić et al. (1990)
Doxorubicin 70 mg/m ² /d1/3w plus vindesine 3 mg/m ² /d1/3w	1990	Phase III trial	First-line	147	18	4	9.9	Borden et al. (1990)
Cisplatin 120 mg/m ² /3w	1987	Phase II trial	≥2 line	26	4	-	-	Sordillo et al. (1987)
Cisplatin 120 mg/m ² /3w	1982	Phase II trial	≥2 line	36	6	-	-	Brenner et al. (1982)
Methotrexate 4 g/m ² /3w	1980	Phase II trial	≥2 line	18	5.6	-	-	Karakousis et al. (1980)

STSs, soft tissue sarcomas; ORR, objective response rate; M-PFS, median progression-free survival; M-OS, median overall survival.

^aM-PFS, was 2.6, 2.9, 2.6, 2.1 months in the patients with adipocytic sarcoma, leiomyosarcoma, synovial sarcoma, and other types of STS, respectively.

In summary, anthracyclines, ifosfamide, trabectedin, gemcitabine, taxanes, dacarbazine and eribulin have certain activities in STSs. Vinca alkaloid agents (vindesine, vinblastine, vinorelbine, vincristine) have important therapeutic effects in specific STS subtypes, such as rhabdomyosarcomas, whereas their activity in other histological subtypes is weak. Other chemotherapeutic drugs (methotrexate, cisplatin, etoposide, pemetrexed) have weak efficacy in STSs and are rarely used.

3 Efficacy of different drugs in different STS histological subtypes

The high heterogeneity of STSs leads to a wide variety of histological subtypes. The rarity of STSs limits the development of large-scale, histologically specific clinical trials. Owing to the differences in the histological subtypes of STSs enrolled in clinical trials, there are differences in the efficacy of the same drug in various

TABLE 8 Representative studies related to chemotherapy for advanced leiomyosarcoma.

Drugs	Years of report	Study types	Setting	Number of patients	ORR	M-PFS (months)	References
Single agents							
Doxorubicin 75 mg/m ² /d1/3w	2022	Phase III trial	First-line	76	13%	6.2	Pautier et al. (2022)
Eribulin 1.4 mg/m ² /d1,8/3w	2022	Phase IV trial	≥2 line	73	7%	2.8	Kawai et al. (2022)
Doxorubicin 75 mg/m ² /3w	2020	Phase III trial	First-line	115	22.6%	6.9	Tap et al. (2020)
Trabectedin 1.5 mg/m ² /d1/3w	2019	Phase III trial	≥2 line	282	10%	4.3	Patel et al. (2019)
Dacarbazine 1,000 mg/m ² /d1/3w	2019	Phase III trial	≥2 line	141	7%	1.6	Patel et al. (2019)
Trabectedin 1.3 mg/m ² /3w	2018	Phase II trial	≥2 line	126	23.5%	4.1	Gadducci et al. (2018)
Trabectedin 1.5 mg/m ² /d1/3w	2016	Phase III trial	≥2 line	152	-	4.3	Demetri et al. (2016)
Dacarbazine 1,000 mg/m ² /d1/3w	2016	Phase III trial	≥2 line	126	-	1.6	Demetri et al. (2016)
Gemcitabine 1,000 mg/m ² /d1,8,15/4w	2012	Phase II trial	≥2 line	43	16%	5.5/6.3 ^a	Pautier et al. (2012)
Eribulin 1.4 mg/m ² /d1,8/3w	2011	Phase II trial	≥2 line	40	5%	2.9	Schoffski et al. (2011)
Eribulin 1.5 mg/m ² /d1/3w	2005	Phase II trial	≥2 line	43	11.6%	-	Le Cesne et al. (2005)
Ifosfamide 12 g/m ² /3d-infusion/4w	2000	Phase II trial	Any-line	38	5%	-	Nielsen et al. (2000b)
Combination regimens							
Doxorubicin 60 mg/m ² /d1/3w plus trabectedin 1.1 mg/m ² /d1/3w	2022	Phase III trial	First-line	74	36%	12.2	Pautier et al. (2022)
Pemetrexed 500 mg/m ² /d1/3w + cisplatin 75 mg/m ² /d1/3w	2021	Phase III trial	≥2 line	10	0%	-	Kim et al. (2021)
Doxorubicin 75 mg/m ² /d1/3w plus olaratumab 20 mg/kg/d1,8/3w in cycle 1 and 15 mg/kg in subsequent cycles	2020	Phase III trial	First-line	119	13.4%	4.3	Tap et al. (2020)
Gemcitabine 100 mg/m ² /d1,8/3w plus pazopanib 800 mg/d	2020	Phase II trial	≥2 line	106	23.8%	6.5	Pautier et al. (2020)
Doxorubicin plus dacarbazine	2020	Retrospective	First-line	107	30.9%	9.2	D'Ambrosio et al. (2020)
Doxorubicin plus ifosfamide	2020	Retrospective	First-line	71	19.5%	8.2	D'Ambrosio et al. (2020)
Doxorubicin	2020	Retrospective	First-line	115	25.6%	4.8	D'Ambrosio et al. (2020)
Gemcitabine 900 mg/m ² /d1,8/3w plus docetaxel 75 mg/m ² /d8/3w	2018	Phase II trial	≥2 line	42	29%	6.9	Gadducci et al. (2018)
Doxorubicin 60 mg/m ² /d1/3w plus trabectedin 1.1 mg/m ² /d1/3w	2015	Phase II trial	First-line	108	48%	8.2/12.9 ^b	Pautier et al. (2015)
Gemcitabine 900 mg/m ² /d1,8/3w plus docetaxel 100 mg/m ² /d8/3w	2012	Phase II trial	≥2 line	40	15%	3.8/4.7 ^c	Pautier et al. (2012)
Doxorubicin 50–75 mg/m ² /d1/3w plus ifosfamide 5 g/m ² /d1/3w	2000	Phase III trial	First-line	112	14%	-	Le Cesne et al. (2000)

ORR, objective response rate; M-PFS, median progression-free survival.

^aM-PFS, was 5.5, 6.3 months in the patients with uterine leiomyosarcoma and nonuterine leiomyosarcoma, respectively.

^bM-PFS, was 8.2, 12.9 months in the patients with uterine leiomyosarcoma and nonuterine leiomyosarcoma, respectively.

^cM-PFS, was 4.7, 3.8 months in the patients with uterine leiomyosarcoma and nonuterine leiomyosarcoma, respectively.

clinical trials. In addition, different STS subtypes respond differently to the same drugs. Therefore, the results of most clinical trials on advanced STSs are not applicable to all histological subtypes. To accurately describe the sensitivity of each STS subtype to different chemotherapeutic drugs, we analyzed the results of the prospective, multicenter clinical trials mentioned earlier in this review and recorded the efficacy of various drugs in different STS subtypes. Because most studies have not reported the remission results for each STS subtype in detail, the available results for some STS subtypes are sparse and limited. Therefore, we supplemented the results of some multicenter retrospective studies on some subtypes.

3.1 Leiomyosarcoma

Leiomyosarcoma can be divided into those with uterine and non-uterine sources. The clinical characteristics of the two types of leiomyosarcoma are slightly different, and currently, the treatment options for leiomyosarcoma from both sources are the same (Pautier et al., 2022). Leiomyosarcoma is the most prevalent STS histotype, with an incidence of 0.5–1/100,000 (Hung et al., 2019; Kim et al., 2019; Gronchi et al., 2020). Thus, there has been a significant inclusion of leiomyosarcoma in most trials of STSs. According to the obtained data, the most effective agent for single-drug chemotherapy of leiomyosarcoma is doxorubicin, with an ORR of 13%–22.6% and a median PFS of 6.2–6.9 months (Table 8) (Tap et al., 2020; Pautier et al., 2022). The second is trabectedin or gemcitabine alone, which can also result in a median PFS of >4 months (Table 8) (Pautier et al., 2012; Demetri et al., 2016; Gadducci et al., 2018). Ifosfamide, dacarbazine, and eribulin alone show mild efficacy against leiomyosarcoma (Table 8). Currently, doxorubicin plus trabectedin is the most effective chemotherapy regimen for the treatment of leiomyosarcoma, with an ORR >36% and a median PFS of >12 months (Pautier et al., 2015). Gemcitabine-based combination chemotherapy also results in a median PFS of >6 months (Table 8). In addition, a retrospective study suggested that doxorubicin plus dacarbazine and doxorubicin plus ifosfamide also achieved better efficacy in advanced leiomyosarcoma (D'Ambrosio et al., 2020).

In summary, doxorubicin-based chemotherapy remains the first-line treatment for advanced leiomyosarcoma, with doxorubicin plus trabectedin achieving the longest median PFS. Gemcitabine-based chemotherapy also has good efficacy in leiomyosarcoma. However, other chemotherapeutic drugs show lower activity against leiomyosarcoma.

3.2 Liposarcomas

Liposarcomas are divided into well differentiated, dedifferentiated, myxoid, round cell, and pleomorphic subtypes. Each histological subtype has a unique clinical presentation and therapeutic response (Lee et al., 2018). Therefore, differences in the histological subtypes of the recruited patients may lead to large differences in the outcomes of different clinical trials. However, owing to the rarity of various histological subtypes of liposarcoma, most clinical trials have not differentiated responses between

different subtypes of liposarcoma. To date, the most effective chemotherapeutic drug for treating advanced liposarcomas is doxorubicin, with a median PFS of 6.7 months (Table 9) (Demetri et al., 2012). Gemcitabine plus docetaxel also shows good efficacy, with a median PFS of 5.6 months (Table 9) (Jones et al., 2019). Trabectedin and eribulin, which have high activity in liposarcoma, achieve a median PFS of only approximately 3 months in clinical trials related to liposarcoma (Table 9), which is significantly lower than that of doxorubicin-based chemotherapy. This may be related to the fact that almost all clinical trials of trabectedin and eribulin in liposarcoma are set at second- or above-line setting. In addition, dacarbazine can only achieve a median PFS of <2 months in liposarcoma (Table 9).

In summary, doxorubicin-based chemotherapy or gemcitabine plus docetaxel is the first recommended option for advanced liposarcomas. In addition, it is worth testing the activity of trabectedin or eribulin alone or in combination with other drugs for advanced liposarcomas in a first-line setting.

3.3 Synovial sarcoma

Synovial sarcoma is a rare histotype of STSs, with an incidence of 0.1–0.5/100,000 (Wibmer et al., 2010; Hung et al., 2019; Aytekin et al., 2020). Clinical trials specifically targeting synovial sarcoma are rare. Extracting detailed treatment data for patients with synovial sarcoma from most clinical trials of STSs is also difficult. Nevertheless, important information can still be obtained. In prospective clinical trials, the currently proven drug with the best efficacy for the treatment of synovial sarcoma is ifosfamide (Table 10) (Nielsen et al., 2000b; Tap et al., 2017). Doxorubicin and eribulin also exhibit certain activities in synovial sarcoma (Table 10). However, gemcitabine, docetaxel, and dacarbazine show only weak activity against synovial sarcoma (Table 10). Retrospective studies confirmed these conclusions (Ferrari et al., 2015; Sanfilippo et al., 2015; Desai et al., 2018; Pender et al., 2018; Stacchiotti and Van Tine, 2018; Carter et al., 2020; Kogushi et al., 2020). A retrospective study demonstrated that trabectedin had activity in synovial sarcoma (Sanfilippo et al., 2015).

3.4 Other STS histological subtypes

The ORR of doxorubicin plus ifosfamide for undifferentiated pleomorphic sarcoma (UPS) is 29% (Le Cesne et al., 2000), that of doxorubicin plus dacarbazine is 26% (Zalupski et al., 1991), that of gemcitabine plus docetaxel is 11%–36% (Maki et al., 2007; Choi et al., 2018; Jones et al., 2019), and that of eribulin is 11% (Kawai et al., 2022).

Clinical trials of chemotherapy for angiosarcoma are rare. The only clinical trial has demonstrated an ORR of 45% for paclitaxel plus bevacizumab for the treatment of advanced angiosarcoma (Bui et al., 2018). Numerous other retrospective studies have demonstrated that doxorubicin- and gemcitabine-based chemotherapies can also achieve efficacy similar to that of paclitaxel in angiosarcoma (Skubitz and Haddad, 2005; Schlemmer et al., 2008; Penel et al., 2012; Stacchiotti et al., 2012; Young et al., 2014; Choi et al., 2018; Watson et al., 2022). In addition,

TABLE 9 Representative studies related to chemotherapy for advanced liposarcoma.

Drugs	Years of report	Study types	Setting	Number of patients	ORR	M-PFS (months)	References
Cabazitaxel 25 mg/m ² /3w	2022	Phase II trial	1–2 line	38	8%	6	Sanfilippo et al. (2022)
Eribulin 1.4 mg/m ² /d1,8/3w	2022	Phase IV trial	≥2 line	64	4.7%	3.2	Kawai et al. (2022)
Trabectedin 1.5 mg/m ² /d1/3w	2019	Phase III trial	≥2 line	102	9%	3	Patel et al. (2019)
Dacarbazine 1,000 mg/m ² /d1/3w	2019	Phase III trial	≥2 line	52	6%	1.5	Patel et al. (2019)
Gemcitabine 900 mg/m ² /d1,8/3w plus docetaxel 75 mg/m ² /d8/3w	2019	Phase II trial	Any-line	15	-	5.6	Jones et al. (2019)
Eribulin 1.4 mg/m ² /d1,8/3w	2017	Phase III trial	≥3 line	71	1.4%	2.9	Demetri et al. (2017)
Dacarbazine 850–1,200 mg/m ² /d1/3w	2017	Phase III trial	≥2 line	72	0%	1.7	Demetri et al. (2017)
Doxorubicin 75 mg/m ² /d1/3w	2012	Phase II trial	First-line	22	-	6.7	Demetri et al. (2012)
Eribulin 1.4 mg/m ² /d1,8/3w	2011	Phase II trial	≥2 line	37	3%	2.6	Schoffski et al. (2011)

ORR, objective response rate; M-PFS, median progression-free survival.

TABLE 10 Representative studies related to chemotherapy for advanced synovial sarcomas.

Drugs	Years of report	Study types	Phase	Number of patients	ORR (%)	M-PFS (months)	References
Eribulin 1.4 mg/m ² /d1,8/3w	2022	Phase IV trial	≥2 line	13	23	3.7	Kawai et al. (2022)
Eribulin 1.4 mg/m ² /d1,8/3w	2011	Phase II trial	≥2 line	19	5	2.6	Schoffski et al. (2011)
Gemcitabine plus docetaxel	2007	Phase II trial	Any-line	9	0	-	Maki et al. (2007)
Doxorubicin 30 mg/m ² /d1–2/3w plus ifosfamide 3.75 g/m ² /d1–2/3w	2003	Phase II trial	First-line	12	42	-	Edmonson et al. (2003)
Ifosfamide 12 g/m ² /3d–infusion/4w	2000	Phase II trial	Any-line	22	36	-	Nielsen et al. (2000b)
Doxorubicin plus ifosfamide	2000	Phase III trial	First-line	29	28	-	Le Cesne et al. (2000)
Doxorubicin plus dacarbazine	1991	Phase III trial	Any-line	8	0	-	Zalupski et al. (1991)

ORR, objective response rate; M-PFS, median progression-free survival.

eribulin is believed to exert activity in angiosarcoma (Kawai et al., 2022).

Doxorubicin plus ifosfamide is the most effective chemotherapy for treating malignant peripheral nerve tumors (MPNTs) (with an ORR of >20%) (Kroep et al., 2011). Doxorubicin and dacarbazine also have therapeutic effects (Zalupski et al., 1991). Gemcitabine, docetaxel, and eribulin are also ineffective against MPNT (Maki et al., 2007; Choi et al., 2018; Kawai et al., 2022).

Doxorubicin plus ifosfamide has a similar efficacy to gemcitabine plus docetaxel in epithelioid sarcoma, and both have moderate activity (Choi et al., 2018; Frezza et al., 2018; Touati et al., 2018).

Gemcitabine plus docetaxel chemotherapy has mild activity in clear cell sarcoma (Cojocaru et al., 2020).

3.5 Specific STS histological subtypes

In terms of chemotherapy, the main specific STS histological subtypes include the rhabdomyosarcoma family and the Ewing sarcoma family of tumors (Granowetter et al., 2009; Gallego et al., 2021; Agaram, 2022). They are more sensitive to chemotherapeutic drugs than other STSs (Chen et al., 2019; Bisogno and Hawkins, 2020; Gallego et al., 2021; Riggi et al., 2021; Setty et al., 2023). However, the sensitive drugs of these specific STS histological subtypes are significantly different from non-specific STS subtypes. Rhabdomyosarcomas can be divided into several subtypes, and the first-line chemotherapy drugs varies among different subtypes (Agaram, 2022; Sparber-Sauer, 2022; Bisogno et al., 2023). The first-line chemotherapy drug for

pleomorphic rhabdomyosarcoma and adult spindle cell rhabdomyosarcoma is usually doxorubicin (Gallego et al., 2021; Gronchi et al., 2021), and for other subtypes include ifosfamide, vincristine, actinomycin D, doxorubicin, cyclophosphamide, and vinorelbine (Walterhouse et al., 2014; Walterhouse et al., 2017; Bisogno et al., 2019; Schoot et al., 2022; Bisogno et al., 2023). Ewing sarcoma family of tumors are considered main members of small round cell sarcomas (Rajwanshi et al., 2009; Marino-Enriquez and Fletcher, 2014; Domanski, 2022; Gajdzis et al., 2022). Vincristine, doxorubicin, cyclophosphamide, ifosfamide, and etoposide are the first-line drugs recommended for Ewing sarcomas (Brennan et al., 2022). Vincristine, irinotecan, and temozolomide are the recommended drugs for patients with rhabdomyosarcoma or Ewing sarcoma with recurrent or frontline chemotherapy failure (Defachelles et al., 2021; Xu et al., 2023). Trabectedin, gemcitabine, taxanes, dacarbazine and eribulin show ineffective or uncertain efficacy against rhabdomyosarcomas or Ewing sarcomas (Etcubanas et al., 1985; Baruchel et al., 2012; Mora et al., 2017; Oesterheld et al., 2020; Kawai et al., 2022).

In addition to the STS subtypes described above, dozens of other STS subtypes require chemotherapy in advanced stages. However, no specific prospective clinical trial has confirmed the chemotherapeutic efficacy of these STS subtypes. Generally, most silent STS subtypes are treated based on data from nonspecific STS clinical trials (Tables 1–7).

4 Discussion

We conducted this review to provide a reference for the selection of chemotherapeutic drugs for advanced STSs. In this study, we comprehensively reviewed the results of representative clinical trials related to chemotherapy for STS over the past 30 years and supplemented with some retrospective studies. Numerous clinical trial results have shown that doxorubicin is the most effective drug and remains the mainstay of chemotherapy for advanced STSs. In addition, ifosfamide, trabectedin, gemcitabine, taxanes, dacarbazine, and eribulin have certain activities in STSs and are commonly used in the real world. Vinca alkaloid agents (vindesine, vinblastine, vinorelbine, vincristine) have important therapeutic effects in special STS subtypes, such as rhabdomyosarcomas and Ewing sarcomas, whereas their activity in other histological subtypes is weak. Other chemotherapeutic drugs (methotrexate, cisplatin, etoposide, pemetrexed) have weak efficacy against STSs and are rarely used. Sensitive chemotherapeutic drugs vary for each STS histotype. Doxorubicin-based chemotherapy is the most effective treatment for leiomyosarcoma, liposarcoma, UPS, angiosarcoma, MPNT, and epithelioid sarcoma. Ifosfamide is the most effective chemotherapeutic drug for synovial sarcoma. Gemcitabine plus docetaxel shows good efficacy against many STS subtypes. However, except for the few histological subtypes mentioned above, other STS subtypes have become the silent majority, and few large sample size studies have focused on and reported the chemotherapeutic efficacy of these STSs in detail.

With an increase in drugs used for second- or above-line treatment, a better understanding of histotype-oriented therapy,

and improved supportive care in oncology, the survival period of patients with advanced STSs has increased over the past decade (Kollar et al., 2019; Smrke et al., 2020; Stricker et al., 2023). The number of treatment lines for these patients is increasing, as is the demand for sensitive chemotherapeutic drugs. This study has important reference value for drug selection in multiline therapy of STSs. In addition, with the widespread and in-depth application of targeted drugs in STSs, the selection of specific chemotherapeutic drugs based on different histological subtypes in combination with targeted drugs is inevitable to achieve better therapeutic effects. This study provides important reference value for the selection of chemotherapeutic drugs for these combined regimens.

STSs are characterized by a low incidence rate and high heterogeneity compared with other cancers. The low incidence rate has led to a considerable number of STS histological subtypes not being studied in depth and has also led to a delay in the research and development of chemotherapeutic drugs related to STSs. Almost no important chemotherapeutic drugs for STSs have emerged in the last decade. The high heterogeneity of STSs has led to significant differences in the outcomes of clinical trials of chemotherapy in different STSs, leading to an inability to accurately compare the efficacy of different drugs in STSs. For example, although many studies have confirmed that different histological subtypes of STSs have different sensitivities to chemotherapy, the results of an important clinical trial showed that in a population of patients with high-risk STSs, there was no benefit of neoadjuvant histotype-tailored chemotherapy regimens over the standard doxorubicin plus ifosfamide chemotherapy (Gronchi et al., 2017; Gronchi et al., 2020). To eliminate the influence of the low incidence rate and high heterogeneity of STSs on judging the efficacy of chemotherapeutic drugs, significant work needs to be carried out, including the following: 1) Clinical trials targeting different STS subtypes should be conducted as much as possible, whereas clinical trials targeting nonselective STSs should be conducted to reduce the effects of high heterogeneity. 2) Detailed histotype data should be reported for clinical trials of STSs. We found that many clinical trials did not report the histotype outcomes, leading to difficulties in histotype studies. Reporting detailed histotype data is a fundamental requirement for these clinical trials. 3) The evaluation criteria are unified. Early evaluation of the chemotherapeutic efficacy of STSs often uses ORR while ignoring other indicators. The ORR does not represent the median PFS and OS. Therefore, the reference value for early clinical trials is limited. Currently, the number of drugs and lines for advanced STSs has increased significantly, and there is a significant error in using the median OS as the main evaluation index. We recommend using the median PFS and 3- or 6-month PFS rates as the main evaluation indicators.

We conducted extensive searches and reviews to include all the relevant studies. However, our approach does not represent a complete review of the literature. For example, some studies may have been omitted because we only included studies published in English and excluded most of the retrospective studies. However, efforts have been made to ensure the inclusion of key studies on the treatment of advanced STSs.

In conclusion, anthracyclines are the most important systemic treatment for advanced STSs. Ifosfamide, trabectedin, gemcitabine, taxanes, dacarbazine, and eribulin exhibit certain activities in STSs. Other chemotherapeutic drugs have weak efficacy against STSs and are rarely used. Depending on the histological subtypes, it is necessary to select specific second- or above-line chemotherapeutic drugs.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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Oral fibroblasts rescue osteogenic differentiation of mesenchymal stem cells after exposure to Zoledronic acid in a paracrine effect

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Background: Medication-related osteonecrosis of the jaw is a serious complication that develops in oncologic patients treated with Zoledronic acid. Although used for over 30 years, the influence of Zoledronic acid on bone has been thoroughly investigated, mainly on osteoclasts. While decreasing osteoclast differentiation and function, for many years it was thought that Zoledronic acid increased osteoblast differentiation, thus increasing bone volume. Moreover, despite the influence of soft tissue on the bone healing process, the impact of zoledronic acid on the interaction between soft tissue and bone was not investigated.

Aim: Our goal was to investigate the influence of Zoledronic Acid and soft tissue cells on osteogenic differentiation of mesenchymal stem cells (MSCs).

Materials and methods: Osteogenic differentiation of MSCs was examined after exposure to Zoledronic Acid. To determine the influence of soft tissue cells on MSCs' osteogenic differentiation, conditioned media from keratinocytes and oral fibroblasts were added to osteogenic medium supplemented with Zoledronic Acid. Proteomic composition of keratinocytes' and fibroblasts' conditioned media were analyzed.

Results: Zoledronic Acid decreased osteogenic differentiation of MSCs by seven-fold. The osteogenic differentiation of MSCs was restored by the supplementation of fibroblasts' conditioned medium to osteogenic medium, despite Zoledronic acid treatment. Five osteogenic proteins involved in the TGF β pathway were exclusively identified in fibroblasts' conditioned medium, suggesting their role in the rescue effect.

Conclusion: Oral fibroblasts secrete proteins that enable osteogenic differentiation of MSCs in the presence of Zoledronic Acid.

Abbreviations: ZA, zoledronic acid; MRONJ, medication related osteonecrosis of the jaw; MSCs, mesenchymal stem cells; PDL, periodontal ligament; BM, bone marrow; CMFib, fibroblasts conditioned medium; CMKr, keratinocytes conditioned medium.

KEYWORDS

osteonecrosis, Zoledronic acid, fibroblasts, mesenchymal stem cells, bone

Introduction

Medication-related osteonecrosis of the jaw (MRONJ) is a severe complication of oral infection or oral surgical procedure which develops in patients treated with Zoledronic Acid (ZA). The clinical manifestation of MRONJ includes the presence of exposed bone in the oral cavity for more than 8 weeks, in patients with previous or current use of bone-modifying agents and no history of radiation therapy to the head and neck region (Ruggiero et al., 2022). ZA is considered the most potent drug that causes MRONJ. The occurrence of MRONJ, reported to be 1%–9% in oncologic patients treated with ZA, is relatively high considering that ZA is commonly used to prevent skeletal complications associated with a variety of malignancies, e.g., lung, renal, breast, and prostatic cancers, and multiple myeloma (Yarom et al., 2019).

Surgical resection of the jaw is the main treatment for stage 3 MRONJ and sometimes is implicated in resistant stage 2 cases (Ruggiero et al., 2022). Therefore, prevention of MRONJ is of paramount importance (Yarom et al., 2019; Ruggiero et al., 2022).

Bone remodeling is regulated by a dialogue between osteocytes that reside within the mineralized matrix of bone and cells situated in the soft tissue external or internal to the mineralized matrix, i.e., mesenchymal stem cells (MSCs), osteoblasts, and osteoclasts (Bonewald, 2011). Since first reported in 2003 (Marx, 2003), studies of MRONJ had focused on the inhibitory effect of ZA on osteoclasts via impairing the mevalonic acid pathway (Russell, 2011; Huang et al., 2019; Wang et al., 2020). Soft tissue toxicity is another adverse effect of ZA that has been shown to interfere with bone repair (Reid et al., 2007; Mozzati et al., 2013). Several studies had shown that ZA has toxic effects on epithelial cells, fibroblasts, and endothelial cells (Saracino et al., 2012; Mozzati et al., 2013), thereby interfering with the normal process of wound healing. Scheper et al. (Scheper et al., 2009; Scheper et al., 2010) were the first to show that low concentrations of ZA released from bone can negatively affect the oral mucosal tissues. The exact ZA concentration in the alveolar bone remains unclear. Early studies used 5 μ M ZA as the closest concentration to accumulate in the alveolar bone (Scheper et al., 2009) and recent studies use 5 μ M and 10 μ M as subtoxic concentrations (Zara et al., 2015; di Vito et al., 2020).

Our research hypothesis was that ZA impacts the osteogenic differentiation of MSCs but the detrimental effect of ZA is modulated by soft tissue cells, i.e., fibroblasts and keratinocytes. The aim of the study was to investigate the influence of keratinocytes and fibroblasts, the main cellular components of the soft tissue, on MSCs' osteogenic differentiation after exposure to ZA.

Materials and methods

Soft tissue cells culture

Fibroblasts cell culture

Three types of primary human oral fibroblasts were used in this study: 1) primary human gingival fibroblasts (ATCC, PCS201-018,

Manassas, VA); 2) primary human lining mucosa fibroblasts, which were obtained from the buccal lining mucosa of the anterior mandible; and 3) primary human masticatory mucosa fibroblasts, which were obtained from the hard palate (Kabakov et al., 2021). Cells from passages 3–5 were cultured in a 10 mm plate at a density of 2×10^6 cells per plate (Corning, Glendale, AZ, United States). Cells were cultured in Dulbecco's Modified Eagle medium (DMEM, Biological Industries, Beit Haemek, Israel) high glucose (gingival fibroblasts) or low glucose (lining/masticatory fibroblasts), supplemented with 10% FBS, 1% penicillin–Streptomycin – Amphotericin B Solution (PSA), then 1% L-Glutamine. Cells were incubated at 37°C and 5% CO₂.

Keratinocytes cell culture

Human Keratinocytes cells (HaCaTs cell line) were seeded at a density of 1×10^6 cells/10 mm plate and cultured in DMEM high glucose supplemented with 10% FBS, 1% PSA, and 1% L-Glutamine until 80% confluence. Cells were incubated at 37°C and 5% CO₂. HaCats were cultured, and expanded according to a previously published protocol (Gamady et al., 2003; Tamari et al., 2019).

Bone cells culture: mesenchymal stem cells culture

Two types of primary human MSCs were used in this study: 1) Primary human bone marrow-derived mesenchymal stem cells (BM-MSCs) (Srouji et al., 2011) and 2) Primary human periodontal ligament derived stem cells (PDL-MSCs) (Somerman et al., 1988). Cells were used from passages 3 to 5 and were cultured in alpha MEM medium (Biological Industries, Beit Haemek, Israel) supplemented with 10% FBS, 1% PSA, and 1% L-Glutamine. Cells were incubated at 37°C and 5% CO₂.

All growth media types (DMEM High, DMEM Low and alpha MEM α) supplemented with 10% FBS, 1% PSA, and 1% L-Glutamine, are referred as “growth medium” in this study.

Conditioned medium preparation

1.5×10^6 cells were seeded in a 10 mm culture plate and cultured with 10 mL medium until reaching 80% confluence. Medium was changed to fresh medium and following 24 h incubation 10 mL was collected and stored at –20°C.

5 μ M ZA solution preparation

190 μ L from 4 mg/5 mL ZA (Actavis Italy SpA, Milan, Italy) was diluted in 60 μ L growth medium to create stock solution no. 1. 200 μ L from stock solution 1 were further diluted in 1,800 mL of growth medium to create stock solution 2. 500 μ L from stock solution 2 were diluted in 9.5 mL growth medium to create 10 mL of 10 μ M ZA solution, and 250 μ L from stock solution 2 diluted in 9.5 mL of growth medium created 9.75 mL of 5 μ M ZA solution.

Osteogenic differentiation of MSCs

BM and PDL MSCs were cultured in osteogenic medium composed of alpha MEM medium, supplemented with 10^{-7} M dexamethasone, 5×10^{-5} M Ascorbic Acid (Sigma-Aldrich, St. Louis, MO), and 10^{-2} mM β Glycerol phosphate disodium salt.

Analysis of osteogenic differentiation: alkaline phosphatase staining

Following 6 days of culture in osteogenic medium, solutions were replaced by BCIP (5-bromo-4-chloro-3-indolyl phosphate; #11383221001) and NBT (nitro blue tetrazolium chloride; #11383213001; Sigma-Aldrich, St. Louis, MO) which were used as an insoluble substrate for the detection of alkaline phosphatase. Substrate solution was removed, and then the cells were washed with phosphate-buffered saline (PBS) and imaged.

Analysis of osteogenic differentiation: Alizarin red staining

Following 14 days of culture in osteogenic medium, cells were fixed using 4% paraformaldehyde. A solution of 2% alizarin red (Sigma-Aldrich, St. Louis, MO) was added to each well, and the cells were incubated at 37°C for 30 min in the dark. The alizarin red solution was removed, after which cells were washed four times with dH_2O and imaged.

Analysis of osteogenic differentiation: von Kossa reaction

Following 14 days of culture in osteogenic medium, cells in each group were fixed with 4% paraformaldehyde for 15 min at room temperature and washed three times with distilled water. Next, the cells were exposed to 5% silver nitride solution under UV radiation for 30 min at room temperature. The wells were then washed two times with distilled water and observed in a light microscope.

Preparation of conditioned media mixed with osteogenic media solution supplemented with 5 μM ZA

PDL and BM MSCs were cultured in osteogenic medium for 6 and 14 days. 10 μM ZA solution was prepared as previously described (instead of diluting ZA in growth medium, it was diluted in osteogenic medium) and mixed with soft tissue cells conditioned media in 1:1 volume ratio to achieve a final concentration of 5 μM ZA.

Quantification of ALP staining, Alizarin red, and von Kossa staining

Each experiment was repeated three times (three different biological repetitions). In each experiment 10 wells were included

for each group (technical repeats). Samples were visually inspected using a light microscope (Nikon, Tokyo 108-6290, Japan) using $\times 4$, $\times 10$, $\times 20$ and $\times 40$ objectives. The same setting of color balance, brightness and contrast were used for each image. 15 photographs were taken from each well in $\times 10$ magnification. Quantification made using ImageJ software (National Institute of Health (NIH)) according to their instructions (O'Brien et al., 2016).

Proteomics

Keratinocytes and gingival fibroblasts were cultured in growth medium until 80% confluence. Medium was replaced with starvation medium (0% FBS). After 24 h, 10 mL of the conditioned medium was collected from each type of cells in aliquots of 1 mL and stored at -20°C for proteomics.

Proteolysis

Medium samples were supplemented with: 8M Urea, 400 mM Ammonium bicarbonate and 10 mM DTT. Protein amount was estimated using Bradford readings. 10 μg protein from the sample were reduced with DTT (60°C for 30 min), modified with 8.8 mM iodoacetamide in 400 mM ammonium bicarbonate (in the dark, room temperature for 30 min) and digested in 2M Urea, 25 mM ammonium bicarbonate with modified trypsin (Promega) at a 1:50 enzyme-to-substrate ratio, overnight at 37°C. An additional second digestion with trypsin was done for 4 h at 37°C.

Mass spectrometry analysis

The eluted peptides were desalted using C18 tips (Homemade stage tips) dried and re-suspended in 0.1% formic acid.

The peptides were resolved by reverse-phase chromatography on $0.075 \times 30\text{-mm}$ fused silica capillaries (J&W) packed with Reprosil reversed phase material (Dr Maisch GmbH, Germany). The peptides were eluted with linear 60 min gradient of 5%–28% 15 min gradient of 28%–95% and 15 min at 95% acetonitrile with 0.1% formic acid in water at flow rates of 0.15 $\mu\text{L}/\text{min}$. Mass spectrometry was performed by Q-Exactive plus mass spectrometer (Thermo) in a positive mode (m/z 300–1800, resolution 70,000 for MS1 and 17,500 for MS2) using repetitively full MS scan followed by high collision dissociation (HCD, at 25 normalized collision energy) of the 10 most dominant ions (>1 charges) selected from the first MS scan. The AGC settings were 3×10^6 for the full MS and 1×10^5 for the MS/MS scans. The intensity threshold for triggering MS/MS analysis was 1.7×10^4 . A dynamic exclusion list was enabled with exclusion duration of 20 s.

The mass spectrometry data was analyzed using Proteome Discoverer 2.4 software with Sequest (Thermo) algorithms against human database with 1% FDR.

Minimal peptide length was set to six amino acids and a maximum of two mis-cleavages was allowed. Semi quantitation was done by calculating the peak area of each peptide based its extracted ion currents (XICs), and the area of the protein is the average of the three most intense peptides from each protein.

The additional statistical analysis was done by Perseus 1.6.15.0.

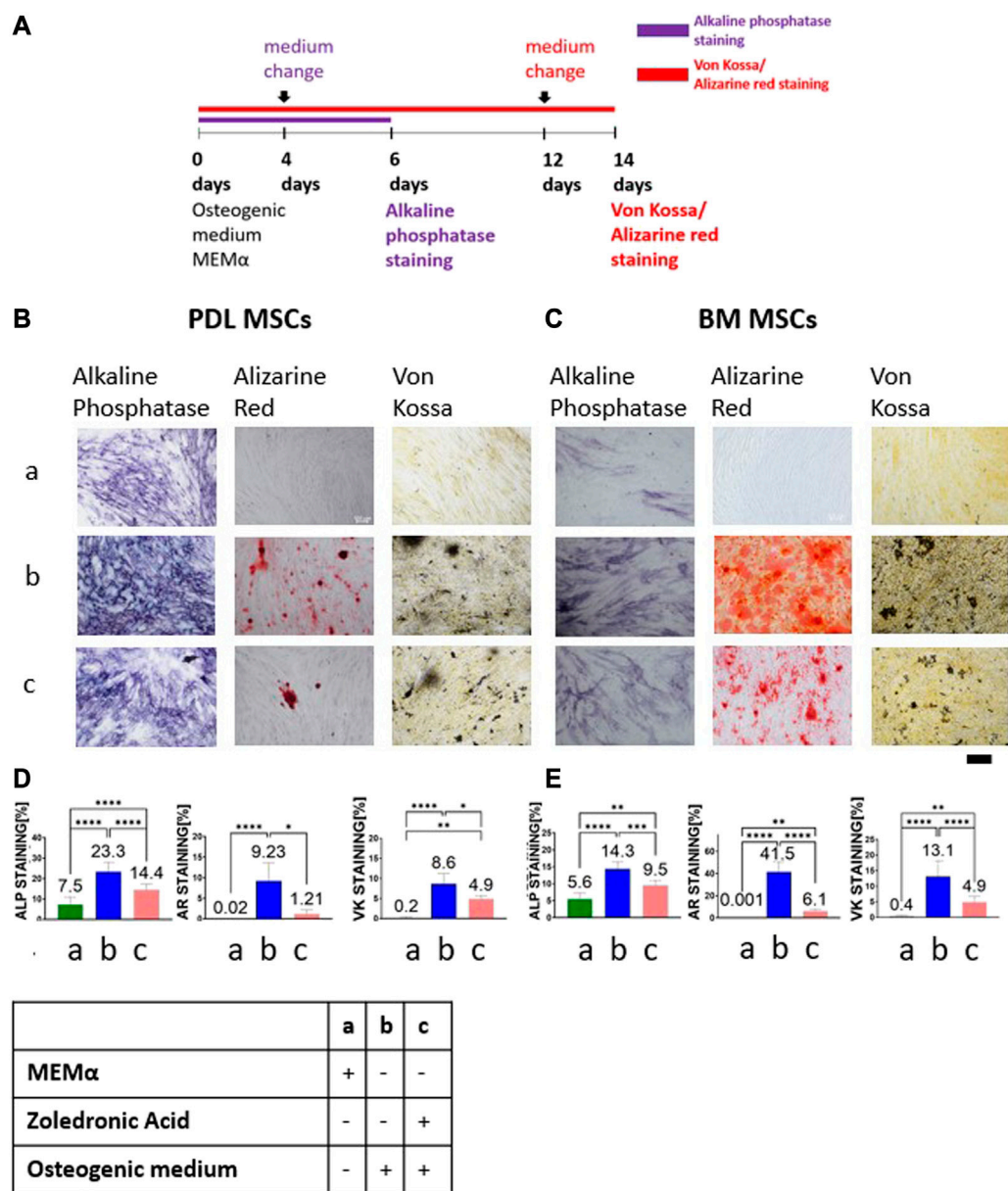


FIGURE 1
ZA decreased MSCs osteogenic differentiation as detected by alkaline phosphatase staining, Alizarin Red and von Kossa staining. Study timeline is shown in (A) Representative light microscope images of PDL and BM MSCs (x10 magnification) with Alkaline phosphatase staining, Alizarin Red and von Kossa staining shown in (B,C). Scale bar represents 400 μm. Statistical analysis of the percentage of the staining for PDL MSCs (D) and BM MSCs (E). MEMα showed the weakest staining and osteogenic medium the strongest staining. 5 μM ZA added to osteogenic medium significantly reduced the percentage of the staining as compared to osteogenic medium in both cell types, in all staining methods. Statistical analysis was performed using one way ANOVA and *post hoc* Tukey tests. **p* < 0.05, ***p* < 0.001, *****p* < 0.0001.

Statistics

Experiments in this study repeated three times to ensure reproducibility. Data were presented as the means ± SE/SD. The significance of the results obtained from control and treated groups were analyzed by GraphPad Prism 9.0 (GraphPad software Inc.) using parametric and non-parametric statistical tests. Differences were considered significant when *p*-value < 0.05.

Results

ZA significantly reduced osteogenic differentiation of MSCs

BM and PDL MSCs were cultured in osteogenic medium and growth medium for 6 days (Figure 1A). On day 4, treatments were changed as follow:1) MEMα as negative control; 2) osteogenic

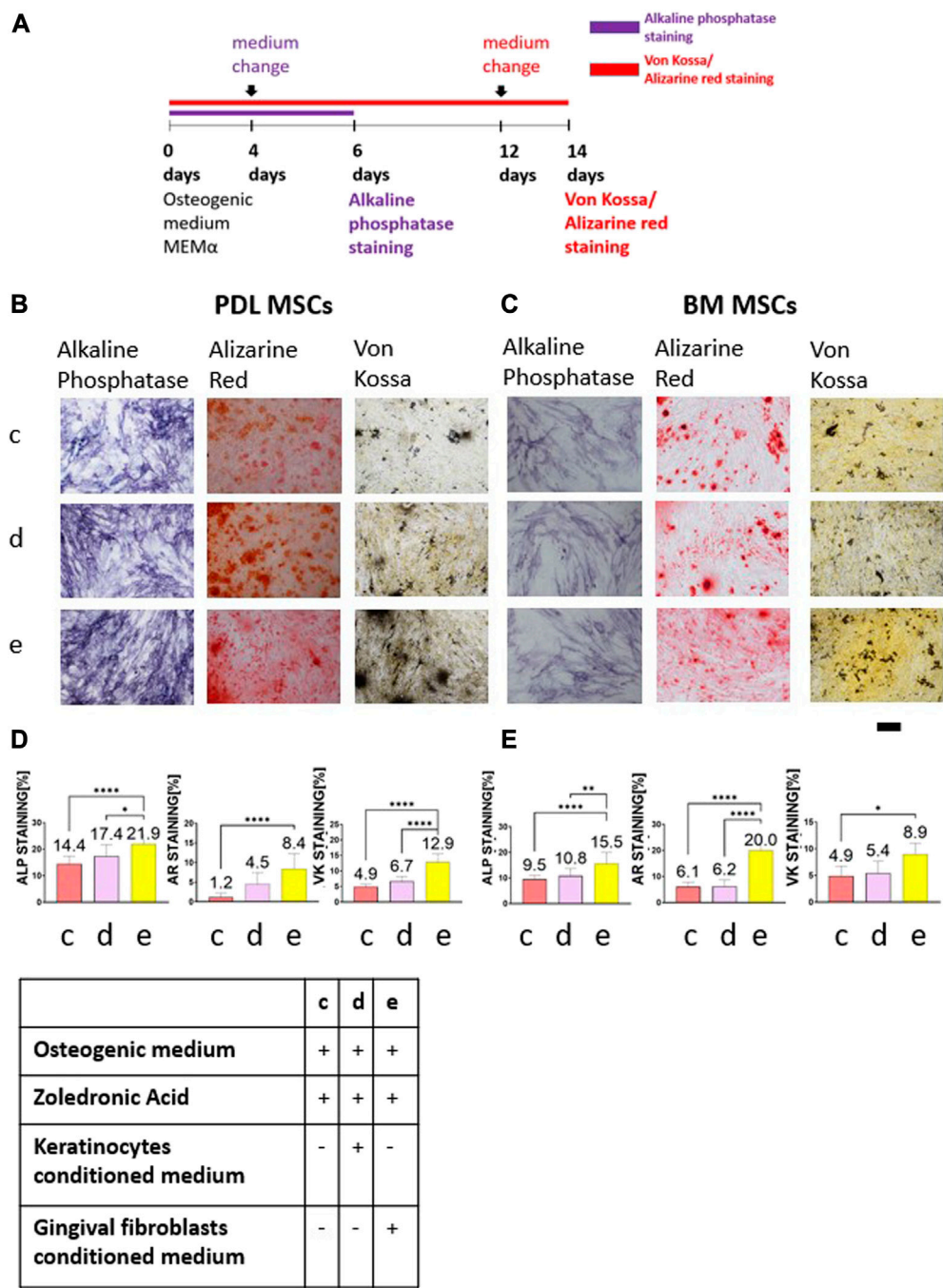


FIGURE 2 The rescue effect of primary human gingival fibroblasts conditioned medium on osteogenic differentiation after ZA exposure was detected by Alkaline phosphatase staining, Alizarin Red and von Kossa staining. Timeline is shown in (A). Representative light microscope images of PDL (B) and BM (C) MSCs with Alkaline phosphatase staining, Alizarin Red and Von Kossa staining (x10 magnification). Scale bar represents 400 μ m. Statistical analysis of the percentage of the staining for PDL-MSCs (D) and BM-MSCs (E). Cells which were exposed to 5 μ M ZA solution showed the weakest staining. Keratinocytes conditioned medium did not influence the staining for both MSCs types in all staining methods. Overall, fibroblasts conditioned medium significantly increased the staining. Statistical analysis was performed using one way ANOVA and *post hoc* Tukey tests. **p* < 0.05, ***p* < 0.001, ****p* < 0.0001.

differentiation media as positive control; and 3) 5 μ M ZA solution based on osteogenic medium. Figures 1B, C show representative images of the staining from the three groups. The statistical analysis for each of the staining is shown in Figures 1D, E.

ALP staining was quantified on day 6. The weakest ALP staining was demonstrated in the MEM α group (PDL-MSC: 7.5% $\pm$ 3.52; BM-MSC: 5.6% $\pm$ 1.7). The strongest staining was observed in the osteogenic medium group (PDL-MSC:

23.3% $\pm$ 4.64; BM-MSC: 14.3% $\pm$ 2.1). 1:1 5 μ M ZA solution based on osteogenic medium group showed a significantly lower percentage staining as compared to the osteogenic medium group (PDL-MSC: 14.4% $\pm$ 2.93; BM-MSC: 9.5% $\pm$ 1.47, p < 0.0001).

BM and PDL-MSCs were cultured in an osteogenic medium and growth medium for 14 days (Figure 1A). On day 12, medium was changed to: 1) MEMa as negative control; 2) osteogenic differentiation media, positive control; and 3) 1:1 5 μ M ZA solution based on osteogenic medium.

The MEMa group showed no Alizarin Red staining (almost 0% in both types of MSCs) as opposed to the osteogenic medium group, which showed the highest percentage staining (PDL-MSC: 9.22% $\pm$ 4.35; BM-MSC: 41.5% $\pm$ 8.94, p < 0.0001). The 1:1 5 μ M ZA solution based on osteogenic medium group showed significant decrease in staining as compared to osteogenic medium (PDL-MSC: 1.2% $\pm$ 0.96; BM-MSC: 6.1% $\pm$ 1.78, p < 0.0001). Similarly, no von Kossa staining was detected in the MEMa group for both types of MSCs (PDL-MSC: 0.2% $\pm$ 0.22; BM-MSC: 0.4% $\pm$ 0.32). The osteogenic medium group showed the highest staining (PDL-MSC: 8.6% $\pm$ 2.58; BM-MSC: 13.1% $\pm$ 5.08). 1:1 5 μ M ZA solution based on osteogenic medium significantly decreased the percentage of staining in both types of MSCs (PDL-MSC: 4.9% $\pm$ 0.84; BM-MSC: 4.9% $\pm$ 1.83, p < 0.0001).

Conditioned medium from primary human gingival fibroblasts restored osteogenic differentiation

BM and PDL MSCs were cultured in osteogenic medium for 6 or 14 days (Figure 2A). Two days before the end of the experiments, medium was changed to: 1) keratinocytes conditioned medium with osteogenic medium and 5 μ M ZA solution; 2) gingival fibroblasts conditioned medium with osteogenic medium and 5 μ M ZA solution; and 3) osteogenic medium with 5 μ M ZA solution. All solutions were prepared in a 1:1 ratio. Figures 2B, C show representative images of the staining from the three groups and the statistical analysis is presented in Figures 2D, E.

The cells cultured in osteogenic medium supplemented with 5 μ M ZA solution showed the weakest alkaline phosphatase staining (PDL-MSCs: 14.4% $\pm$ 2.93; BM-MSCs: 9.5% $\pm$ 1.47). The addition of keratinocytes conditioned medium to osteogenic medium supplemented with 5 μ M ZA solution did not change the percentage of the staining (PDL-MSC: 17.4% $\pm$ 4.32; BM-MSC: 10.8% $\pm$ 2.83). The addition of fibroblasts conditioned medium to osteogenic medium supplemented with 5 μ M ZA solution significantly increased the percentage of the staining for both cell types (PDL-MSC: 21.9% $\pm$ 3.81; BM-MSC: 15.5% $\pm$ 4.46; p < 0.0001).

Analysis of Alizarin Red staining after 14 days revealed the lowest percentage of staining in the osteogenic medium supplemented with 5 μ M ZA solution group (PDL-MSC: 1.2% $\pm$ 0.96; BM-MSC: 6.1% $\pm$ 1.78). The addition of keratinocytes conditioned medium to the osteogenic medium supplemented with 5 μ M ZA solution did not influence the percentage of the staining (PDL-MSC: 4.5% $\pm$ 2.86; BM-MSC: 6.2% $\pm$ 2.46). However, addition of fibroblasts conditioned medium to the osteogenic medium supplemented with 5 μ M ZA solution significantly

increased the staining in both types of MSCs (PDL-MSC: 8.4% $\pm$ 3.83; BM-MSC: 20% $\pm$ 6.22, p < 0.0001).

The same results were obtained using von Kossa staining. The lowest percentage staining was demonstrated in the osteogenic medium supplemented with 5 μ M ZA solution group (PDL-MSC: 4.9% $\pm$ 0.84; BM-MSC: 4.9% $\pm$ 1.83). The addition of keratinocytes conditioned medium to the osteogenic medium supplemented with 5 μ M ZA solution did not influence the staining (PDL-MSC: 6.7% $\pm$ 1.57; BM-MSCs: 5.4% $\pm$ 2.22). Increase of von Kossa staining was observed in both MSCs types after addition of fibroblasts conditioned medium to the osteogenic medium supplemented with 5 μ M ZA solution (PDL-MSC: 12.9% $\pm$ 2.52, p < 0.0001; BM-MSC: 8.9% $\pm$ 2.11, p < 0.05).

Conditioned medium from primary human lining mucosa and primary human masticatory mucosal fibroblasts restored MSCs osteogenic differentiation

Conditioned medium was collected from primary human gingival fibroblasts, primary human masticatory mucosal fibroblasts, and primary human lining mucosal fibroblasts. Conditioned medium from each cell type was mixed with ZA and osteogenic medium to create 5 μ M ZA solution. Control group contained cells cultured in 5 μ M ZA solution based on osteogenic medium. Figure 3A describes alkaline phosphatase, Alizarin Red and von Kossa staining timeline. Figures 3B, C show representative images of the staining from the four groups: osteogenic medium supplemented with 5 μ M ZA solution; human gingival fibroblasts conditioned medium diluted 1:1 with osteogenic medium supplemented with 5 μ M ZA solution, masticatory mucosal fibroblasts conditioned medium diluted 1:1 in osteogenic medium supplemented with 5 μ M ZA solution and lining mucosal fibroblasts conditioned medium diluted 1:1 with osteogenic medium supplemented with 5 μ M ZA solution. The statistical analysis is presented in Figures 3D, E.

The weakest Alizarin Red staining of PDL-MSCs was achieved in the osteogenic medium supplemented with 5 μ M ZA solution group (1.2% $\pm$ 0.96). Addition of conditioned media from three types of fibroblasts increased Alizarin Red staining: human gingival fibroblasts was 8.4% $\pm$ 3.8, p < 0.0001; masticatory mucosal fibroblasts was 32.5% $\pm$ 8.06, p < 0.0001; and lining mucosal fibroblasts was 29.7% $\pm$ 4.55, p < 0.0001. Likewise, in BM-MSCs the weakest Alizarin Red staining was detected in the osteogenic medium supplemented with 5 μ M ZA solution group (6.1% $\pm$ 1.78) while addition of fibroblasts' conditioned medium increased Alizarin Red staining (20% $\pm$ 6.22, 18% $\pm$ 7.26, and 23% $\pm$ 6.22 in human gingival fibroblasts, masticatory mucosal fibroblasts and lining mucosal fibroblasts respectively, p < 0.0001). Using von Kossa staining, similar results were found. The weakest von kossa staining of PDL-MSCs was achieved in the osteogenic medium supplemented with 5 μ M ZA solution group (4.9% $\pm$ 0.84). Addition of fibroblasts' conditioned medium increased von Kossa staining 12.9% $\pm$ 2.53, < 0.0001; 15.2% $\pm$ 4.16, p < 0.0001; and 12.9% $\pm$ 4.53, respectively, p < 0.0001, in human gingival fibroblasts, masticatory mucosal fibroblasts and lining mucosal fibroblasts respectively. The same pattern was observed in BM-MSCs.

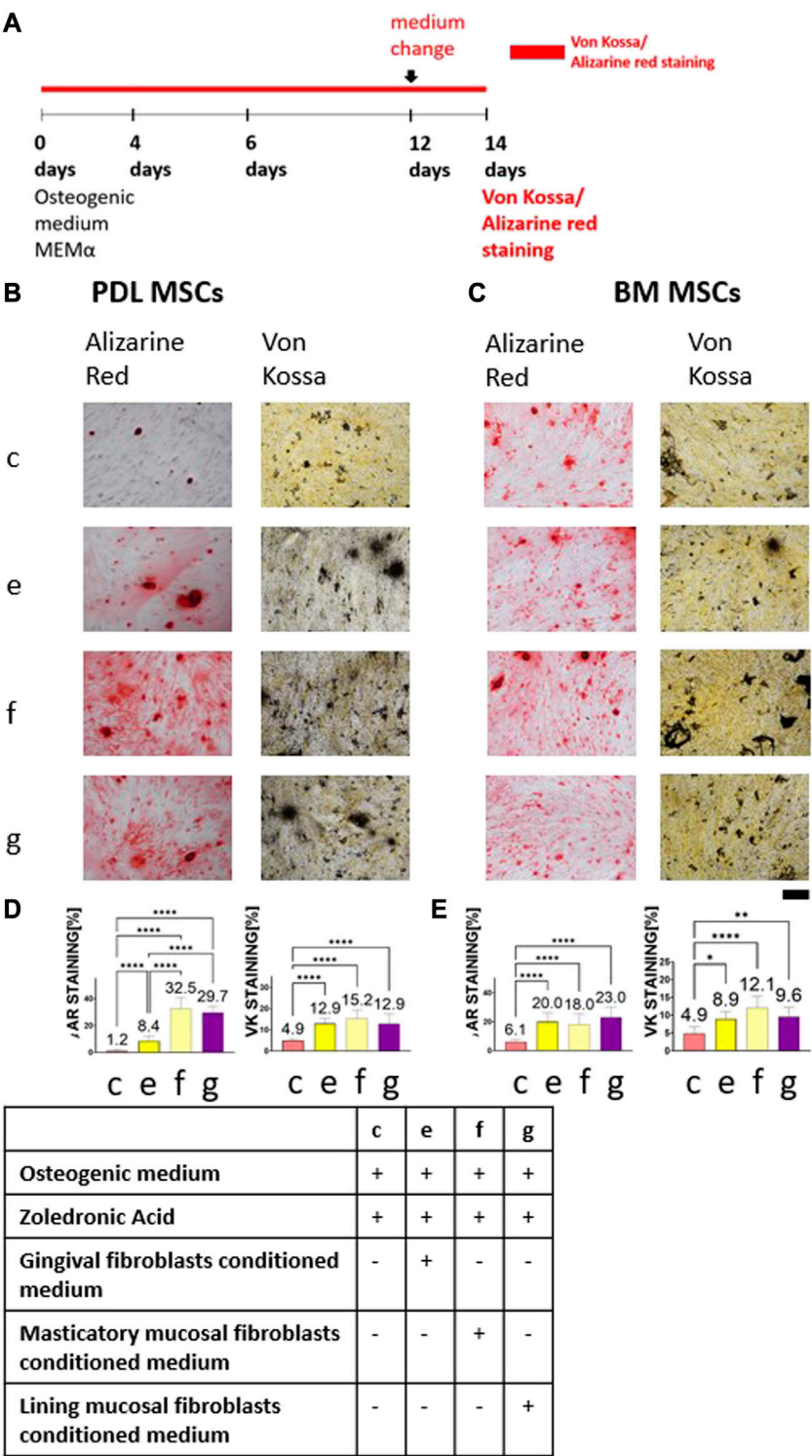
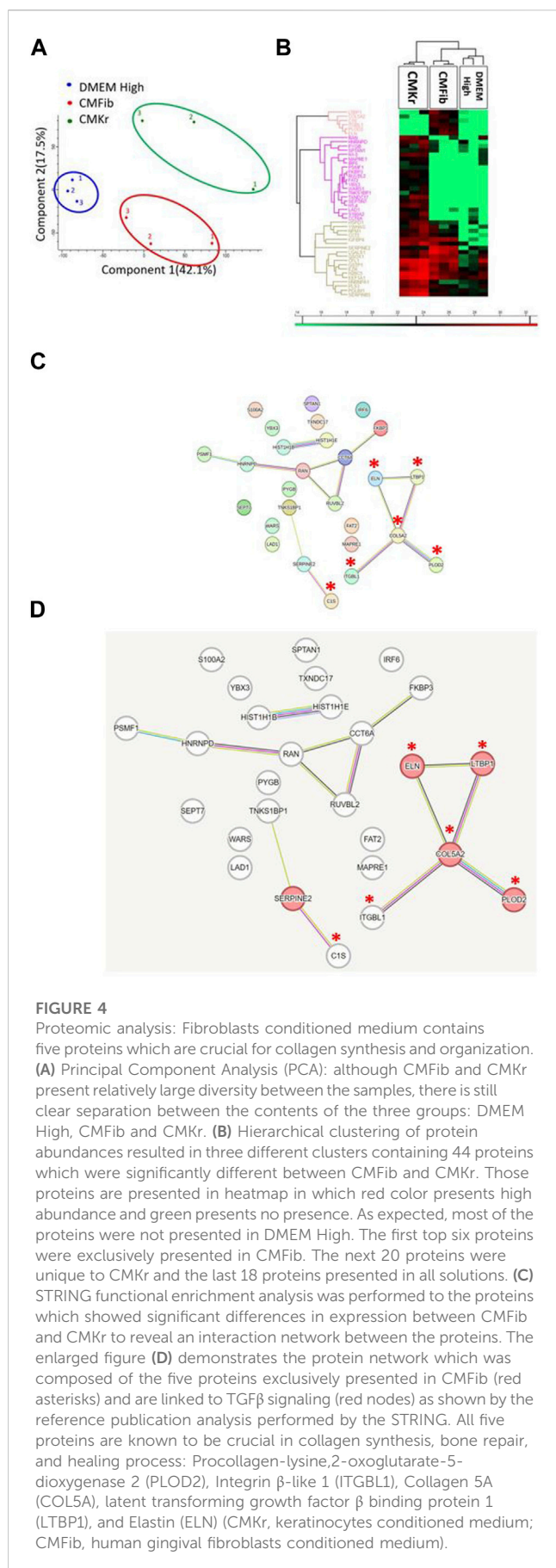


FIGURE 3
The rescue effect of human gingival fibroblasts conditioned medium on MSCs osteogenic differentiation after ZA exposure was achieved by masticatory mucosal fibroblasts and lining mucosal fibroblasts as detected by Alizarin Red and von Kossa staining. Experiment timeline is shown in (A). Representative light microscope images of PDL (B) and BM (C) MSCs with Alizarin Red and von Kossa staining (x10 magnification). Scale bar represents 400 μ m. Statistical analysis of the percentage of the staining for PDL MSCs (D) and BM MSCs (E). Cells which were exposed to 5 μ M ZA solution showed the weakest staining in both MSCs types and in all staining methods. Conditioned media obtained from three types of fibroblasts significantly increased the staining. Statistical analysis was performed using one way ANOVA and *post hoc* Tukey tests. **p* < 0.05, ***p* < 0.001, *****p* < 0.0001.



Conditioned medium from gingival fibroblasts contained specific proteins which are known to induce osteoblastic differentiation

Conditioned media from primary human gingival fibroblasts and keratinocytes were collected after 24 h of culture in starvation. For each type of cells, three samples from different cell passage were used for proteomic analysis (Figure 4). A principal component analysis of the individual samples revealed that DMEM High, fibroblasts conditioned medium, and keratinocytes conditioned medium were separated into three distinct groups (Figure 4A). This was also the case for a hierarchical cluster analysis of protein abundances (Figure 4B), which resulted in three clusters containing 44 proteins in which abundances were distinct between the two conditioned media. The first top six proteins were exclusively presented in the fibroblasts conditioned medium. The next twenty proteins were unique to keratinocytes conditioned medium and the last eighteen proteins were presented in all solutions. A STRING functional enrichment analysis of the proteins which showed significant differences in expression between fibroblasts conditioned medium and keratinocytes conditioned medium (excluding proteins in DMEM) was performed to reveal an interaction network between the proteins. Proteins which were found exclusively in the fibroblasts conditioned medium are marked with red asterisks (Figure 4C). The red nodes represent proteins which participate in the TGF β signaling according to the reference publication analysis performed by the STRING (Figure 4D) (Szkłarczyk et al., 2019). Protein exclusively found in fibroblasts conditioned medium include Procollagen-lysine, 2-oxoglutarate-5-dioxygenase 2 (PLOD2), Integrin β -like 1 (ITGBL1), collagen 5A (COL5A), latent transforming growth factor β binding protein 1 (LTBP1), and Elastin (ELN). All five proteins are known to be related to collagen synthesis and organization during bone remodeling and healing process.

Discussion

MRONJ is a severe complication of oncologic patients who receive high dosage of ZA. In the last 20 years, the pathobiology of MRONJ has focused on the striking osteoclast inhibition by ZA (Russell, 2011). However, its influence on all actors of bone homeostasis had not been studied until now. For years it was speculated that ZA stimulates osteoblasts' proliferation and inhibits osteocytes apoptosis or does not influence osteoblasts at all (Rogers et al., 2000; Maruotti et al., 2012; Chen et al., 2018).

Although not the first time that an inhibiting effect of 5 μ M ZA on MSCs is demonstrated (di Vito et al., 2020), our study highlights the critical role of the surrounding soft tissue, specifically fibroblasts, in maintaining osteogenic differentiation in the presence of ZA.

As on MSCs and osteoblasts, the effect of ZA on soft tissue cells viability is also dose dependent (Scheper et al., 2009; Scheper et al., 2010). 1 μ M ZA is considered a threshold concentration

above which osteoblastic proliferation and differentiation is impaired (Zara et al., 2015; di Vito et al., 2020). The impact on soft tissue cells increases as concentrations rise. It significantly impaired soft tissue cells function in a wound scratch assay (Yuan et al., 2019).

BM MSCs are considered the gold standard for *in vitro* studies of bone differentiation. PDL MSCs present similar characteristics and demonstrate an osteogenic differentiation potential as well (Choi et al., 2011; Liu et al., 2014; Abedian et al., 2020). MSCs produce ALP shortly after the beginning of their osteogenic differentiation. Hence, the ALP activity reaction represents the first stages of osteogenic differentiation. Alizarin Red is a low cost and easy staining which demonstrate calcium nodules, which appear at the mineralization stage of the osteogenic differentiation. However, Alizarin Red tend to be washed easily from the well, and Von Kossa reaction which also detects calcium deposits was used to confirm the findings after Alizarin Red staining. Using all methods verified that the osteogenic differentiation could be completed *in vitro* and strengthened our results (Hashemibeni et al., 2013; Lee et al., 2017; Jeon et al., 2018).

Our study showed a smaller osteogenic differentiation potential of PDL MSCs, which exhibited less mineral deposition as demonstrated in weaker Alizarin Red and von Kossa staining. This finding is consistent with another study which found that in addition to weaker Alizarin Red and alkaline phosphatase staining, there was also lower expression of alkaline phosphatase and RunX2 in gene and protein levels (Liu et al., 2014). A 5 μ M ZA solution significantly decreased the osteogenic differentiation in the initial and mineralization stages. It is possible that ZA decreased the expression of key markers genes, such as alkaline phosphatase and RunX2 which mark the initial phase and Osteocalcin which marks the mineralization phase (Choi et al., 2011; Basso et al., 2013). Moreover, the most significant finding was that the addition of fibroblasts' conditioned medium to MSCs with ZA reversed this effect and enabled osteogenic differentiation. This finding demonstrated a paracrine effect of fibroblasts on osteoblasts progenitor cells.

Masticatory mucosal fibroblasts significantly increased PDL MSCs osteogenic differentiation as compared to gingival fibroblasts, as detected by Alizarin Red staining. There was also an increase in differentiation as compared to lining mucosal fibroblasts, although not statistically significant. Masticatory mucosal fibroblasts exhibit a genetic profile which enables a higher degree of plasticity as compared to lining mucosal fibroblasts (Kabakov et al., 2021).

The ability of human gingival fibroblasts secretome to accelerate wound healing through its anti-inflammatory and pro-angiogenic content has already been studied (Ahangar et al., 2020). Whereas keratinocytes express genes, essential for their role as mucosal barrier, fibroblasts express unique genes which are related to the extracellular matrix (Lee et al., 2013). Similarly, our proteomic analysis found five exclusive proteins in fibroblasts conditioned medium which are critical to collagen synthesis. Collagen5A (COL5A) is highly involved in the formation of both endochondral and intramembranous ossification (Wu et al., 2010). Procollagen-lysine,2-oxyglutarate and 5-dioxygenase (PLOD2) were demonstrated to be specifically

upregulated during the late stage of osteoblastic differentiation *in vitro* (Uzawa et al., 1999). Integrin β -like 1 (ITGBL1) is highly expressed in bone remodeling via TGF β pathway and mediation of RunX2 activation (Li et al., 2015). Latent transforming growth factor β (LTBP1) regulates the bioactivity of TGF β (Dallas et al., 2000; Dallas et al., 2002). In addition, a recent study showed its crucial role in mandibular growth process (Xiong et al., 2020). Elastin (ELN) was found to have the greatest power to predict *in vivo* bone formation (Twine et al., 2014). All five proteins, exclusively found in fibroblasts' conditioned medium, are known to be connected to bone repair via the TGF β signaling pathway as presented by the STRING analysis. TGF β is an important bone differentiation inducer, and its altered levels were observed in impaired wound healing *in vitro* and *in vivo* (Kim et al., 2017; Berberich et al., 2020).

Conclusion

Our study confirmed paracrine effect of fibroblasts on osteoblasts progenitors, which rescued osteogenic differentiation after exposure to ZA. More study is needed to explore the influence of direct contact between fibroblasts and osteoblasts progenitors. Moreover, we present an *in vitro* study, which is very limited. Several *in vivo* MRONJ models showed preventive and therapeutic effects of MSCs and endothelial progenitor cells conditioned media on MRONJ (Kikuri et al., 2010; Matsuura et al., 2016; Doppelt et al., 2020). MSCs, and to much greater extent endothelial progenitor cells, are very difficult to obtain and expand because of their low numbers in blood and bone marrow. Fibroblasts are easily extracted from oral mucosa and the procedure of a connective tissue graft, which can be used to seal a socket after an extraction and is commonly performed in periodontology. Therefore, the findings in this study should be examined in an *in vivo* model to confirm its clinical implications.

Clinical relevance

Scientific rationale for study

Medication Related Osteonecrosis of the Jaw (MRONJ) occurs mainly in oncologic patients and can lead to severe mutilation, which dramatically decreases quality of life. As a result, dentists are reluctant to perform necessary procedures in high-risk patients.

Principal findings

Our study found the ability of fibroblasts' secretome to rescue osteogenic differentiation of MSCs is probably due to its unique content, which included pro-osteogenic proteins.

Practical implications

Connective tissue grafts, which contain oral fibroblasts, can be used to seal extraction socket in order to induce bone differentiation, thus promoting healing and preventing MRONJ.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

TB prepared the research design, conducted the experiments, analyzed the data, performed the statistical analysis, and wrote the manuscript. OD-F analyzed the data and drafted and critically revised the manuscript. BC drafted and critically revised the manuscript. HZ-G prepared the research design, analyzed the data, revised the statistical analysis, and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphar.2023.1172705/full#supplementary-material>

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m7G-related genes predict prognosis and affect the immune microenvironment and drug sensitivity in osteosarcoma

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Background: Osteosarcoma (OS), a primary malignant bone tumor, confronts therapeutic challenges rooted in multidrug resistance. Comprehensive understanding of disease occurrence and progression is imperative for advancing treatment strategies. m7G modification, an emerging post-transcriptional modification implicated in various diseases, may provide new insights to explore OS pathogenesis and progression.

Methods: The m7G-related molecular landscape in OS was probed using diverse bioinformatics analyses, encompassing LASSO Cox regression, immune infiltration assessment, and drug sensitivity analysis. Furthermore, the therapeutic potential of AZD2014 for OS was investigated through cell apoptosis and cycle assays. Eventually, multivariate Cox analysis and experimental validations, were conducted to investigate the independent prognostic m7G-related genes.

Results: A comprehensive m7G-related risk model incorporating eight signatures was established, with corresponding risk scores correlated with immune infiltration and drug sensitivity. Drug sensitivity analysis spotlighted AZD2014 as a potential therapeutic candidate for OS. Subsequent experiments corroborated AZD2014's capability to induce G1-phase cell cycle arrest and apoptosis in OS cells. Ultimately, multivariate Cox regression analysis unveiled the independent prognostic importance of CYFIP1 and EIF4A1, differential expressions of which were validated at histological and cytological levels.

Conclusion: This study furnishes a profound understanding of the contribution of m7G-related genes to the pathogenesis of OS. The discerned therapeutic potential of AZD2014, in conjunction with the identification of CYFIP1 and EIF4A1 as independent risk factors, opens novel vistas for the treatment of OS.

KEYWORDS

m7G, immune infiltration, prognosis, osteosarcoma, AZD2014, drug sensitivity

Introduction

Osteosarcoma (OS) is the most commonly diagnosed malignant bone tumor characterized by the presence of intratumoral osteogenesis (Wylie, 2004). Its annual incidence in the general population ranges from two to three cases per 1,000,000 individuals, but notably peaks at eight to eleven cases per

1,000,000 individuals among children and adolescents (Ritter and Bielack, 2010). However, the introduction of multi-agent chemotherapy has improved the 5-year overall survival rate for OS, achieving approximately 60%–70% long-term survival (Gill and Gorlick, 2021), and the prognosis for patients with metastatic or relapsed disease remains devastating, with a mere 5-year overall survival rate of 20% (Link et al., 1986; Aljubran et al., 2009; Meyers et al., 2011). Moreover, the emergence of chemotherapy resistance in OS has hindered therapeutic advancements over the past decades (Gill and Gorlick, 2021). Molecularly, several crucial factors contribute to the suboptimal outcomes in OS treatment. First, the lack of sensitive markers for subtyping patients with poor prognosis hampers personalized treatment strategies. Second, the intricate immunological microenvironment of OS remains poorly understood, limiting our ability to harness immune-based therapies effectively. Last, the complex genetic landscape of OS presents challenges in identifying and targeting key molecular drivers for therapeutic intervention (Meltzer and Helman, 2021). Consequently, there is a pressing need to gain novel insights into the molecular genetics of OS as such discoveries hold immense potential in optimizing early detection methods, advancing treatment modalities, and enhancing prognostic predictions for OS patients.

N7-methylguanosine (m7G) is a significant post-transcriptional modification, which means that a methyl group is added to the seventh N position of RNA guanine by methylation transferase (Guy and Phizicky, 2014; Sloan et al., 2017; Song et al., 2020). Through the methylation modification of RNA, m7G influences the production, maturation, and decomposition of RNA by triggering various biological and pathological reactions (Song et al., 2020). Recently, increasing evidence has shown that m7G modification involves the oncogenesis and progression of various cancers and plays the role of a double-edged sword (Chen Y. et al., 2022; Luo et al., 2022). For example, m7G-related genes serve as tumor promoters in various cancers, such as glioma, hepatocarcinoma, esophageal squamous cell carcinoma, non-small-cell lung cancer, and head and neck squamous cell carcinoma, but exhibit an anti-tumor effect in certain cancers (Pandolfini et al., 2019; Tian et al., 2019; Chen et al., 2021; Li et al., 2021; Chen J. et al., 2022; Han et al., 2022). Recently, increasing research focused on the role of m7G in the tumor microenvironment (TME) (Huang et al., 2022a; Li Z. et al., 2022; Gao et al., 2022; Zeng et al., 2023). Certain studies have exhibited that m7G-related genes shape the TME by affecting the distribution of immune cells (Wang et al., 2017; Chen J. et al., 2022; Dong et al., 2022; Wu et al., 2022). Furthermore, studies have found that m7G was associated with response to chemotherapeutic drugs, including cisplatin and docetaxel (Okamoto et al., 2014). As such, exploring the mechanism of m7G during the occurrence and development of OS may promote the advancement of OS treatment.

Herein, we utilized the bioinformatic analysis to explore the role of m7G-related genes in the molecular landscape of OS. Additionally, we constructed a prognostic model and its relation with immune infiltration and chemotherapy sensitivity. Furthermore, an integrated nomogram was established to quantitatively predict OS patients' prognosis. More importantly, through drug sensitivity analysis, we found that AZD2014 may serve as the potential sensitive drug for OS, and AZD2014 can induce the G1-phase cell cycle arrest and apoptosis of OS cells, serving as a potential therapeutic drug for OS patients.

Eventually, we identified CYFIP1 and EIF4A1 as two independent risk factors of OS through multivariate Cox regression analysis and verified the differential expression of CYFIP1 and EIF4A1 at histological and cytological levels, offering new therapeutic targets for OS.

Materials and methods

Data collection and processing

The RNA sequencing data of OS patients with corresponding clinical information in TARGET-OS and GSE21257 datasets were separately downloaded from Therapeutically Applicable Research to Generate Effective Treatments (TARGET; <https://ocg.cancer.gov/programs/target>) and the Gene Expression Omnibus (GEO; <https://www.ncbi.nlm.nih.gov/geo/>) databases. Additionally, the mRNA expressions of OS patients in these two cohorts were merged and batch-corrected via the “sva” package. The m7G-related genes were collected based on the published literature (Regmi et al., 2022). Detailed information on the 98 m7G-related genes is shown in Supplementary Table S1.

Screening of prognostic m7G-related genes and unsupervised consensus clustering

Univariate Cox regression analysis was performed to identify the prognostic m7G-related genes of TARGET-OS patients. The “NMF” package was applied to identify different m7G-related clusters based on the expressions of prognostic m7G-related genes. The “ns” algorithm was used as a clustering measure, and the cophenetic coefficient was applied to decide the best clustering. After the best cophenetic coefficients were selected, a heatmap was depicted using the “consensusmap” function. The “limma” package was adopted to determine differentially expressed genes (DEGs) among different m7G-related clusters with a Foldchange >1.5 and *p*-value <0.05.

Construction and verification of a prognostic model based on m7G-related genes

The TARGET-OS dataset was used as the training cohort to construct a prognostic model. The least absolute shrinkage and selection operator (LASSO) analysis was used to further narrow the preliminarily screened prognostic m7G-related genes. The final prognostic model can be expressed as follows: risk score = $\sum \text{Coef}_{\text{mRNAi}} * \text{Expression}_{\text{mRNAi}}$, in which $\text{Expression}_{\text{mRNAi}}$ represents the expression level of each prognostic m7G-related gene and $\text{Coef}_{\text{mRNAi}}$ represents the coefficient of the corresponding prognostic m7G-related genes in the LASSO-Cox regression model. Based on the prognostic model, the risk score of each OS patient was calculated, and OS patients were separated into high- and low-risk groups based on the medium risk score. In addition, OS patients included in the GSE21257 dataset were set as a verification cohort to validate the prediction performance of the constructed prognostic model. Furthermore, the prediction performance of the constructed prognostic model was validated in the merged dataset.

Construction of an integrated nomogram

We utilized the “survival,” “survminer,” and “rms” packages in R to develop an integrated nomogram. Multivariate Cox regression models were performed to identify the independent factors associated with the prognosis of OS patients. Following the result of multivariate analysis, a Cox proportional hazard (PH) model was applied to develop a nomogram to predict the survival probability of OS patients of the merged dataset. The receiver operating characteristic (ROC) curve, calibration curve, and decision curve analysis (DCA) curve were performed to assess the predictive accuracy and clinical usefulness.

Functional analyses and mechanism exploration

The underlying effect of m7G-related genes on the occurrence and development of OS was investigated through functional enrichment analysis. First, DEGs between the merged datasets' high- and low-risk groups were identified with the R package “limma.” Subsequently, the functional enrichment analyses, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses, were completed to explore underlying pathways. In addition, a protein–protein interaction (PPI) network based on identified DEGs was constructed on the Metascape website (<http://metascape.org/gp/index.html>) to screen hub genes and hub modules. Subsequently, the Gene Set Enrichment Analysis (GSEA) algorithm was applied to explore the activity variation of KEGG analysis. Moreover, the GSVA algorithm was applied to explore the activity variation of biological process (BP) terms in GO analysis.

Immune infiltration and drug sensitivity analysis

The Estimation of Stromal and Immune cells in Malignant Tumor tissues using Expression data (ESTIMATE) algorithm was performed to calculate the stromal score, immune score, ESTIMATE score, and tumor purity in the high- and low-risk groups. Moreover, MCP counter algorithms were used to estimate the proportion of immune cells. The Genomics of Drug Sensitivity in Cancer (GDSC), developed by the Sanger Institute in the UK, is considered the most prominent public resource for drug sensitivity of tumor cells at present, collecting the sensitivity and response of tumor cells against drugs. The “limma,” “ggpubr,” and “oncopredict” packages were utilized to perform drug sensitivity between the high- and low-risk groups in the merged dataset and to screen potential therapeutic drugs for OS, with $p < 0.01$ as the screening criterion.

Tissue, cell lines, and reagents

OS samples and adjacent normal tissues were collected from patients undergoing hinge knee arthroplasty in the Orthopedics Department of Xiangya Hospital. The Ethics Committee of Xiangya Hospital of Central South University approved this study, and

informed consent was obtained from all the participants or their legal guardians.

The human OS cell lines U2OS and MG63 cells were purchased from the Procell Life Science&Technology Company. Under 37°C and 5% CO₂, U2OS cells were maintained in high-glucose DMEM, while MG63 cells were cultured in MEM, and all mediums were added with 10% fetal bovine serum and 1% streptomycin/penicillin.

AZD2014 was purchased from the Selleck Company (Houston, TX, USA). Dimethyl sulfoxide (DMSO) was utilized to dissolve the AZD2014 powder to prepare a 50 mM stock solution stored at −80°C. The stock solution was diluted with the appropriate assay medium in the subsequent experiment, while 0.1% DMSO was used as the vehicle control.

Immunohistochemical analysis

The pathological tissues and adjacent normal tissues of OS patients were fixed in 4% paraformaldehyde, embedded in paraffin, and then, sliced into slides for immunohistochemistry (IHC). Subsequently, deparaffinization, dehydration, and antigen reparation were performed for each slide. To block the endogenous peroxidase activity, the slides were incubated with 3% hydrogen peroxide solution at room temperature for 10 min. After rinsing with PBS, the slides were hatched for 1 hour at room temperature with the goat serum (ZLI-9022, ZSGB-Bio, China). Then, the slides were hatched with *EIF4A1* primary antibody (R383037, ZenBio, China) and *CYFIP1* primary antibody (ab156016), which were diluted into 1:100, respectively, at room temperature overnight. After rinsing in PBS for three cycles for 5 min/times, the slides were hatched with an antibody booster and anti-rabbit secondary antibody (PV-9000, ZSGB-Bio, China) for 20 min at room temperature, respectively. Finally, the signals of sections were developed using 3,3'-diaminobenzidine tetrahydrochloride, and all slides were stained with hematoxylin.

RNA extraction and quantitative PCR

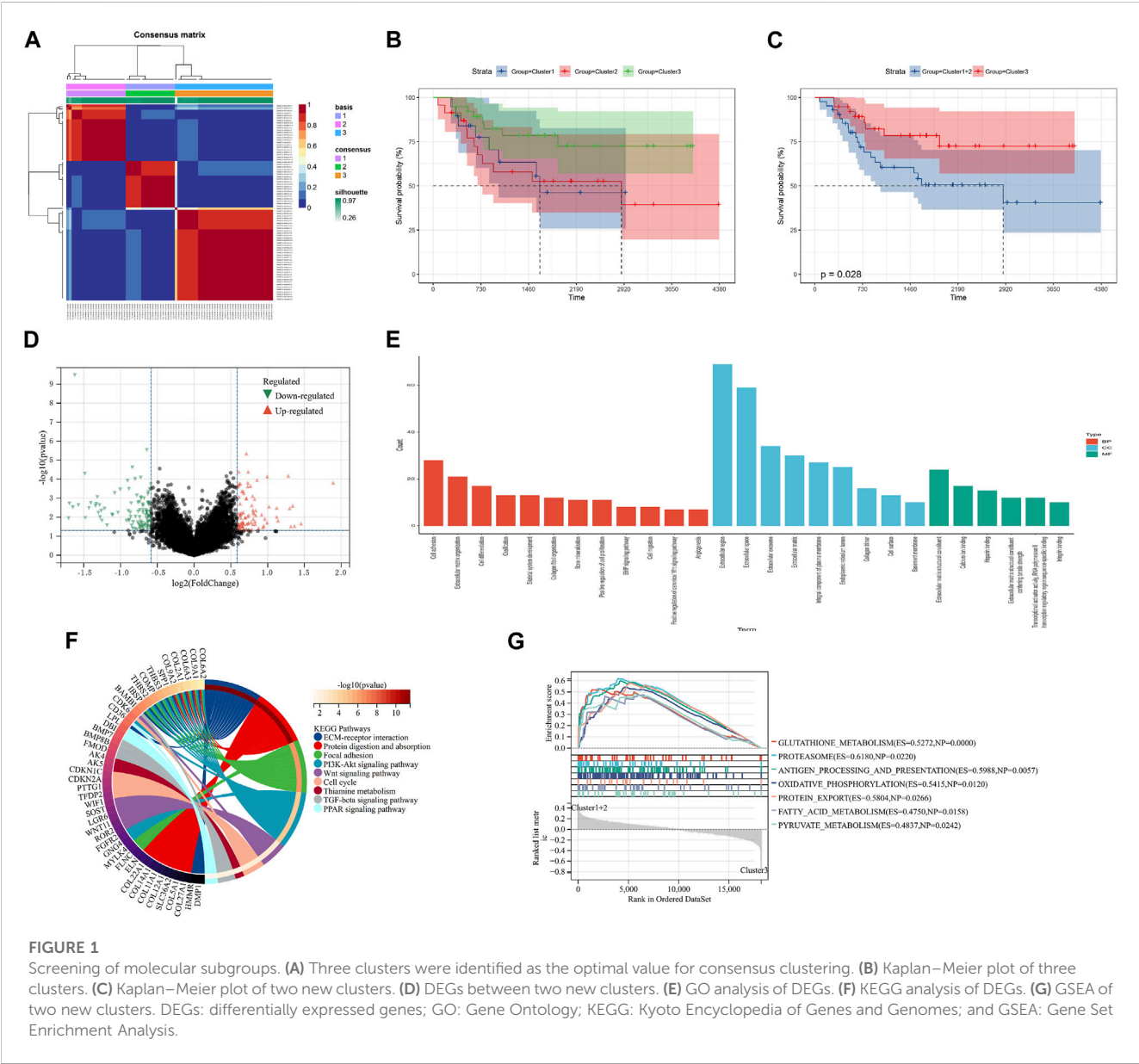
Total RNA was extracted from hFOB1.19, U2OS, and MG63 cell lines by using the AG RNAex Pro RNA extraction kit (AG, Changsha, China) and utilized to synthesize cDNA with the Reverse Transcription Kit (AG, Changsha, China). qPCR analysis was carried out on the ABI7500 system using the TB Green Premix Pro Taq HS qPCR Kit (AG, Changsha, China). Last, we used the $\Delta\Delta C_q$ method to calculate the relative expression levels of each sample, and the results were expressed as $2^{-\Delta\Delta C_q}$. GAPDH were used for normalization in the qPCR experiment.

Cell cycle propidium iodide staining assay

For the cell cycle assay, cells were seeded in the six-well plates (5×10^5 /well) overnight and then treated with AZD2014 at the concentrations of 2.5, 5.0, and 10.0 μ M. After 24 h, cells were collected and rinsed in PBS. We then fixed these samples in 70% ethanol overnight at 4°C. Subsequently, 0.5% propidium iodide (PI) (Multisciences Biotech Co., Ltd.) added with 0.01% RNase was used

TABLE 1 Prognostic genes generated using univariate Cox analysis.

Sig_genes	Full name	Category	Gene card ID	p-value	HR (95% CI for HR)
<i>CYFIP2</i>	Cytoplasmic FMRI interacting protein 2	Protein coding	GC05P157267	0.0077	1.40 (1.10–1.80)
<i>IGF2BP2</i>	Insulin-like growth factor 2 mRNA-binding protein 2	Protein coding	GC03M185643	0.02	1.40 (1.00–1.90)
<i>ALKBH1</i>	AlkB homolog 1. Histone H2A dioxygenase	Protein coding	GC14M077672	0.019	0.31 (0.12–0.83)
<i>NUDT1</i>	Nudix hydrolase 1	Protein coding	GC07P002242	0.034	1.70 (1.00–2.70)
<i>FTO</i>	FTO alpha-ketoglutarate-dependent dioxygenase	Protein coding	GC16P053853	0.014	0.29 (0.11–0.78)
<i>EIF4A1</i>	Eukaryotic translation initiation factor 4A1	Protein coding	GC17P007572	0.021	2.50 (1.20–5.50)
<i>EIF4E3</i>	Eukaryotic translation initiation factor 4E family member 3	Protein coding	GC03M071675	0.014	0.31 (0.12–0.79)
<i>NUOT16</i>	Nudix hydrolase 16	Protein coding	GC03P131381	0.0019	0.30 (0.14–0.64)
<i>CYFIP1</i>	Cytoplasmic FMRI interacting protein 1	Protein coding	GC15M022867	0.0014	0.23 (0.09–0.56)



for staining. Cell cycle analysis was performed on the flow cytometer (NovoCyte, ACEA).

Cell apoptosis assay

For the apoptosis assay, cells were seeded in six-well plates (2×10^5 /well) with AZD2014 at the concentrations of 2.5, 5.0, and 10 μ M for 48 h. DMSO was used to treat the vehicle control group. The cells and supernatants were collected and washed twice with ice-cold PBS. The annexin V/PI kit (Multisciences Biotech Co., Ltd.) was used to detect apoptosis, according to the manufacturer's instructions.

Statistical analysis

Statistical tests were performed using R software (version 4.2.1). Continuous data were expressed as the mean and standard deviation, while categorical data were expressed as count and percentage. Univariate, LASSO, and multivariate Cox analyses were performed to identify independent prognostic factors and construct an integrated nomogram, including predictable clinical traits and risk scores. The performance and clinical usefulness of the model were assessed by the calibration curve, timeROC, and timeDCA. All tests were two sided. The statistical significance was shown as follows: p -value < 0.05 (*), p -value < 0.01 (**), and p -value < 0.001 (***).

Results

Identification of prognostic m7G-related genes and m7G-related clusters

Ninety-eight m7G-related genes were obtained from the previous literature (Supplementary Table S1), and nine m7G-related genes were identified as the prognosis-related genes through univariate cox regression analysis: *CYFIP2*, *IGF2BP2*, *ALKBH1*, *NUDT1*, *FTO*, *EIF4A1*, *EIF4E3*, *NUDT16*, and *CYFIP1* (all $p < 0.05$) (Table 1). Based on the expression of nine identified m7G-related genes, we classified the patients into clusters to explore the impact of m7G RNA modification on OS patients. The cophenetic plot signified that dividing the patients into three clusters is the best clustering choice (Figure 1A; Supplementary Figure S1). Then, we used the Kaplan–Meier curve for these three clusters and found that the prognosis of Cluster 3 was better than that of Cluster 1 and Cluster 2 (Figure 1B, total p -value: 0.056, total HR: 0.65, 95CI% [0.41, 1.02]; Cluster1–Cluster2: p -value 0.91; Cluster1–Cluster3: p -value 0.08; and Cluster1–Cluster3: p -value 0.04). Subsequently, considering the similar prognoses between Cluster 1 and Cluster 2, we merged them into one group, plotted the Kaplan–Meier curve for the new clusters, and found the distinguished prognosis between these two clusters (p -value: 0.028, HR: 0.41, 95CI% [0.19, 0.93]) (Figure 1C). To explore the underlying mechanism that results in different prognoses, we performed the differentially expressed analysis and functional enrichment analyses between the clusters. As a result, 195 DEGs were identified, of which 106 were downregulated and 89 were upregulated (Figure 1D). GO

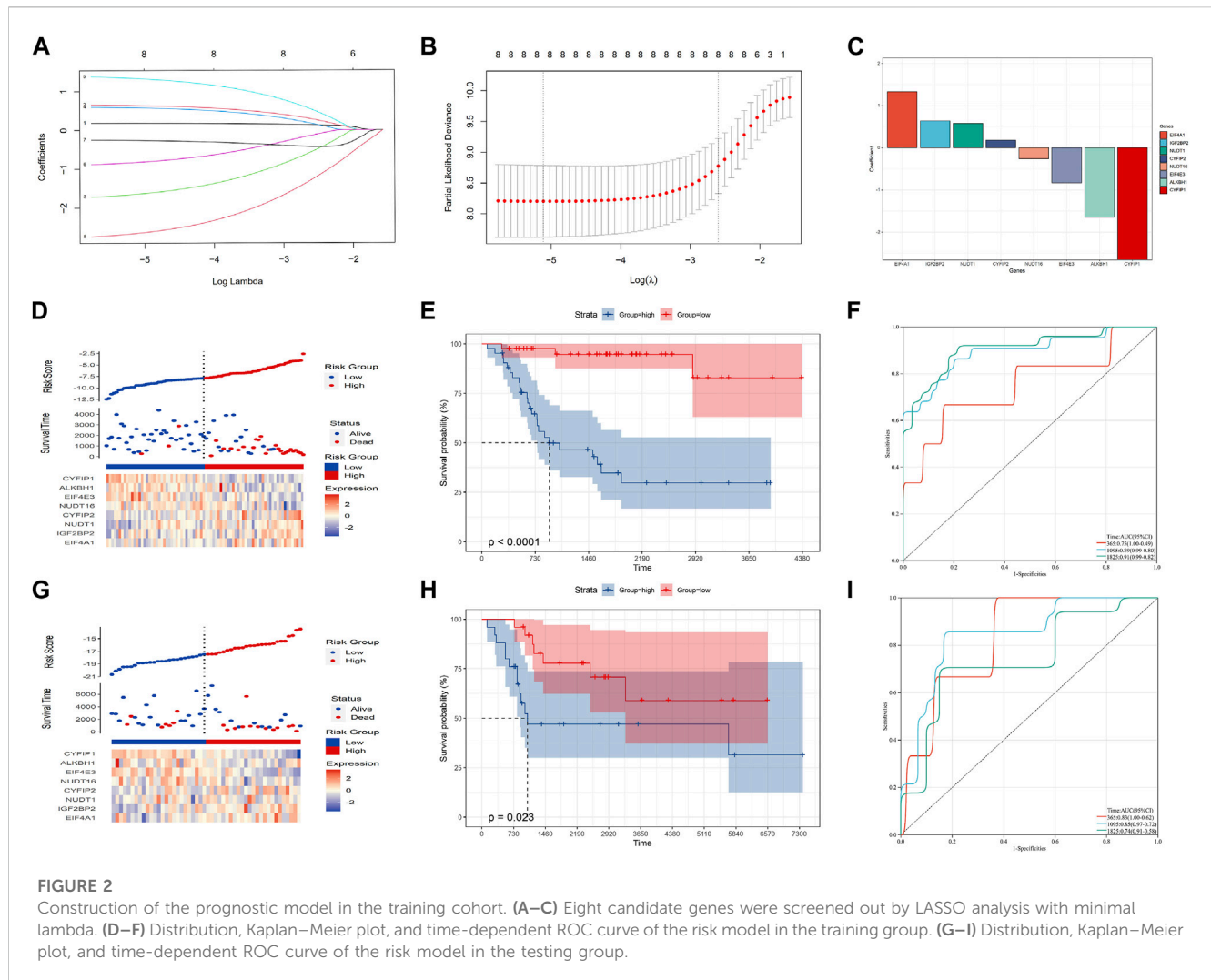
analysis's biological process (BP) was mainly enriched in cell adhesion, extracellular matrix organization, and cell differentiation. GO analysis's cellular component (CC) was mainly enriched in the extracellular region, extracellular space, and extracellular exosome. GO analysis's molecular function (MF) was mainly enriched in extracellular structural constituent, calcium-ion binding, and heparin binding (Figure 1E). KEGG analysis was mainly enriched in ECM–receptor interaction, protein digestion and absorption, focal adhesion, and the PI3K–Akt signaling pathway (Figure 1F). Furthermore, the GSEA was primarily enriched in glutathione metabolism, proteasome, oxidative phosphorylation, and fatty acid metabolism (Figure 1G). Therefore, the differences in cell adhesion and extracellular matrix metabolism of GO and KEGG enrichment analyses might affect OS invasiveness and metastasis ability, while metabolism-related pathways, such as glutathione metabolism, oxidative phosphorylation, and fatty acid metabolism, in GSEA may represent the different metabolism status among OS clusters, leading to the differential prognosis of OS patients. To sum up, m7G modification may be associated with extracellular matrix organization, focal adhesion, ECM–receptor interaction, and cell metabolism, which may affect the invasion and metabolism-related ability of OS cells, resulting in different prognoses among clusters.

Construction and validation of a prognostic model

LASSO analysis was used to narrow down the prognostic m7G-related genes and construct a prognostic model based on the TARGET-OS dataset. Then, eight m7G-related genes were included in the final model, and the prognostic model could be expressed as follows: risk score = $0.18 \times CYFIP2 + 0.64 \times IGF2BP2 - 1.65 \times ALKBH1 + 0.58 \times NUDT1 + 1.33 \times EIF4A1 - 0.83 \times EIF4E3 - 0.26 \times NUDT16 - 2.66 \times CYFIP1$ (Figures 2A–C). Subsequently, OS patients were separated into high- and low-risk groups based on the medium risk score, and the prognosis of the low-risk group was significantly better than that of the high-risk group (Figures 2D, E; p -value: < 0.0001 , HR: 13.01, 95CI% [3.89, 43.53]). The timeROC curve with the area under the curve (AUC) of 1-, 3-, and 5-year being 0.75, 0.89, and 0.91, respectively, signified the high prediction efficiency of the constructed prognostic model (Figure 2F). Meantime, the prognostic model's predictive ability was tested in the GSE21257 dataset, suggesting the prognosis of the low-risk group was also better than that of the high-risk group. Moreover, the AUC of 1-, 3-, and 5-year ROC was 0.83, 0.85, and 0.74, respectively (Figures 2G–I; p -value: 0.023, HR: 2.82, 95CI% [1.11, 7.14]). Therefore, our prognostic model may exhibit excellent performance in predicting the prognosis of OS patients.

Construction of an integrated nomogram

In order to improve the quantitative predictive ability of the risk model, we merged two cohorts (Supplementary Figure S2; Figures 3A–C; p -value: 0.0047, HR: 2.34, 95CI% [1.28, 4.28]) and integrated the clinical characteristics into the risk model to establish a nomogram. The result of multivariate Cox regression analysis indicated that the risk score (1.6×10^{-3}), metastasis (1.6×10^{-3}), and primary site (0.03) were the independent factors affecting the prognosis of OS patients, while the relationship between gender/age

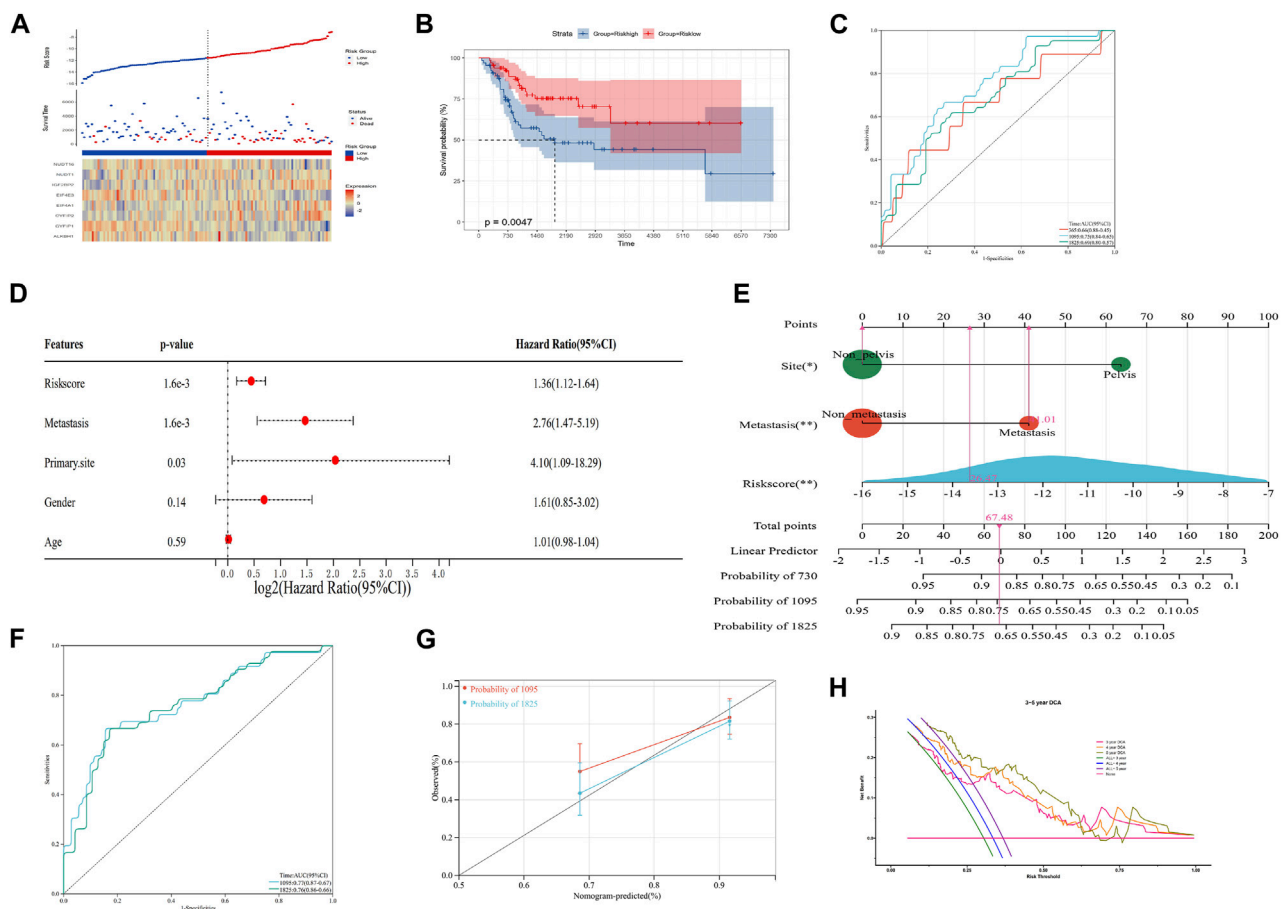


and the prognosis of OS patients was not significant (Figure 3D). Subsequently, we constructed an integrated nomogram including the risk score, metastasis, and primary site (Figure 3E). From the nomogram, each item can obtain its corresponding score and the total score and its corresponding survival rate can be obtained after adding the score of all items. The timeROC curve with a 3- and 5-year AUC being 0.77 and 0.76, respectively, indicated the predictive accuracy of the nomogram, and the results of the 3- and 5-year calibration plots confirmed this (Figures 3F, G). Furthermore, the 3-, 4-, and 5-year timeDCA curves indicated the excellent clinical usefulness of the nomogram (Figure 3H). Therefore, our integrated nomogram may serve as a viable quantitative predictor of the prognosis of OS patients.

Mechanism exploration and functional enrichment analyses

To explore the mechanism of our m7G-related prognostic model in OS, we performed differentially expressed analysis between the high- and low-risk groups. Then, 110 DEGs were identified, of which

69 were downregulated and 41 were upregulated (Figure 4A). GO function enrichment analysis indicated that DEGs were enriched in the immune-related processes and extracellular matrix metabolism (Figure 4B, C). Additionally, the KEGG enrichment analysis signified that DEGs were closely associated with some immune-related diseases and corresponding signaling pathways (Figure 4D). The PPI analysis further identified seven hub modules, which mainly involved the immune response and extracellular matrix metabolism (Figure 4E). Moreover, the GSEA and GSVA enrichment analyses were conducted to identify the expression pattern of the involved BP. The results revealed that immune-related processes, including antigen presentation, the B-cell receptor signaling pathway, and natural killer cell-mediated cytotoxicity, were highly expressed in the low-risk group, compared with the high-risk group. In contrast, the metabolism-related processes, including nitrogen metabolism, alpha linolenic acid metabolism, and linoleic acid metabolism, showed the opposite trend (Figure 4F, G). These function enrichment analyses synergistically suggested that m7G-related DEGs were closely associated with immunity disorders, extracellular matrix organization, and cellular metabolism in OS patients, which may be the underlying mechanism affecting the prognosis of OS patients.



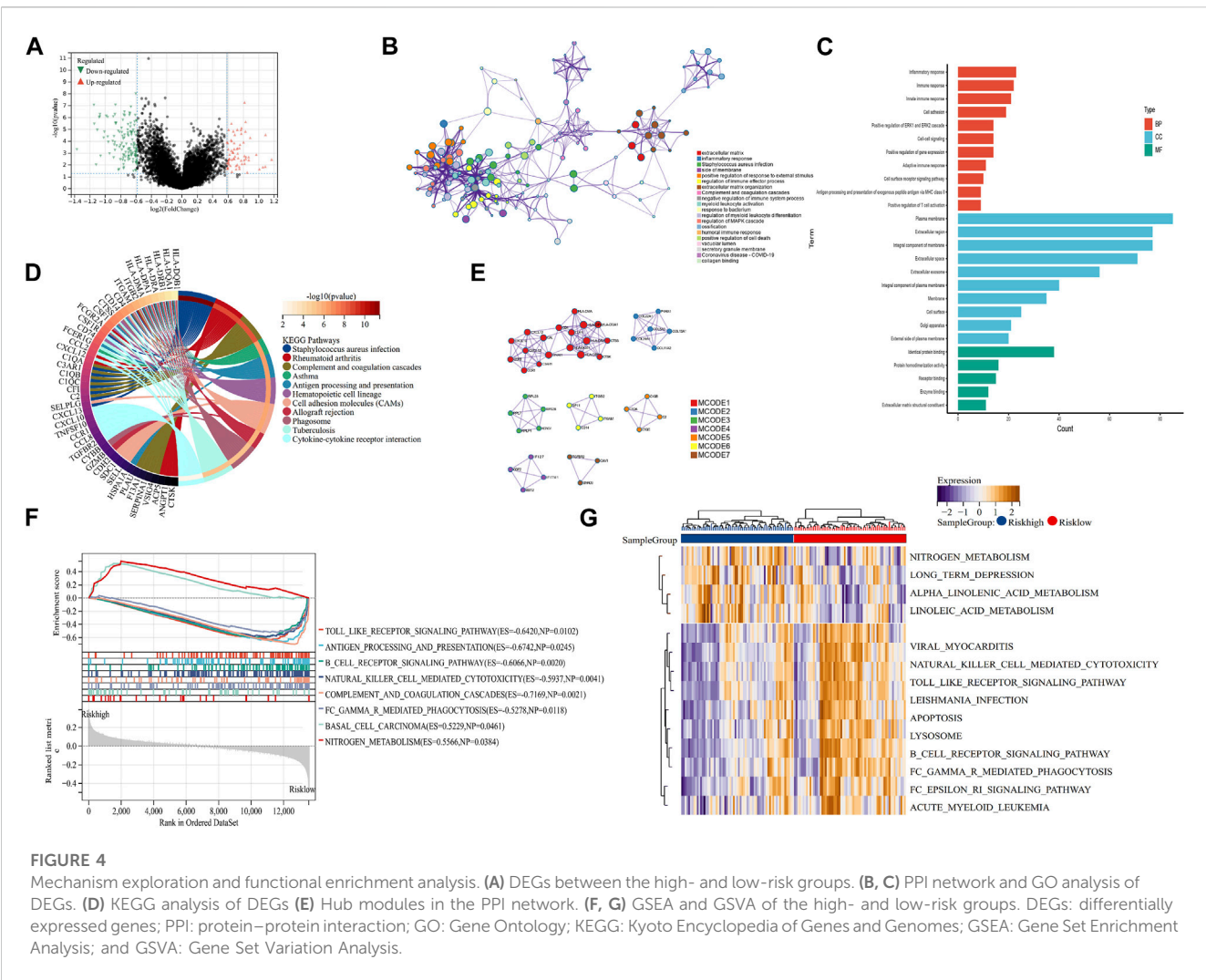
Immune infiltration analysis and drug sensitivity analysis

To evaluate the impact of m7G modification on the OS immune microenvironment, we used ESTIMATE and MCP counter analyses to assess the infiltration of immune cells. Then, we found that the low-risk group's stromal score, immune score, and estimate score were significantly higher. In contrast, the tumor purity of the low-risk group was significantly lower than that of the high-risk group (Figure 5A, B). Additionally, the MCP counter further confirmed the risk score was associated with the immune microenvironment and revealed the infiltration of T cells, cytotoxic lymphocytes, B lineage, monocytic lineage, neutrophils, and fibroblasts was distinctly higher in the low-risk group (Figure 5C). To determine the possible small molecules targeting m7G-related genes and further improve the clinical value of the prognostic model, we performed the drug sensitivity analysis by comparing IC₅₀ between high- and low-risk groups. The results indicated that the high-risk group was more sensitive to AZD2014 (6.1×10^3), AZD5153 (8.2×10^3), acetalax (8.8×10^3), and dactolisib

(8.9×10^3) in targeting m7G-related genes than the low-risk group (Figures 5D–G). AZD2014, the most significant sensitive drug in our drug sensitivity analysis, was included in the verified experiment.

AZD2014 blocks OS cell division and induces apoptosis

AZD 2014, an mTOR inhibitor, has been reported as an anti-proliferative drug against various cancers. To explore the impact of AZD2014 on OS, we performed the cell cycle assay and the apoptosis assay. The results of the cell cycle assays showed that the increase in AZD2014 expanded the proportion of G1-phase cells but decreased the proportion of S- and G2-phase cells, suggesting that AZD2014 can induce the G1-phase arrest in OS cells (Figure 6A, B). Subsequently, we analyzed the impact of AZD2014 on OS-cell apoptosis and found an increase of annexin V (+) OS cells followed by the high concentrations of AZD 2014, indicating that AZD2014 induced OS cell apoptosis as well (Figure 6C, D). As such, our results indicated that AZD2014 induced the



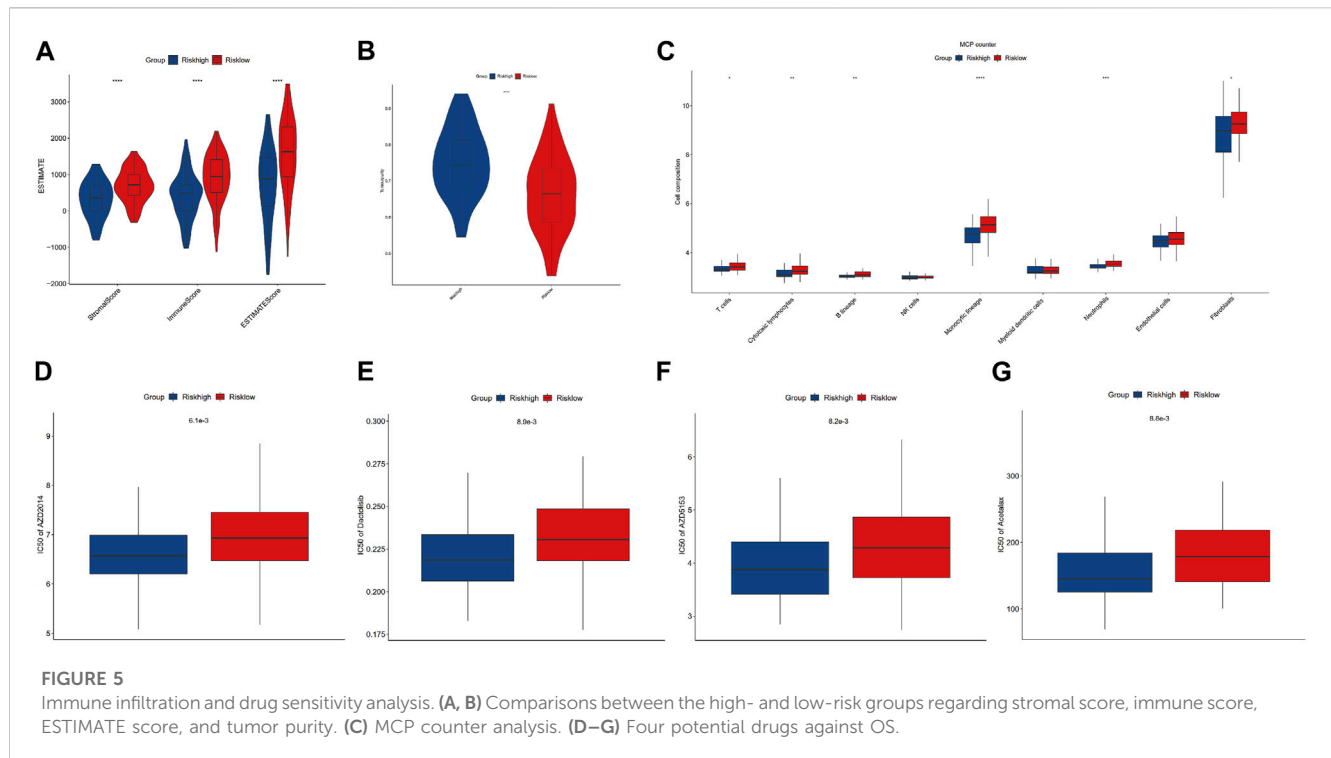
G1-phase cell cycle arrest and apoptosis of OS cells, which may serve as a potential drug for OS.

Verification of the m7G-related signature in OS

To explore the independent prognostic factors of OS, we performed multivariate Cox regression analysis among eight m7G-related signatures included in the prognostic model and found that *CYFIPI* and *EIF4A1* served as the independent prognostic factors of OS patients (Figure 7A). Subsequently, we carried out qPCR and IHC to validate our bioinformatic results. The result of qPCR illustrated that in comparison with osteoblasts, the expression of *CYFIPI* was significantly lower, while that of *EIF4A1* was higher in OS cells (Figure 7B). Meanwhile, IHC demonstrated that compared to the adjacent normal tissue, the expression of *CYFIPI* was significantly lower, while that of *EIF4A1* was evidently higher in OS tissue (Figure 7C). Taken together, our histological and cytological experiments validated the low expression of *CYFIPI* and high expression of *EIF4A1* in the OS status, which is consistent with our bioinformatic results, signifying the therapeutic potential of *CYFIPI* and *EIF4A1*.

Discussion

Over the past decades, OS treatments have been stagnant because of the increase in chemotherapeutic resistance (Lin et al., 2021). Treatment advance requires further understanding of OS pathogenesis, progression, and drug resistance mechanism. Recently, m7G, a post-transcriptional modification, has been found to be involved in the oncogenesis, progression, and drug resistance of various cancers (Luo et al., 2022). Notably, previous studies have revealed the significance of m7G modification, such as *METTL1*-mediated tRNA modification, in driving oncogenic transformation and promoting resistance to specific drugs like lenvatinib (Orellana et al., 2021; Huang et al., 2023). Given the pivotal role of m7G modification in disease processes, researchers have focused on identifying and studying genes involved in the regulation of this modification to unravel their impact during various disease states (Huang et al., 2022a; Li X. Y. et al., 2022). Similarly, our study aimed to employ bioinformatic analysis to explore the prognostic significance of m7G-related genes in OS patients. Through univariate Cox and LASSO regression analyses, we identified eight m7G-related genes, including *EIF4A1*, *IGF2BP2*, *NUDT1*, *CYFIPI2*, *NUDT16*, *EIF4E3*, *ALKBH1*, and *CYFIPI1*, and



developed a prognostic model based on their expression patterns. Subsequently, we calculated a corresponding risk score using these m7G-related genes, which could potentially serve as a valuable prognostic and chemosensitive predictive tool for OS. Furthermore, we investigated the impact of AZD 2014, a drug of interest, on the cell cycle and apoptosis of OS cells. Our findings demonstrated that AZD2014 induces the G1-phase cell cycle arrest and apoptosis in OS cells. These observations suggest that the identified m7G-related genes may not only serve as indicators of drug sensitivity but also have clinical implications for predicting the outcomes of OS patients.

With the increasing understanding of genetics, epigenetic regulation has obtained significant attention in various biological and pathological processes (McKusick, 1970). Among the crucial RNA modifications involved in epigenetic regulation, m7G modification has emerged as a subject of growing interest (Dai et al., 2021). Numerous studies have demonstrated the wide-ranging involvement of m7G modification in the pathogenesis and development of various diseases, particularly cancers (Chen Y. et al., 2022). For example, overactive *METTL1* can promote the methylation and maturation of m7G in let-7 miRNA, a tumor suppressor miRNA, thereby inhibiting the metastasis of lung cancer cells (Pandolfini et al., 2019). Conversely, the disruption of m7G modification resulting from *METTL1* knockout leads to global translation defects of oncogenes and the loss of typical malignant transformation markers, thereby inhibiting the occurrence and development of intrahepatic cholangiocarcinoma (Dai et al., 2021). However, unlike other tumor types, the study of m7G modification in OS remains largely unexplored. In this study, we focused on clustering OS patients based on the prognostic value of m7G-related genes determined by univariate Cox regression analysis. Our findings revealed a significant association between m7G modification and the prognosis of OS patients. To quantitatively

assess the impact of m7G modification on OS patients, we constructed a prognostic model. The discriminatory power of this model was validated in both training and testing groups, and its performance was further evaluated in the merged group using timeROC curves. Furthermore, the independence of the prognostic model was demonstrated through multivariate Cox regression analysis in the merged cohort. Additionally, we identified metastasis and the primary tumor site as independent factors influencing the prognosis of OS patients. To enhance the accuracy of prognosis prediction in OS patients, we developed a nomogram that integrates the risk score derived from the prognostic model with clinical characteristics, including metastasis and primary sites. The calibration plots for the 3- and 5-year outcomes, as well as the timeROC curves, demonstrated the efficacy and accuracy of the nomogram in predicting patient prognosis. Furthermore, the timeDCA curve indicated the excellent clinical utility of this nomogram, further supporting its potential as a valuable predictive tool for clinicians. Consequently, our study highlights the importance of m7G modification in OS and presents a robust prognostic model and nomogram that incorporate m7G-related genes and clinical characteristics for accurate prognostic prediction. The findings underscore the potential clinical usefulness of this approach in guiding treatment decisions and improving patient outcomes in OS.

Notably, our prognostic model revealed an interesting association between the expression of m7G-related genes in OS patients and their immune status. Patients with better prognoses displayed more active immune statuses, suggesting a potential correlation between m7G modification and immune responses in OS. In recent years, increasing evidence has emphasized the intricate interplay and coevolution between tumor cells, immune components, and the tumor stroma, underscoring their significant roles in cancer pathogenesis, progression, and treatment (Gill and Gorlick, 2021;

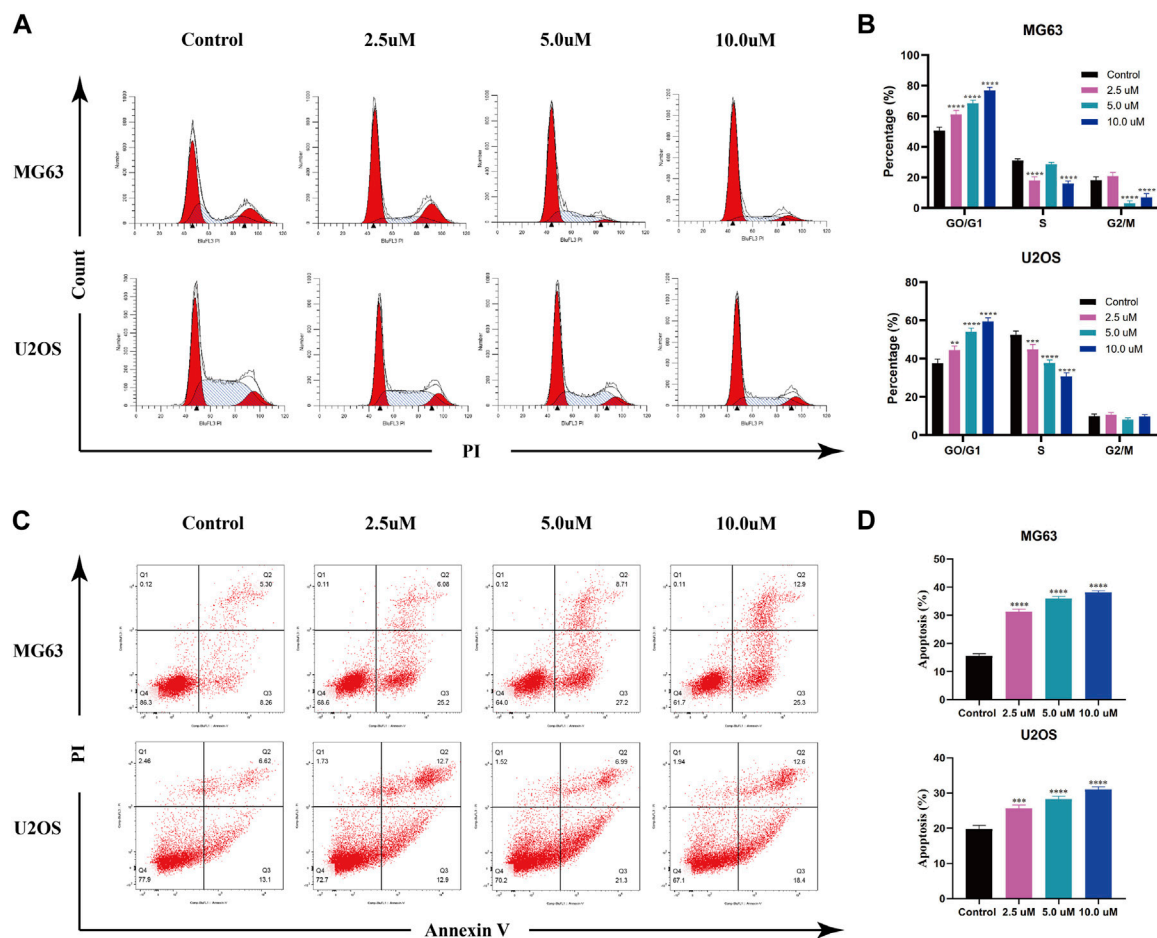


FIGURE 6

AZD2014 blocks OS cell division and induces apoptosis. (A, B) Impact of AZD2014 on the OS cell cycle; (C, D) impact of AZD2014 on OS cell apoptosis.

Heymann et al., 2021; Huang et al., 2022b; Huang R. et al., 2022). Previous studies have highlighted the involvement of tumor stroma in various processes, including neovascularization, inherent features for tumor homing, microvesicle secretion, paracrine cross-feeding, and immune modulation, all contributing toward tumor progression (Cortini et al., 2017). Additionally, immune cells, such as myeloid-derived suppressor cells, regulatory T cells, and tumor-associated macrophages, have been identified as key players in regulating tumorigenesis and tumor growth (Xia et al., 2022). To explore the impact of m7G modifications on the TME, we used ESTIMATE and MCP counter analyses. The results confirmed that the low-risk group, as defined by our m7G-related risk scores, exhibited higher levels of immune infiltration compared to the high-risk group. Furthermore, functional enrichment analysis of DEGs and PPI network hub modules provided additional evidence that the immune score of the low-risk group was higher than that of the high-risk group. Collectively, these findings suggested that m7G-related risk scores could serve as a valuable reference for guiding immunotherapeutic strategies in OS. Therefore, our findings underscored the significance of considering m7G-related gene expressions as potential indicators for immunotherapies, further emphasizing the interplay between epigenetic modifications, immune responses, and OS pathogenesis.

The emergence of chemotherapeutic resistance and the propensity for metastasis and recurrence pose significant challenges in the treatment of OS, necessitating the exploration of novel therapeutic options. In our research, we identified four potential drugs that hold promise for OS treatment: AZD 2014, AZD5153, acetalax, and dactolisib. Previous studies have demonstrated the anti-tumor effects of AZD5153, acetalax, and dactolisib in OS (Gobin et al., 2014; Sun et al., 2020; Sheard et al., 2021). However, the impact of AZD2014 on OS remains to be elucidated. AZD 2014, also known as vistusertib, is an ATP-competitive *mTOR*1/2 inhibitor with broad-spectrum anti-cancer properties, exhibiting a strong anti-proliferative activity (Zheng et al., 2015; Pi et al., 2021). The *PI3K/Akt/mTOR* signaling pathway has been implicated in the occurrence and development of OS, suggesting that targeting this pathway may hold promise for OS treatment (Gill and Gorlick, 2021). Additionally, recent studies have indicated a link between m7G modifications and the *mTOR* pathway in the pathogenesis and progression of certain cancers (Chen J. et al., 2022; Han et al., 2023). In our study, we demonstrated that AZD2014 induces the G1-phase cell cycle arrest and apoptosis in OS cells through cell cycles and apoptosis assays. These findings are consistent with the results obtained by Pi et al. (2021), who observed similar effects of

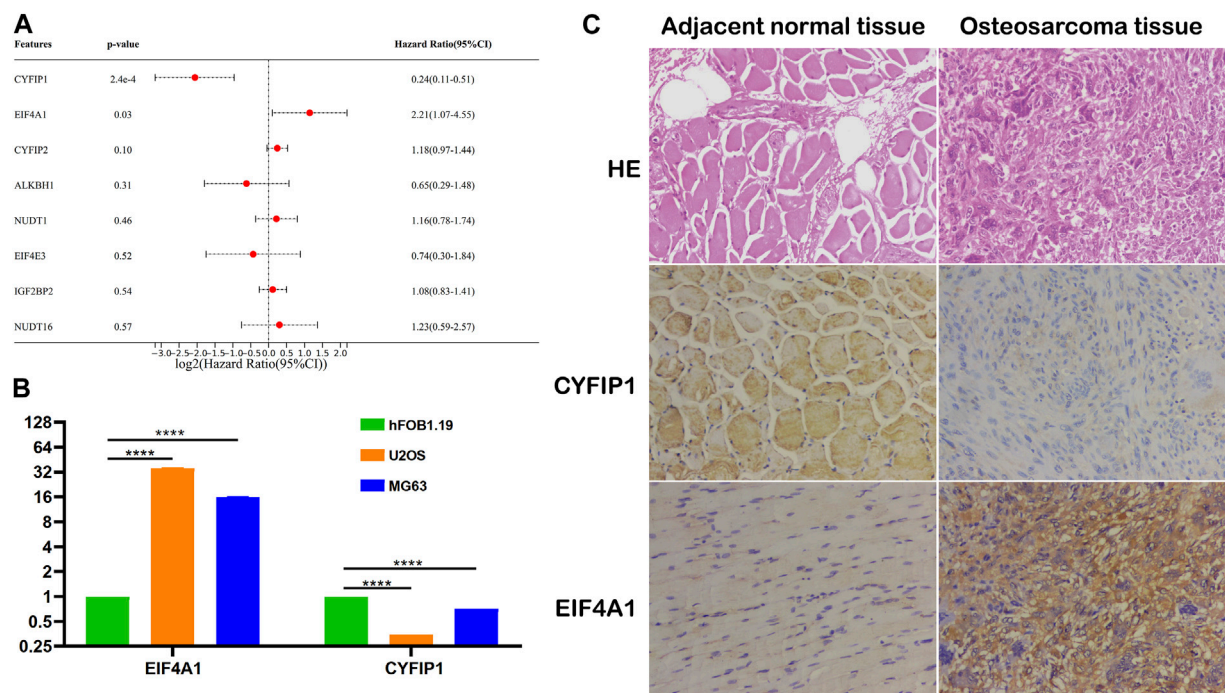


FIGURE 7

Validation of the m7G-related signatures. (A) Multivariate Cox regression analysis of the m7G-related signatures; (B) qPCR of CYFIP1 and EIF4A1 between osteoblasts and OS cells; and (C) IHC of CYFIP1 and EIF4A1 between OS tissue and adjacent normal tissue. qPCR: quantitative PCR; IHC: immunohistochemistry.

AZD2014 in ovarian cancer. Collectively, our results suggested that AZD2014 may represent a promising therapeutic option for OS treatment, warranting further investigation.

Finally, our study identified *CYFIP1* and *EIF4A1* as two independent risk factors for OS through multivariate Cox regression analysis. We further confirmed the differential expression of *CYFIP1* and *EIF4A1* at both histological and cytological levels. *CYFIP1*, also known as the *SRA1* protein, is a component of the *CYFIP1-EIF4E-FMR1* complex, which binds to the mRNA cap and regulates translational repression (De Rubeis et al., 2013). Recent studies have demonstrated the significance of *CYFIP1* in cancer development, particularly in promoting invasion (Silva et al., 2009). *EIF4A1* is an ATP-dependent RNA helicase that participates in the assembly of the *EIF4F* complex that is essential for cap recognition and mRNA binding to ribosomes (Schmidt et al., 2023). During protein translation, *EIF4A1* unwinds RNA secondary structures in the 5'-UTR of mRNA, facilitating the efficient binding of small ribosomal subunits and subsequent scanning for initiator codons (Taubert et al., 2020). Increasing evidence has highlighted the oncogenic role of *EIF4A1* in various cancers, including prostate, pancreatic, and breast cancer (Modelska et al., 2015; Ma et al., 2019; Wang et al., 2022). Given the significant involvement of *CYFIP1* and *EIF4A1* in cancer pathogenesis, including their potential roles in invasion and protein translation, targeting these molecules may hold promise as a therapeutic approach for OS. Developing therapies that specifically modulate the functions of *CYFIP1* and *EIF4A1* could provide new avenues for improving the treatment outcomes of OS patients.

Undoubtedly, there are several limitations that should be acknowledged in the present study. First, the rare incidence of OS

poses challenges in recruiting a large cohort for comprehensive analysis, which may limit the generalizability of the findings. Future studies should aim to collaborate with multiple research centers or utilize international databases to overcome this limitation. Second, it is important to acknowledge that the RNA-seq and clinical data utilized in this study were predominantly sourced from TCGA and GEO databases, which primarily represent European and North American populations. This may introduce inherent selection bias and limit the applicability of the findings to other ethnic populations (Tomczak et al., 2015). Therefore, it is crucial to validate the results in diverse cohorts that encompass different ethnic backgrounds and geographical regions. Furthermore, the bioinformatic analysis performed in this study relies on publicly available databases, which may contain inherent limitations and inconsistencies. The accuracy and reliability of the results are contingent on the quality and comprehensiveness of the data sources. Therefore, caution should be exercised in the interpretation and application of the findings. Last, the current study is based on bioinformatic analysis and *in vitro* experiments, which provide valuable insights into the role of m7G-related genes in OS. However, further *in vivo* experiments and clinical studies are warranted to validate the findings and assess the clinical feasibility of the identified m7G-related risk model and potential therapeutic targets.

Conclusion

In summary, our study sheds light on the potential importance of m7G-related genes in OS. Through the establishment of a

prognostic model and investigation of drug responses, we obtained valuable insights into the role of m7G modification in the pathogenesis of OS and its impact on treatment outcomes. Particularly, our findings suggest that AZD2014 holds promise as a potential therapeutic agent for OS. Moreover, we identified *CYFIP1* and *EIF4A1* as independent risk factors for OS. These findings contribute to a better understanding of the molecular mechanisms underlying OS and may pave the way for the development of targeted therapies. Further research and clinical validation are warranted to explore the full therapeutic potential of m7G-related genes in OS.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#); further inquiries can be directed to the corresponding author.

Author contributions

WL and ZL selected the topic and revised the manuscript. ZL, YY, ZW, and WZ conducted the experiments, wrote the manuscript, and made figures as well as tables. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Argon-helium knife cryoablation plus programmed cell death protein 1 inhibitor in the treatment of advanced soft tissue sarcomas: there is no evidence of the synergistic effects of this combination therapy

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Background: Effective treatment for advanced soft tissue sarcomas (STSs) is necessary for improved outcomes. Previous studies have suggested that cryoablation can have a synergistic effect with programmed cell death protein-1 (PD-1) inhibitor in the treatment of malignancy. This study aimed to clarify the efficacy and safety of argon-helium knife cryoablation in combination with PD-1 inhibitor in the treatment of STSs.

Methods: Retrospectively collected and analyzed the clinical data of patients with advanced STS who underwent cryoablation and PD-1 inhibitor between March 2018 and December 2021.

Results: This study included 27 patients with advanced STS. In terms of target lesions treated with cryoablation, 1 patient achieved complete response, 15 patients had partial response (PR), 10 patients had stable disease, and 1 patient had progressive disease. This corresponded to an overall response rate of 59.3% and a disease control rate of 96.3%. In terms of distant target lesions untreated with cryoablation, only two patients had a PR compared to the diameter of the lesion before ablation. The combination therapy was relatively well tolerated. None of the patients experienced treatment-related death or delayed treatment due to adverse events.

Conclusion: Cryoablation combined with PD-1 inhibitors in the therapy of advanced STS is safe and can effectively shrink the cryoablation-target lesion. However, there is no evidence of the synergistic effects of this combination therapy.

KEYWORDS

sarcoma, argon-helium knife, cryoablation, PD-1 inhibitor, immunotherapy

1 Introduction

Soft tissue sarcomas (STSs) are rare heterogeneous mesenchymal tumors with more than 70 histological subtypes (1). These malignancies occur in less than 6 in 100,000 people and account for less than 2% of all adult cancers (2). Nevertheless, there are tens of thousands of new cases of STSs in China annually (3). The most important treatment for STS is surgical resection. However, more than 50% of STSs will eventually experience metastasis. Approximately 90% of advanced STS metastasizes to lung. Other common sites of metastasis include the liver, bone, and brain (4, 5). The limited availability of effective treatments for metastatic STS results in a median overall survival of 1–1.5 years for this malignancy (6, 7). Therefore, additional effective treatments are required for advanced STS.

Cryoablation is a mature technique for local tumor treatment with decades of history (8). Argon-helium knife cryoablation is performed by inserting a cryoprobe that can freeze to -150°C rapidly into the tumor and then rapidly freezing the tumor. The probe is then rapidly rewarmed to $20\text{--}40^{\circ}\text{C}$. Rapid temperature changes can lead to coagulation necrosis of tumor tissue within a certain range (9). Recently, cryoablation is widely used in the adjuvant treatment of various malignancies (8). Studies have preliminarily demonstrated the efficacy and safety of argon-helium knife cryoablation in the treatment of metastatic STSs (10–13). However, these studies are already outdated. In recent years, programmed cell death protein-1 (PD-1) inhibitor has been gradually used in the treatment of advanced STS (14, 15). Some studies have suggested that cryoablation combined with PD-1 inhibitor can achieve better efficacy (16, 17). However, reports on the efficacy and safety of cryoablation plus PD-1 inhibitor in STSs are rare.

As a large sarcoma center, we have significant experience in the diagnosis and treatment of sarcomas (18–20). In recent years, we have treated many patients with advanced STS with cryoablation plus PD-1 inhibitor. The clinical data of these patients were retrospectively collected and analyzed in this study, to evaluate the safety and efficacy of cryoablation plus PD-1 inhibitor in STSs. This study will provide a reference for the clinical treatment and research of advanced STSs.

2 Methods

2.1 Patients and eligibility criteria

In this retrospective study, all patients with advanced STS were treated with argon-helium knife cryoablation in combination with a PD-1 inhibitor between March 2018 and March 2022. The eligibility criteria were as follows: (1) treatment with argon-helium knife cryoablation plus a PD-1 inhibitor, (2) histologically proven STS, (3) measurable lesions, (4) multiple metastases, and (5) complete clinical data.

This study was approved by the Medical Ethics Committee of Henan Cancer Hospital (Approval No. 2021-526-002), and written consent was obtained from each patient (awareness data were collected for clinical study).

2.2 Treatment

Different patients were treated with different PD-1 inhibitors, including camrelizumab (Hengrui Medicine, China), sintilimab (Innovent Biologics, China), and toripalimab (Junshi Biosciences, China). The drug treatment protocols for these patients were as follows: PD-1 inhibitor was administered intravenously at a dose of 200 mg via a 30-min intravenous infusion, once every 3 weeks. Patients were treated with PD-1 inhibitor until disease progression (PD) or unacceptable adverse events (AEs).

Some patients underwent cryoablation immediately after treatment with PD-1 inhibitor; others were treated with cryoablation for better results after a period of PD-1 inhibitor treatment.

When performing cryoablation, general anesthesia and computed tomography (CT) guidance are used. A percutaneous cryoablation device utilizing 1.7-mm-diameter cryoprobe and the Cryo-Hit™ cryosurgical system (CryoHit type; Galil Medical, Yokneam, Israel) are used during all procedures. The number of needles used was dependent on the size of the target lesion. Based on the size and location of the target lesion, cryoprobes were inserted into the center of the lesion mass under CT guidance. After the probe position is determined, the freeze-thaw cycle is carried out. A single cycle consists of a 10-minute freezing period

during which the local temperature drops to -170°C due to the rapid expansion of argon gas, and a 2-minute rewarming period during which the local temperature rises to 20°C due to the rapid expansion of helium gas. Generally, a procedure consists of two freeze-thaw cycles. In order to obtain effective treatment, the ice hockey area needs to exceed the edge of the lesion by 1 cm or more, and the scope of a single procedure needs to cover more than 80% of the lesion. After cryoablation, vital signs were monitored routinely, and hemostatic agents were administered. If there are symptoms or evidence of infection, antibiotics will be administered.

2.3 Evaluation

The efficacy of the treatment was evaluated using CT or magnetic resonance imaging according to the Response Evaluation Criteria in Solid Tumors version 1.1. During PD-1 inhibitor treatment, patients should be evaluated once in two treatment cycles. Patients are advised to be evaluated once a month after cryoablation. Tumor responses were categorized as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). The overall response rate (ORR) was defined as the sum of the CR and PR rates. The disease control rate (DCR) was defined as the sum of the ORR and SD. AEs were assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.

2.4 Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) (version 21.0, SPSS Inc., USA). Quantitative variables are presented as numerical values (percentages). The figures were drawn using GraphPad Prism 5.0 (GraphPad Software Inc., USA). This analysis was descriptive, and the follow-up period was extended to January 31, 2023.

3 Results

3.1 Patient characteristics

From March 2018 to March 2022, 27 patients with advanced STS who were treated with argon-helium knife cryoablation combined with PD-1 inhibitor were identified (Table 1). There were 12 men and 15 women, with an average age of 39.7 years. All patients had an Eastern Cooperative Oncology Group performance status of 0 or 1. The histological subtype included alveolar soft part sarcoma ($n=5$), undifferentiated pleomorphic sarcoma ($n=5$), leiomyosarcoma ($n=3$), liposarcoma ($n=3$), synovial sarcoma ($n=3$), Ewing sarcoma ($n=2$), fibrosarcoma ($n=2$), angiosarcoma ($n=1$), rhabdomyosarcoma ($n=1$), epithelioid sarcoma ($n=1$), and clear cell sarcoma ($n=1$). The lungs were the most frequent metastatic sites in most patients.

3.2 Efficacy

Different patients were treated with different PD-1 inhibitors. A total of three PD-1 inhibitors were used (Table 1). In terms of target lesions treated with cryoablation, 1, 15, 10, and 1 patients had CR, PR, SD, and PD, respectively (Table 1; Figures 1, 2). This corresponded to an ORR of 59.3% and a DCR of 96.3%. In terms of distant target lesions untreated with cryoablation, only two patients had a PR compared to the diameter of the lesion before ablation (Table 1; Figure 1).

3.3 Safety

In general, argon-helium knife cryoablation combined with PD-1 inhibitor therapy was relatively tolerated (Table 2). Grade 1 or 2 AEs associated with cryoablation included the following: pneumonitis (63.0%) (Figures 2, 3), fever (55.6%), pneumothorax

TABLE 1 Patient demographics and characteristics.

Patient No.	ECOG PS	Histological subtype	Stage	Location of target lesions	PD-1 inhibitor	Best response of the target lesions	Best response of other lesions
1	0	ASPS	IV	Lung	Sintilimab	CR	PR
2	0	ASPS	IV	Lung	Sintilimab	PR	SD
3	0	ASPS	IV	Lung	Toripalimab	SD	SD
4	1	ASPS	IV	bone	Toripalimab	SD	SD
5	1	ASPS	IV	Lung and retroperitoneum	Toripalimab	SD	SD
6	0	UPS	IV	Lung	Camrelizumab	PR	PR
7	1	UPS	IV	Lung	Sintilimab	PR	SD
8	0	UPS	IV	Head	Camrelizumab	PR	PD

(Continued)

TABLE 1 Continued

Patient No.	ECOG PS	Histological subtype	Stage	Location of target lesions	PD-1 inhibitor	Best response of the target lesions	Best response of other lesions
9	1	UPS	IV	Bone	Sintilimab	SD	PD
10	2	UPS	IV	Lung	Camrelizumab	PD	PD
11	1	Leiomyosarcoma	IV	Lung	Sintilimab	PR	SD
12	0	Leiomyosarcoma	IV	Lung	Camrelizumab	PR	SD
13	1	Leiomyosarcoma	IV	Liver	Toripalimab	PR	PD
14	0	Liposarcoma	IV	Lung	Sintilimab	PR	SD
15	1	Liposarcoma	IV	Lung	Sintilimab	PR	PD
16	0	Liposarcoma	IV	bone	Toripalimab	SD	PD
17	0	Synovial sarcoma	IV	Lung	Camrelizumab	PR	SD
18	2	Synovial sarcoma	IV	Lung	Camrelizumab	PR	PD
19	1	Synovial sarcoma	IV	bone	Camrelizumab	SD	PD
20	0	Ewing sarcoma	IV	Lung	Sintilimab	PR	SD
21	1	Ewing sarcoma	IV	Chest wall	Sintilimab	SD	PD
22	0	Fibrosarcoma	IV	Lung	Camrelizumab	PR	SD
23	1	Fibrosarcoma	IV	Lung	Camrelizumab	SD	SD
24	1	Angiosarcoma	IV	Head	Sintilimab	SD	PD
25	1	Rhabdomyosarcoma	IV	Lung	Toripalimab	PR	PD
26	1	Epithelioid sarcoma	IV	Lung	Sintilimab	PR	PD
27	0	Clear cell sarcoma	IV	Lung	Camrelizumab	SD	PD

ECOG PS, Eastern Cooperative Oncology Group performance status; ASPS, alveolar soft part sarcoma; UPS, undifferentiated pleomorphic sarcoma; PD-1, Programmed cell death protein 1; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

(40.7%), pleural effusion (29.6%), cough (25.9%), pain (18.5%), and nerve injury (3.7%). Grade 1 or 2 AEs associated with PD-1 inhibitor included the following: hypothyroidism (18.5%), fatigue (14.8%), increased transaminase increase level (11.1%), pneumonitis (11.1%), rash (11.1%), and diarrhea (7.4%). Grade 3–4 AEs were rare. None of the patients experienced delayed treatment or treatment-related death due to AEs.

4 Discussion

Although there are dozens of subtypes of STSs, most are immunotherapeutic indolent. The results of one of the most influential clinical trials reported in 2017 showed that only 7 of the 40 patients with STS had an objective response (21). Many other studies have found similar results (15). To improve the efficacy of STS immunotherapy, various approaches are now being explored to enhance the immunogenicity of sarcomas (22, 23). Previous studies have suggested tissue and cell destruction caused by cryoablation may increase the expression of tumor antigens, thereby enhancing the ability of the immune system to recognize and attack tumor cells (24, 25). Currently, only one study has reported the safety of

cryoablation combined with PD-1 inhibitor therapy in STSs, but it has not shown significant synergistic efficacy (16).

In this study, 27 patients with STS were received argon-helium knife cryoablation plus PD-1 inhibitor treatment. The results showed that 96.3% (26/27) of the patients experienced the control of the size of target lesions. This suggests that cryoablation is effective in treating STS metastases. However, only 7.4% (2/27) of the patients receiving cryoablation showed a reduction in the diameter of distant lesions. This suggests that cryoablation combined with PD-1 inhibitor treatment is hard to produce synergistic effects as it does in other cancers (26, 27). There are many factors affecting the efficacy of PD-1 inhibitor. First, it may be due to the immune inertia of the sarcoma itself that the local increase in immunogenicity produced by cryoablation is insufficient to activate the body's systemic antitumor immune response. Second, cryoablation inevitably leads to local bleeding, necrosis, and inflammatory reaction (Figures 2, 3). Nevertheless, the inflammatory response inhibits the efficacy of PD-1 inhibitor (28). Therefore, the effect of cryoablation on antitumor immune response is two-sided. It may improve antitumor immune response by improving immunogenicity or inhibit antitumor immune response by triggering inflammatory response. A study that found

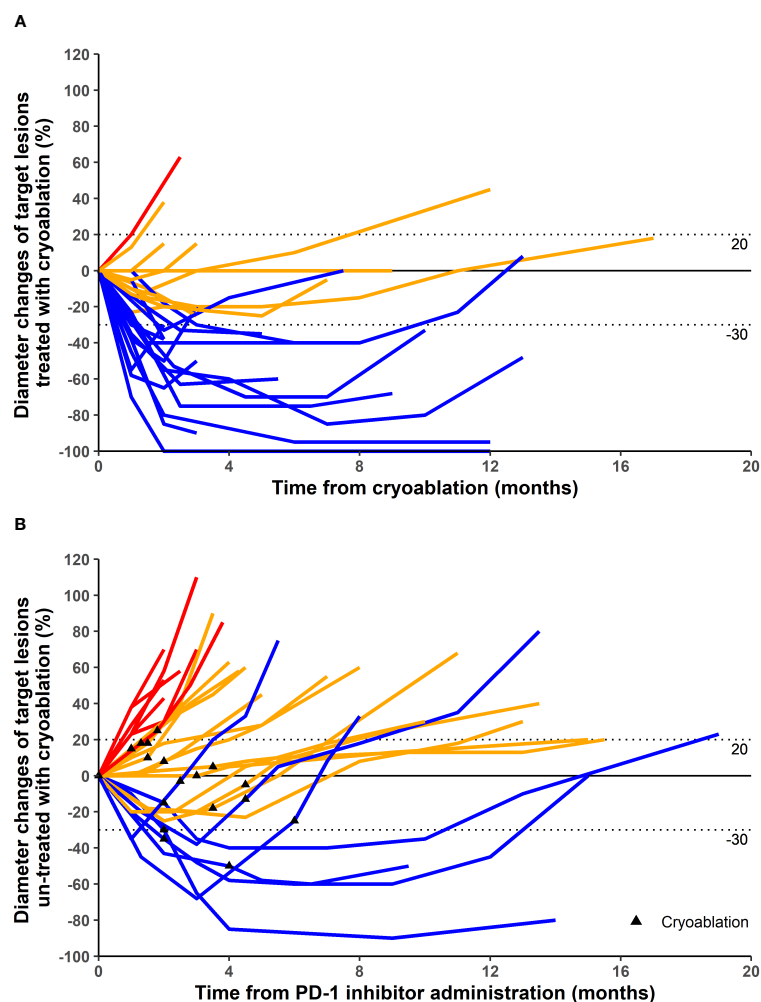


FIGURE 1

Maximum percentage diameter changes from baseline in target lesion treated with cryoablation (A) and untreated with cryoablation (B). Treatment efficacy was evaluated using the Response Evaluation Criteria in Solid Tumors version 1.1. (A) The baseline is the diameter of target lesions before cryoablation. (B) The baseline is the diameter of distant lesions (untreated with cryoablation) before the first dose of programmed cell death protein 1 (PD-1) inhibitor. Some patients underwent cryoablation immediately after treatment with PD-1 inhibitor, and others were treated with cryoablation for better results after a period of PD-1 inhibitor treatment.

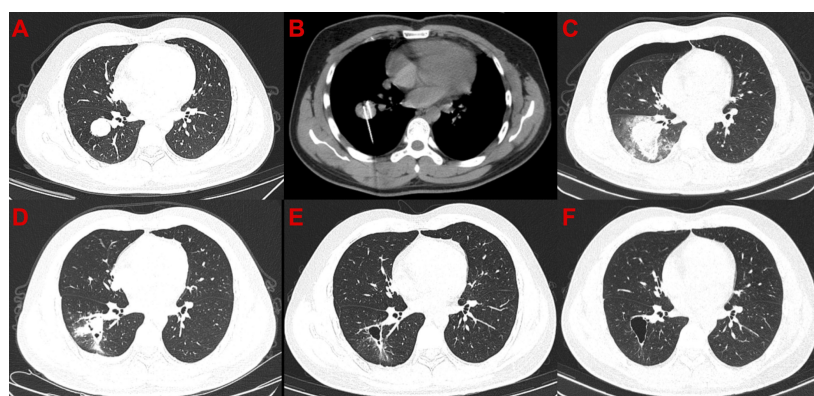


FIGURE 2

Computed tomography images of the only patient with complete response after cryoablation. (A) Before cryoablation. (B) During cryoablation. (C) After cryoablation, local large-scale inflammation and pneumothorax can be observed. (D) One month after cryoablation, pneumothorax disappeared, and the scope of inflammation narrowed. (E) Moreover, 3.5 months after cryoablation, cavity formation was observed in the diseased area. (F) 7.5 months after cryoablation.

TABLE 2 Adverse events.

Adverse events (AEs)	Grade 1-2	Grade 3-4
AEs related to cryoablation		
Pneumonitis	63.0% (17/27)	11.1% (3/27)
Fever	55.6% (15/27)	
Pneumothorax	40.7% (11/27)	3.7% (1/27)
Pleural effusion	29.6% (8/27)	3.7% (1/27)
Cough	25.9% (7/27)	3.7% (1/27)
Pain	18.5% (5/27)	
Nerve injury	3.7% (1/27)	3.7% (1/27)
AEs related to immunotherapy		
Hypothyroidism	18.5% (5/27)	3.7% (1/27)
Fatigue	14.8% (4/27)	3.7% (1/27)
Transaminase increase	11.1% (3/27)	
Pneumonitis	11.1% (3/27)	
Rash	11.1% (3/27)	
Diarrhea	7.4% (2/27)	

Data are presented as percentages (number events/total).

that the freezing rate of cryoablation had a significantly different effect on the immune system may confirm our conjecture (29). Third, adjuvant medication has an important effect. A prior study confirmed that the use of concomitant drugs (steroids, systemic antibiotics, proton pump inhibitors) was associated with worse clinical outcomes when receiving PD-1 inhibitor (30). In this study, we used the abovementioned adjuvant therapy agents in almost every patient. In addition, the combination sequence of cryoablation with PD-1 inhibitors may also be important. A current study has demonstrated that different sequences of chemotherapy combined with PD-1 inhibitors have significantly different effects on efficacy (31). In this study, all patients were treated with PD-1 inhibitor before

cryoablation. This combination sequence may not be conducive to synergistic efficacy. A systematic follow-up study for a valid comparison of PD-1 inhibitor treatment before and after cryoablation can demonstrate which strategy could be clinically beneficial. In conclusion, there are many factors that lead to the lack of synergistic effect between cryoablation and PD-1 inhibitors in this study, and further studies are required. Additionally, it is worth noting that some studies have demonstrated that thermal ablation can promote the release of tumor antigens, thereby driving downstream immune responses (32–34). The differences in the effects of different ablation methods such as thermal ablation or cryoablation on anti-tumor immunity are also worth further research.

In general, cryoablation combined with PD-1 inhibitor has a safety profile, with rare serious complications. The most common complications are pneumonia and pleural effusion after treatment of lung lesions (Figures 2, 3). Patients with these complications are often treated with adjuvant medications, such as steroid hormones or antibiotics. Such adjunctive agents inhibit the antitumor immune response and even lead to a poor prognosis (30). This may be one reason for the absence of synergistic results in this study. Therefore, PD-1 inhibitor should not be considered in patients considering lung cryoablation until steroid hormones or antibiotics are confirmed to be unnecessary.

We acknowledge that the small sample size, retrospective nature, and lack of a control group lower the level of evidence in this study. Although cryoablation does not activate the antitumor immune response, it shrinks the cryoablation-target lesions and improves patients' symptoms, which may be beneficial in prolonging the survival of patients with advanced STS with insufficient effective treatment. Therefore, cryoablation remains an important therapeutic treatment for some advanced STSs, and the interaction mechanism between cryoablation and immunotherapy should be further studied in the future.

In conclusion, cryoablation combined with PD-1 inhibitors in the treatment of advanced STS is safe and can effectively shrink the cryoablation-target lesion. However, there is no evidence of the synergistic effects of this combination therapy.

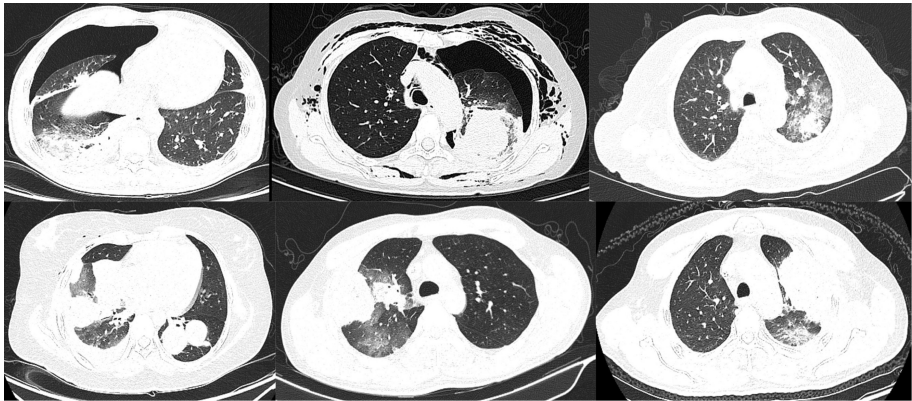


FIGURE 3 Lung inflammation after cryoablation. Nearly all lung lesions developed local pneumonia after cryoablation, and some patients also had pneumothorax. All these inflammations disappeared within 1 month after cryoablation.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Medical Ethics Committee of Henan Cancer Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

JW and ZT were responsible for the conception, design and drafting of the study. DZ, SD, SG, PZ, XW and WY were

responsible for the patient recruitment and clinical investigation. JW, YY, WY and ZT were responsible for the analysis and interpretation of data. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Case report: the dissociated response and clinical benefit of primary leiomyosarcoma of the bone treated with penpulimab plus lenvatinib after failed multi-line therapy

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Leiomyosarcoma occurring in the bone as primary tumor localization is extremely scarce with limited cases described in the literature, accounting for less than 0.7% of all primary bone malignancies. Once distant metastasis occurs, patients have limited treatments and often a somber prognosis, which underscore the need for innovative and effective treatment approaches. The emerging evidence suggests that anti-angiogenic therapy could inhibit angiogenesis and normalize vascular permeability in the tumor microenvironment, which, in turn, would increase immune effector cell infiltration into tumors. Immunotherapy depends on the accumulation and activity of immune effector cells within the tumor microenvironment, and immune responses and vascular normalization seem to be reciprocally regulated. Immunotherapy combined with anti-angiogenic therapy has recently made great progress in the treatment of various types of tumors. However, the effectiveness of the combination treatment in metastatic leiomyosarcoma is undetermined. In this study, we presented a rare case of primary leiomyosarcoma of the bone located in the trochanteric region of the femur, accompanied by multiple distant metastases. After the failure of multi-line therapies including AI regimens as the adjuvant chemotherapy, anlotinib as the first-line therapy, GT regimen as the second-line therapy, and eribulin as the third-line therapy, the patient received combinational therapy with penpulimab plus lenvatinib. The best efficacy for this regimen was a partial response, with a progression-free survival of 8.4 months according to the iRECIST criteria. After a dissociated response was detected without severe toxicities, the patient received local radiotherapy and continued treatment on penpulimab plus lenvatinib and eventually achieved long-term survival benefits with a total of over 60 months of overall survival with good quality of life and ongoing treatment. As our previous retrospective study found that one-third of advanced STS patients could still achieve clinical benefits from rechallenge with multi-targeted tyrosine kinase

inhibitors (TKIs), after the failure of previous TKI therapy, this case provided the potential clinical activity of immunotherapy combined with anti-angiogenic TKI rechallenge in metastatic leiomyosarcoma.

KEYWORDS

leiomyosarcoma, immunotherapy, anti-angiogenic therapy, dissociated response, clinical benefit

Introduction

Leiomyosarcoma (LMS) is a malignant neoplasm derived from either smooth muscle cells or precursor mesenchymal stem cells that eventually differentiate into smooth muscle cells, with an estimated incidence of 10%–20% of all newly diagnosed soft tissue sarcomas (STSs) (Novotny and George, 2021). This tumor can originate from any site of the body, with the retroperitoneum, uterus, and limbs/girdles being more frequently affected (George et al., 2018). LMS that occurred in bones as primary tumor localization is extremely rare and was first reported by Evans and Sanerkin in 1965, with an incidence of less than 0.7% of all primary bone malignancies (Evans and Sanerkin, 1965; Wang and Lucas, 2019). Primary leiomyosarcoma of the bone (PLMSB) usually occurs in the long tubular bones of the lower extremities, with about 70% of cases found in the knee joint (distal femur and proximal tibia) (Wu et al., 2022). It is rarely reported to occur in the hip joint and trochanteric area of the femur. The diagnosis of PLMSB is marked by the absence of either osteoid or chondroid matrix LMS. Clinical features and relevant prognostic factors of PLMSB are not well defined because of the few data obtained mostly from retrospective analyses, small case series, and case reports (Wang and Lucas, 2019). LMS has a predilection for metastasis, with common sites of metastasis including the lung, liver, soft tissues, and bones; metastasis to the pancreas is extremely rare (Rekhi et al., 2011).

Systemic chemotherapy with doxorubicin alone or in combination is still the first-line treatment for unresectable metastatic LMS (Pautier et al., 2022). Despite multiple clinical trials investigating single-agent and combination schemes over the past decades, progression-free survival (PFS) for various therapies remains in the 3–7-month range with median overall survival (OS) at 12–18 months (Miller et al., 2020). Second- and later-line regimens provide small clinical benefits in patients with STSs including LMS (e.g., pazopanib, trabectedin, and eribulin). LMS is a disease of complex cytogenetic and molecular aberrancies and is characterized by a relatively inflamed TME with higher PD-L1 expression, greater immune infiltration, and antigen presentation compared with other sarcoma subtypes (Feng et al., 2023; Lacuna et al., 2023). Despite the potential for immunotherapy in LMS, immune checkpoint inhibitors (ICIs) targeting PD-1, PD-L1, and CTLA-4 have greatly improved oncologic outcomes for several cancer types but show minimal efficacy for LMS (Tawbi et al., 2017). Thus, treatment regimens with higher efficacy are needed to improve the outcomes of patients with metastatic LMS. Immune barrier mediated by tumor angiogenesis is well established, and there is an ever-growing list of immune cells exhibiting the dual capacity of facilitating angiogenesis and immunosuppression (Fukumura et al., 2018). Through directly inhibiting tumor growth and metastasis, anti-angiogenic therapies reprogram the tumor milieu from an immunosuppressive to an immune-permissive microenvironment, which, in turn, increases immune effector cell infiltration into tumors (Lee et al., 2020).

Activated immunity by ICIs can also enhance anti-angiogenesis by decreasing the expression of VEGF and alleviating hypoxia (Lee et al., 2020; Wang et al., 2023). ICIs combined with anti-angiogenic therapy have exhibited favorable outcomes in various types of cancers (Fukumura et al., 2018). Penpulimab is a novel Fc-engineered IgG1 monoclonal antibody against PD-1. By eliminating fragment crystallizable (Fc) receptor-binding activities such as antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP), the agent not only enhances the efficacy of immunotherapy but also exhibits improved safety profiles. Lenvatinib, an oral multi-targeted tyrosine kinase inhibitor (TKI), has potent anti-angiogenic activity, mainly through the inhibition of VEGFR 1–3, PDGFR α , FGFR 1–4, KIT, and RET. To date, little information is available on the efficacy and safety of this combination in metastatic LMS.

Here, we report a rare case of leiomyosarcoma metastatic to the pancreas in a patient with confirmed PLMSB in the trochanteric region of the femur. After progressing on third-line therapy, the patient received the fourth-line therapy with penpulimab plus lenvatinib and eventually achieved long-term benefits with a total of over 60 months of overall survival with a good quality of life and ongoing treatment. This case demonstrated the efficacy and safety of immunotherapy combined with anti-angiogenic therapy for the later-line treatment of metastatic PLMSB and provided the possibility of a rechallenge with TKI.

Case presentation

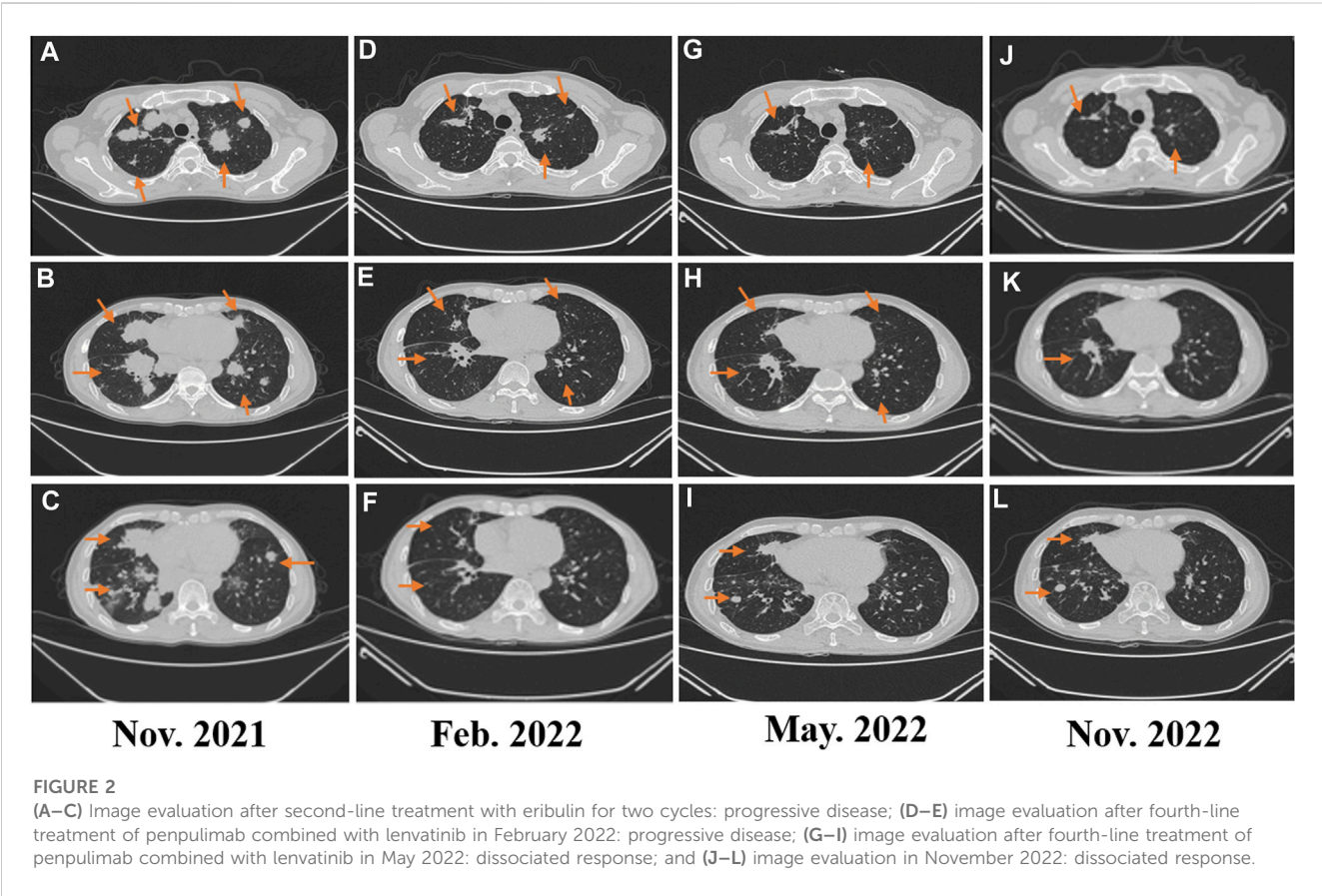
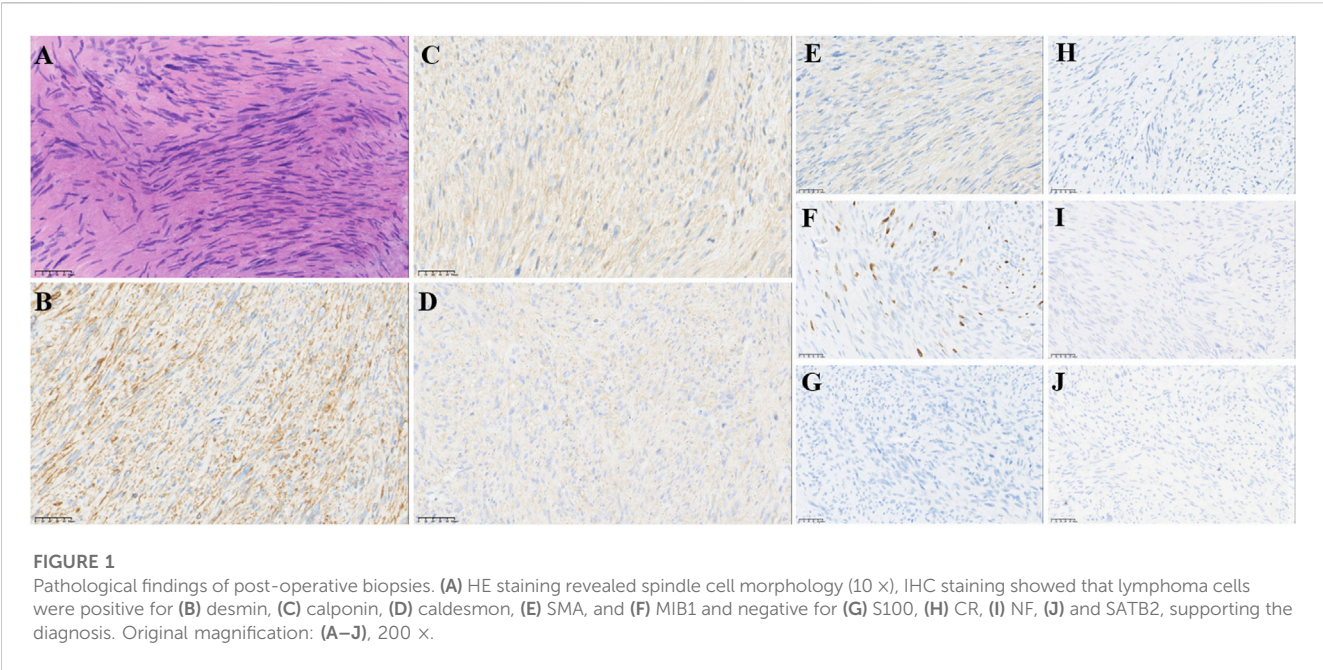
In February 2018, a 49-year-old male presented with an enlarged palpable mass in the proximal right lower extremity, accompanied by persistent dull pain and movement restriction. The patient did not have any previous significant medical conditions. The computed tomography (CT) revealed the shadow of a heterogeneous mass (89 × 42 mm) on the right intertrochanteric region of the femur, and the lesion was obviously strengthened unevenly after enhancement. Metastatic tumors were excluded according to the patient's whole-body imaging findings. In August 2018, an excisional biopsy of the right intertrochanteric space-occupying lesion was performed, and the pathological evaluation demonstrated a right femoral intertrochanteric leiomyosarcoma. After two cycles of neoadjuvant doxorubicin–ifosfamide (AI) chemotherapy comprising doxorubicin 75 mg/m² given at day 1 and ifosfamide at 1.8 g/m² per day over 5 days every 3 weeks, the patient underwent extensive resection of femoral intertrochanteric leiomyosarcoma and total hip arthroplasty under general anesthesia on 5 December 2018. Macroscopic examination of the resected bone segment showed a grayish-yellow mass with a size of 8 cm × 7 cm × 6 cm in the bone marrow cavity and 9 cm from the broken end of talus tissue, which had a pattern of growth replacing the marrow and invading the surrounding soft tissues. The

efficacy assessment of neoadjuvant chemotherapy revealed extensive necrosis in the tumor with a tumor necrosis rate of approximately 80%–90% compared with preliminary pathologic results. The final pathology of the post-operative specimens suggested spindle cell morphology with immunohistochemical results being positive for desmin, calponin, caldesmon, SMA, and MIB1 and negative for S100, CR, NF, and SATB2 and confirmed the diagnosis of left femoral intertrochanteric leiomyosarcoma, grade III (FNCLCC) (Figure 1). Three cycles of adjuvant AI regimen chemotherapy were given after surgery, followed by field adjuvant radiotherapy at a dose of 50 Gy until May 2019. Unfortunately, multiple newly bilateral pulmonary nodules were detected by the routine chest CT scan on April 2020 (Supplementary Figures S1A–D). Subsequently, the patient underwent first-line treatment with anlotinib (12 mg, d1–14) every 3 weeks for seven cycles from June to October 2020 and achieved a PFS of 4 months. Then, the number and size of bilateral pulmonary nodes increased, and the curative effect was evaluated as progression disease (PD) based on the RECIST criteria (Supplementary Figures S1E–H). Thus, the patient accepted second-line chemotherapy of gemcitabine at the dose of 1,000 mg/m² on days 1 and 8, combined with docetaxel at the dose of 75 mg/m² on day 8 for six cycles. After a PFS of 8 months, the patient began complaining of persistent epigastric abdominal sharp pain with the numeric rating scale score of 7, accompanied by fever, nausea, vomiting, abdominal distension, and diarrhea in May 2021. The patient was not presented with melena, swallowing difficulties, or shortness of breath. He was bedridden for the vast majority of the time with an ECOG performance status score of 3, which severely compromised his life quality. The follow-up imaginological examination revealed new metastases in bilateral erector spinae, left internal abdominal oblique muscle, L1 and L4 vertebra, and pancreas, along with acute necrotizing pancreatitis and pancreatic pseudocyst (Figures 3A–E). At that time, the laboratory examination showed the abnormal elevation of serum amylase (up to 182 IU/L) and lipase (up to 325.4 IU/L) (Figure 4A). After broad-spectrum antibiotics and other supportive treatments, the patient's abdominal pain was significantly alleviated, and the levels of both serum amylase and lipase gradually declined but remained persistently above the normal reference range (Figure 4A). Subsequently, the patient began to try third-line therapy with eribulin at the dose of 1.4 mg/m² administered intravenously on days 1 and 8 every 21 days from September 2021. However, the patient did not benefit from eribulin, with the disease being judged to progress after only two cycles (Figures 2A–C, 3F–J). In December 2021, the patient received the combined treatment with penpulimab (200 mg IV infusion every 3 weeks), a humanized anti-PD-1 IgG1 antibody, plus the multikinase inhibitor lenvatinib (8 mg/day). The accompanying treatment plan consisted of denosumab (120 mg) injections every 4 weeks to prevent skeletal-related events. After two cycles, the physical conditions and life quality of the patient had improved significantly, with pain fading away, weight restoration, and the ECOG performance status score improving to 1. The level of serum amylase and lipase demonstrated a constant downward trend with complete normalization (14.39 U/mL) by the end of his six cycles of treatment (Figure 4A). A CT re-examination in February 2022 indicated that tumors had shrunk along with the absorption of the inflammatory exudation around the pancreas and the recession of the pancreatic pseudocyst, and the efficacy evaluation was partial response (PR) (Figures 2D–F, 3K–O). The PFS for this regimen was 8.4 months according to the iRECIST criteria.

Interestingly, repeated CT and MRI scans in May 2022 pictured a mixed response with a new pulmonary lesion observed in the posterior basal segment of the right lung but a regression in the size of the rest of the target lesions, which suggested an immune-unconfirmed progressive disease (iUPD) according to the iRECIST criteria. In August 2022, the previous new nodule of the lung continued to grow, but the rest of the lesions remained stable, which indicated immune-confirmed progressive disease (iCPD), with a pattern of dissociated response (DR) (Figures 2G–I, 3P–T). Given the clinical benefit of previous combination therapy, after a multi-disciplinary treatment (MDT) discussion and a thorough risk communication with the patient, he was treated with radiotherapy for the new pulmonary lesion (30 Gy in 10 fractions) in September 2022 while continuing penpulimab plus lenvatinib treatment. The latest imaging assessment in November 2022 revealed that all lesions were all reduced or stable, with the exception of a new metastasis in the right lobe of the liver (Figures 2J–L, 3U–Y). The patient was planned to be treated with SBRT for the hepatic metastatic lesion. Until now, the patient is still alive and undergoes treatment with lenvatinib plus penpulimab, with the OS time being extended to over 60 months and counting. Importantly, during the course of combination therapy, he had no other adverse events related to anti-angiogenic therapy or immunotherapy except hypothyroidism and osteoarthritis, which were controlled well by levothyroxine sodium tablets and short-term treatment of prednisone, respectively. In order to intuitively reflect the patient's prognosis and clinical curative effect, we listed the entire treatment process with timeline in Figure 4B.

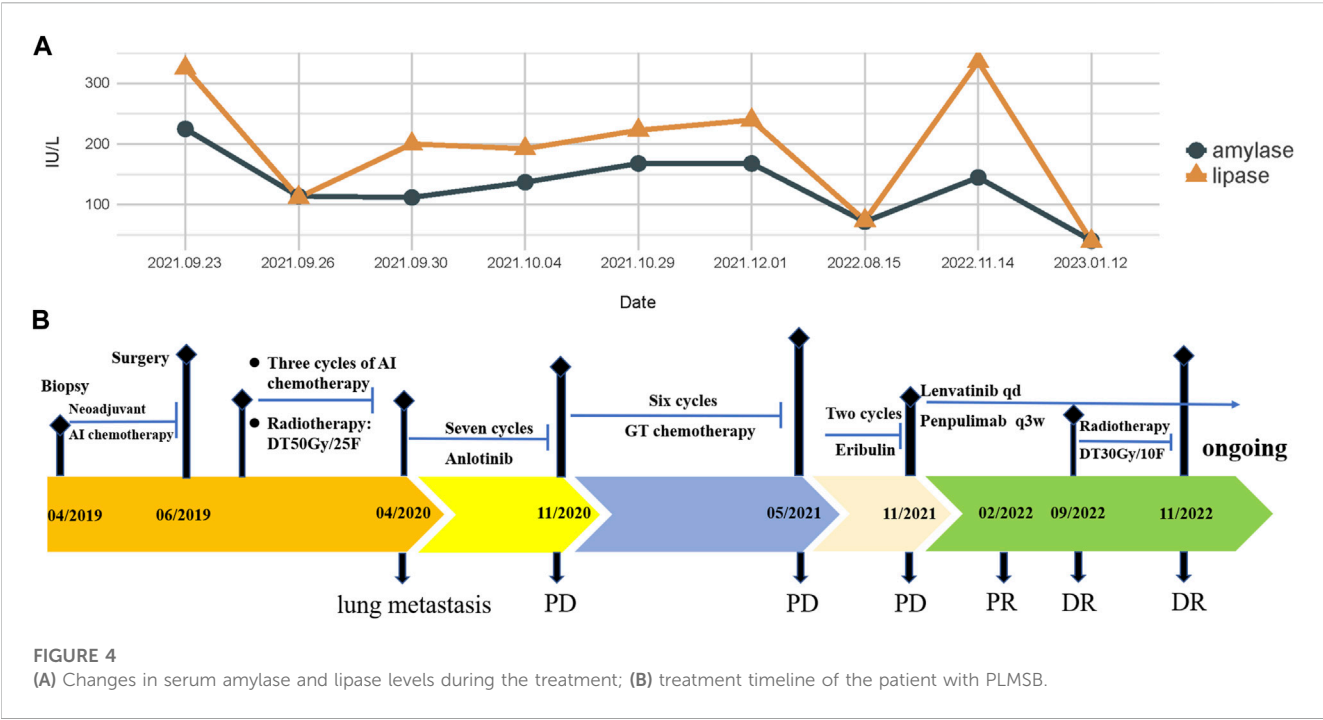
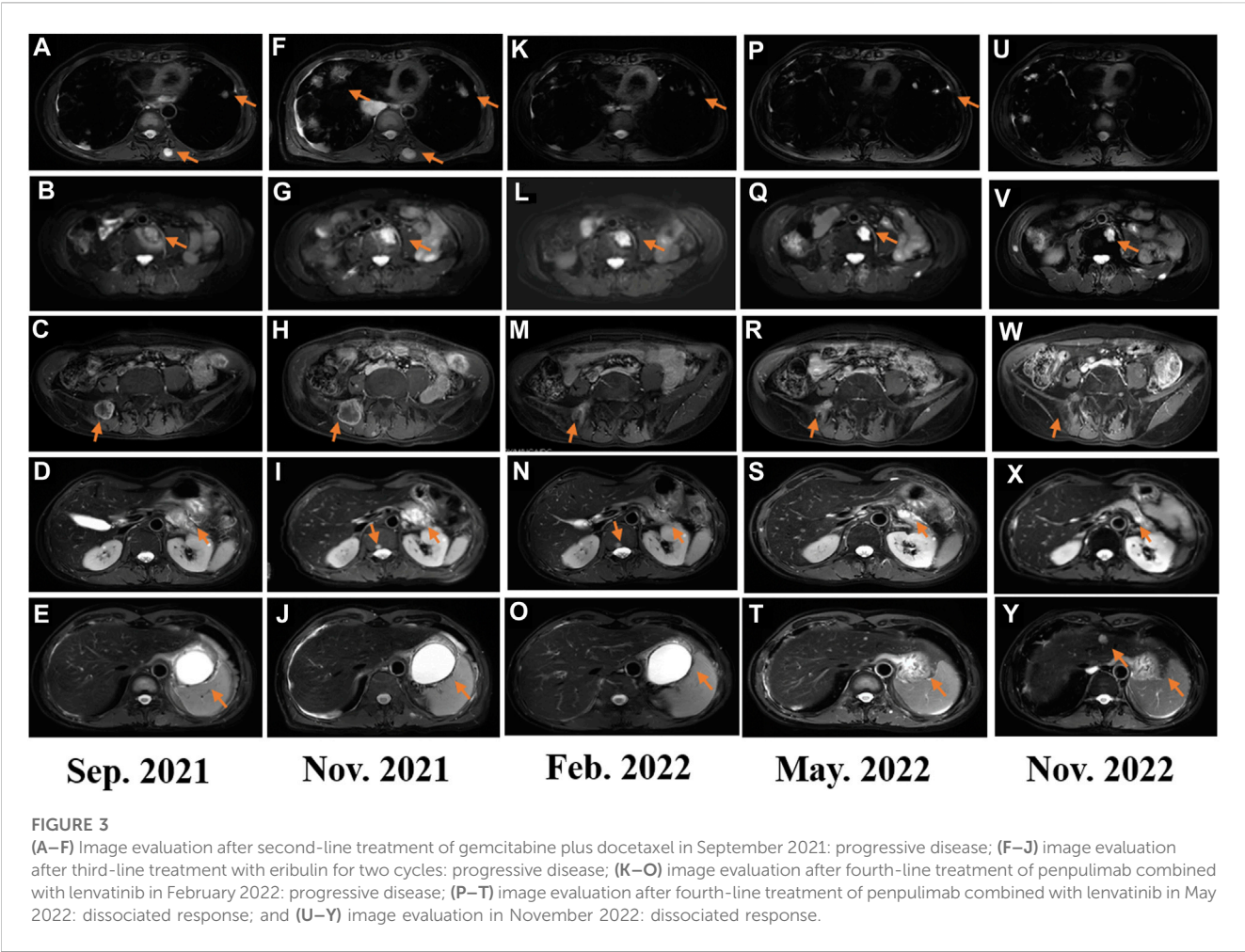
Discussion

Primary LMSB is a rare and highly invasive leiomyosarcomas, first described in 1965, with an incidence of less than 0.7% among all primary bone malignancies (Wang and Lucas, 2019). PLMSB generally has non-specific clinical and radiologic presentations (Wang and Lucas, 2019). The histopathologic features of PLMSB are identical to those originating from other more prevalent anatomical sites, characterized by smooth muscle differentiation and the absence of malignant osteoid formation (Wang and Lucas, 2019). The diagnosis of PLMSB is a challenge, which must be distinguished from metastatic leiomyosarcoma from other sites, fibroblastic osteosarcoma, primary undifferentiated pleomorphic sarcoma (UPS) of bone, and metastatic sarcomatoid carcinoma (George et al., 2018). Fibroblastic osteosarcoma tissues are arranged as fibrosarcomatoid structures, and the identification of focal malignant osteogenesis and the absence of myogenic tumor markers aid in distinguishing osteosarcoma from PLMSB (Wang and Lucas, 2019). In addition, SATB2 is a relatively specific marker for osteosarcoma that may be utilized to differentiate between osteosarcoma and PLMSB (Wang and Lucas, 2019). UPS is typically an exclusion diagnosis, and myogenic markers such as desmin and SMA can be particularly valuable in differentiating UPS from PLMSB (Matushansky et al., 2009). Metastatic sarcoma is morphologically similar to PLMSB but has unique immunohistochemical profiles, with the expression of p63 and PAX8 and lack of myogenic markers like desmin and SMA



(Wang et al., 2021). The lesion reported in this case presented as a spindle cell sarcoma by microscopy, and immunohistochemistry showed positive staining for desmin, calponin, caldesmon, SMA, and MIB1 and negative staining for S100, CR, NF, and SATB2. Metastatic tumors were excluded according to the patient's medical

history and whole-body imaging findings. Based on the absence of malignant osteogenesis and positive myogenic markers, diseases mentioned previously were ruled out, and a definite diagnosis of PLMSB in the right intertrochanteric region of the femur was finally reached.



Patients with PLMSB tended to have a worse prognosis than those with soft tissue LMS (Kobayashi et al., 2022). Due to its low prevalence, data on optimal treatment for PLMSB are limited. When possible, surgery with negative surgical margins remained the primary treatment modality, independent of origin sites (Wang and Lucas, 2019). No clear survival benefit has been demonstrated with the use of adjuvant chemotherapy or radiotherapy (Zhang et al., 2022). However, due to possible differences in biological behavior, chemotherapy and/or radiotherapy are still recommended, especially for cases that failed to completely resect the lesion (Sun et al., 2022). Local recurrence is relatively uncommon, while distant metastases are more likely to develop early, generally in the first year, regardless of the initial tumor grade (Rekhi et al., 2011). The most frequent metastatic sites are the lung and the axial skeleton, while metastases to the pancreas are not common and few cases have been reported (Rekhi et al., 2011). In our case, the patient developed multiple metastases including the pancreas within 8 months after extended resection and adjuvant AI regimen chemotherapy and radiotherapy. For advanced STS/LMS, anthracycline-based chemotherapy is the cornerstone therapy, with a median overall survival of approximately 1 year (Novotny and George, 2021). After the failure of first-line chemotherapy, there is no evidence to indicate which regimen is optimal for second-line therapy and beyond. Some chemotherapeutic agents like gemcitabine, eribulin, and trabectedin have exhibited certain efficacy for LMS, but their significance in improving OS remains controversial (Meyer and Seetharam, 2019). Some tyrosine kinase inhibitors, including pazopanib, regorafenib, and sunitinib among others, have demonstrated efficacy in STS/LMS. Moreover, multi-targeted TKI anlotinib against VEGFR1, VEGFR2/KDR, VEGFR3, FGFR1-3, PDGFR- α , and c-Kit has been licensed in China for the treatment of advanced STS after the failure of anthracycline-based chemotherapy based on phase II (Chi et al., 2018a) and phase IIb studies (ALTER0203) (Chi et al., 2018b). The subgroup analysis from a phase III study in LMS patients demonstrated the superior efficacy of eribulin compared to dacarbazine, with a median PFS of 2.2 months versus 2.6 months, a median OS of 12.7 months versus 13.0 months, and an ORR of 5% versus 7%, respectively (Blay et al., 2019). Given these premises, we selected the AI regimen, anlotinib, and GT regimen as adjuvant chemotherapy and first- and second-line treatments for this patient, respectively. The patient experienced the progression of pancreatic metastases that presented as severe acute pancreatitis while receiving second-line therapy. He was managed conservatively with supportive treatment, followed by two cycles of eribulin salvage chemotherapy. Regrettably, the patient did not benefit from single eribulin, with the therapeutic evaluation being PD.

In general, there is little evidence that immunotherapies work for LMS, barring a few case reports. The SARC028 trial revealed that the objective response rate (ORR) was 17.5% among patients with undifferentiated pleomorphic sarcoma (4/10), liposarcoma (2/10), and synovial sarcoma (1/10), while no response was observed in the LMS subgroup (Tawbi et al., 2017). The poor outcome was in line with another phase II study confirming the lack of efficacy of single nivolumab in uterine LMS (Ben-Ami et al., 2017). Many studies have shown that the combination of anti-angiogenic agents and ICI might enhance the presence and activation of CTLs in the TME to further enhance the anti-tumor effect, emerging as a novel treatment strategy (Roulleaux Dugage et al., 2021). A phase 2 trial (NCT02636725) revealed that pembrolizumab plus axitinib had preliminary activity and

manageable toxicity in patients with advanced STSs, with a 3-month PFS rate of 65.6% (Wilky et al., 2019). Another phase II trial (NCT03798106) showed encouraging activity in advanced STS, achieving an ORR of 28.3% (Kim et al., 2021). Results of a single-center retrospective analysis enrolling 61 patients with advanced STSs also showed that the median PFS was significantly prolonged after ICI-TKI combination compared to ICI alone, with 50% (8/16) of LMS patients achieving SD (You et al., 2021). A phase 2 trial (NCT04551430) revealed that cabozantinib combined with ipilimumab and nivolumab was superior to cabozantinib for the treatment of non-translocation STSs in DCR and PFS, with LMS being the most frequent responding histology (Van Tine et al., 2023). The patient initiated combinational therapy with penpulimab plus lenvatinib, and the efficacy evaluation was PR after 3 months. Different from other monoclonal antibodies against PD-1 which all use IgG4 subtypes, penpulimab is an IgG1 monoclonal antibody engineered to completely eliminate Fc γ receptor binding and Fc-mediated effector functions that can impair anti-tumor activity, exhibiting better stability, less host cell protein residue, and more favorable safety profiles (Tawbi et al., 2017). Lenvatinib, a multi-targeted TKI inhibitor, has potent anti-angiogenic activity, mainly through the inhibition of VEGFR 1–3, PDGFR α , FGFR 1–4, KIT, and RET [5]. However, another phase 2 trial (NCT04784247) evaluating the efficacy of lenvatinib and pembrolizumab in selected sarcomas suggested there were no responses in the LMS cohort with a poor PFS (Avutu et al., 2023). Due to the intra-/intertumor heterogeneity of LMS, it is not sufficient to guide combination approaches with current biomarkers such as PD-L1, TILs, and TMB. Ectopic lymphoid aggregates, termed as tertiary lymphoid structures (TLSs), have been recently demonstrated to be related to higher response to immunotherapy and superior prognosis in various types of cancers including STSs, independent of CD8⁺ T-cell density and PD-L1 status (Schumacher and Thommen, 2022; Wang et al., 2022). Anti-angiogenic immune-modulating therapies have been proven to induce tumoral high-endothelial venules with T-cell-enriched TLSs, which exhibit an improved tumor response (Hua et al., 2022). The predictive value of TLS in STSs has been validated prospectively. The PEMBROSARC trial suggested a significant improvement in the ORR and 6-month non-progression rate in the TLS-positive STS cohort compared to the previously unselected cohort (Italiano et al., 2022). The SPARTO study (NCT05210413) is ongoing to evaluate spartalizumab combined with pazopanib in solid tumors including TLS-positive STSs. In addition, the CONGRATS study (NCT04095208), still recruiting, includes STS patients with a sarcoma enriched with TLS to evaluate the combination of nivolumab and relatlimab, with results expected in the near future. This case not only proved the superior clinical activity of ICIs and anti-angiogenic therapy but also displayed the possibility of TKI rechallenge with lenvatinib after the failure of anlotinib therapy in advanced LMS. The median time interval between initial TKI treatment and TKI rechallenge was 14 months. The long TKI treatment interval with other types of therapy might change the tumor microenvironment and make tumors restore the sensitivity to anti-angiogenic therapy. Our previous retrospective study also found that 34.6% of advanced STS patients could still achieve clinical benefits from rechallenge with multi-targeted TKI after the failure of previous TKI therapy, with a median OS of 11.7 months and a median PFS of 3.3 months (Liu et al., 2021). Another explanation for the efficacy of TKI rechallenge might be the differences in targets and affinities among the multi-targeted TKIs. Apart from different targets, lenvatinib and anlotinib have different

affinities with VEGFR. It is well known that multi-targeted TKI therapy increased the expression of PD-L1 and tumor mutation burden, and TKI-resistant clones made tumor cells more sensitive to combination immunotherapy (Isomoto et al., 2020). It was assumed that the TKI-sensitive clone might re-populate and dominate the tumor cell population after prolonged exposure to ICIs, causing immunotherapy resistance while restoring TKI sensitivity (Lam et al., 2021). The mechanism of TKI efficacy in this rechallenge setting needs further exploration.

After more than 8 months of treatment with penpulimab and lenvatinib, the patient was confirmed to have progressive disease based on the iRECIST criteria but with a pattern of dissociated response (DR). This atypical response pattern is analogous to mixed responses in settings of chemotherapy and targeted therapy, which was defined as the simultaneous coexistence of responding and non-responding lesions within the same patient (Borcoman et al., 2019). The incidence of DR in cancer patients receiving systemic chemotherapy and targeted therapy has been previously reported to range from 13.9% to 39.0% (Guan et al., 2022). DR has also been found in patients receiving combination immunotherapy, like PD-1/PD-L1 inhibitors combined with chemotherapy, targeted therapy, or radiotherapy, with a DR rate of 12.5% in mesothelioma, 13.2% in NSCLC, 14.3% in endometrial carcinoma, and 30.3% in RCC (Guan et al., 2022). To date, the DR rate in STS has been rarely reported in the literature. In contrast to PR/complete response, DR is considered as an unfavorable prognostic factor for patients receiving targeted therapy or chemotherapy. However, in almost all studies regarding the response pattern of immune-related DR, patients with DR had a prolonged OS or increased clinical benefit compared to those who achieved true PD (39). Sato et al. (2021) revealed that DR had significantly longer OS compared to those showing PD (46.9 versus 8.2 months) in advanced NSCLC patients treated with nivolumab. Furthermore, a durable clinical benefit was observed in approximately 20%–50% of patients with DR after the continuation of immunotherapy (Humbert et al., 2020). Importantly, DR cannot simply be considered as a true tumor progression and does not represent real-acquired resistance to ICIs (Borcoman et al., 2019). Although no clear recommendations exist in DR setting, immediately discontinuing immunotherapy or switching to other systematic therapies may not be an early alternative strategy (Borcoman et al., 2019). If possible, local treatments of progressing lesions should be discussed in selected patients with good clinical conditions (Borcoman et al., 2019). Liniker et al. (2016) demonstrated the feasibility of salvage radiotherapy to lesions progressing on the PD-1 blockade in advanced melanoma, achieving an overall response rate of 45%. This patient received local radiotherapy to the single metastatic lesion of the lung and liver, respectively, while continuing treatment with penpulimab plus lenvatinib and eventually obtained a long-term and high-quality survival benefits.

In summary, we presented a rare case of PLMSB in the right intertrochanteric region of the femur. After the failure of multi-line therapies, this case not only proved the superior clinical activity of immunotherapy combined with anti-angiogenic therapy in PLMSB but also showed the possibility of TKI rechallenge. Localized therapy of progressive lesions after DR was an important treatment strategy that could be beneficial to advanced LMS patients. So far as we know, no similar therapeutic

regimens and strategies have been reported in PLMSB, and our findings provided new insights into therapeutic options for advanced PLMSB, which still need to be tested in clinical trials with larger samples.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Ethics statement

The study was approved by Ethics Committee on Biomedical Research, West China Hospital of Sichuan University. Written informed consent was obtained from the individual for the publication of any potentially identifiable images or data included in this article.

Author contributions

BW and YD designed the study. BW and YH acquired data. Analysis and interpretation of data were carried out by JL, HZ, and XY. Writing and revision of the manuscript were carried out by YD and YJ. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphar.2023.1239699/full#supplementary-material>

SUPPLEMENTARY FIGURE S1

(A–D) Multiple newly bilateral pulmonary nodules were detected by the routine chest CT scan on April 2020; (E–H) the progression bilateral pulmonary lesions noted after the first-line treatment with anlotinib for seven cycles (October 2020).

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Apatinib plus chemotherapy for non-metastatic osteosarcoma: a retrospective cohort study

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Background: Effective adjuvant therapy for osteosarcoma is necessary for improved outcomes. Previous studies demonstrated that apatinib plus doxorubicin-based chemotherapy may improve the efficacy of neoadjuvant therapy. This study aimed to clarify the effectiveness and safety of apatinib plus doxorubicin and cisplatin (AP) as neoadjuvant therapy for osteosarcoma.

Methods: The clinical data of osteosarcoma patients who underwent neoadjuvant therapy and surgery between August 2016 and April 2022 were retrospectively collected and analyzed. Patients were divided into two groups: the apatinib plus AP (apatinib + AP) group and the methotrexate, doxorubicin, and cisplatin (MAP) group.

Results: This study included 42 patients with nonmetastatic osteosarcoma (19 and 23 patients in the apatinib + AP and MAP groups, respectively). The 1- and 2-year disease-free survival rates in the apatinib + AP group were higher than those in the MAP group, but the difference was not significant ($P=0.165$ and 0.283 , respectively). Some adverse events were significantly more common in the apatinib + AP group than in the MAP group, including oral mucositis (grades 3 and 4) (52.6% vs. 17.4%, respectively, $P=0.023$), limb edema (47.4% vs. 17.4%, respectively, $P=0.049$), hand-foot syndrome (31.6% vs. 0%, respectively, $P=0.005$), proteinuria (26.3% vs. 0%, respectively, $P=0.014$), hypertension (21.1% vs. 0%, respectively, $P=0.035$), and hypothyroidism (21.1% vs. 0%, respectively, $P=0.035$). No drug-related deaths occurred. There was no statistically significant difference in the incidence of postoperative complications between the groups ($P>0.05$).

Conclusion: The present study suggests that apatinib + AP may be a promising candidate for neoadjuvant therapy for osteosarcoma, warranting further validation in prospective randomized controlled clinical trials with long-term follow-up.

KEYWORDS

apatinib, doxorubicin, cisplatin, osteosarcoma, neoadjuvant therapy, adverse events

1 Introduction

Osteosarcoma is one of the most common osteogenic malignancies. There are 2,000–3,000 newly diagnosed cases annually in China (1, 2). The standard treatment for non-metastatic osteosarcoma is preoperative chemotherapy (neoadjuvant chemotherapy), surgery, and postoperative adjuvant chemotherapy (2–4). The purpose of neoadjuvant chemotherapy is to 1) eliminate small metastases that may exist; 2) shrink the tumor, increase the rate of limb salvage, and reduce the recurrence rate of osteosarcoma; and 3) determine the effect of chemotherapy to facilitate the formulation of plans for postoperative chemotherapy, thus improving the curative effect (5, 6). The drug regimen used as neoadjuvant chemotherapy has been studied repeatedly and in detail. The currently recognized regimen is a combination of methotrexate, adriamycin (doxorubicin), and cisplatin (MAP), or doxorubicin and cisplatin (AP) (6, 7). Approximately 50% of patients initially diagnosed with non-metastatic osteosarcoma develop metastasis and ultimately do not survive, even after receiving neoadjuvant chemotherapy (6, 8, 9). Therefore, new therapeutic drugs and methods are urgently required.

Apatinib was marketed in 2014 in China as the first domestically produced multi-target tyrosine kinase inhibitor (TKI) (10). Targets that are inhibited by apatinib include VEGFR1, VEGFR2, c-RET, c-KIT, and c-SRC (11, 12). Several studies have demonstrated that apatinib can inhibit the proliferation, invasion, and migration of osteosarcoma cells *in vitro* (13–15) and is effective in treating patients with advanced osteosarcoma (16).

Our previous study has demonstrated that apatinib ameliorates doxorubicin-induced migration and cancer stemness of osteosarcoma cells (17). This suggests that apatinib combined with neoadjuvant chemotherapy may achieve better outcomes in non-metastatic osteosarcoma than the current standard regimen. However, no reports have confirmed this. Some of our patients with osteosarcoma were treated with apatinib plus neoadjuvant chemotherapy in clinical practice. In the present study, we retrospectively collected and analyzed the clinical data of these patients and summarized the effectiveness and safety of this treatment regimen.

2 Materials and methods

2.1 Patient

All osteosarcoma patients included in this retrospective study were treated between August 2016 and April 2022. This study was approved by the Institutional Review Board of Henan Cancer Hospital and was conducted in accordance with the guidelines and principles of the Declaration of Helsinki. All patients included in the present study met the following criteria: 1) pathologically confirmed osteosarcoma; 2) no evidence of distant metastasis; 3)

received apatinib + AP or MAP neoadjuvant and postoperative adjuvant therapy; 4) underwent limb salvage surgery or amputation.

2.2 Treatment protocol

Based on the different drugs received, patients were divided into two groups: the apatinib + AP group and the MAP group. In the MAP group, patients received preoperative chemotherapy comprising two 5-week cycles of doxorubicin 37.5 mg/m²/day for 2 days, cisplatin 60 mg/m²/day for 2 days, and methotrexate 12 g/m² (18). Surgery was scheduled after two cycles of preoperative chemotherapy. The patients received another four cycles of treatment postoperatively, similar to preoperative chemotherapy.

Some patients chose to receive apatinib plus AP treatment (apatinib + AP group). In this group, all patients received preoperative therapy consisting of cisplatin 60 mg/m² and doxorubicin 37.5 mg/m² per day on days 1–2. Each patient received six cycles of chemotherapy, which were repeated every 3 weeks. Surgery was scheduled after two cycles of chemotherapy. Postoperatively, the patients received another four cycles of treatment, similar to preoperative chemotherapy. Patients in parallel received 500 mg (those with body surface area [BSA] ≥1.5 m²) or 250 mg (those with BSA <1.5 m²) apatinib per day, starting on day 3. Apatinib was interrupted during chemotherapy and interrupted for 2 weeks postoperatively and then continued until 1 year postoperatively.

Adverse events (AEs) were determined according to the Common Terminology Criteria for Adverse Events version 4.0. For patients who could not tolerate AEs, the dose of apatinib was reduced to 250 mg/day or 125 mg/day.

Limb salvage surgery or amputation was performed after two cycles of preoperative therapy. All surgeries were aimed at achieving complete resection of the primary lesion. Apatinib was interrupted for 2 weeks postoperatively and then continued until 1 year postoperatively.

2.3 Evaluation

The Response Evaluation Criteria in Solid Tumors (version 1.1) was used to evaluate the effectiveness of neoadjuvant treatment. Tumor responses were categorized as complete response (CR), partial response (PR), stable disease (SD), or progressive disease. The disease control rate (DCR) was defined as the sum of the rates of CR, PR and SD. The differences in alkaline phosphatase (ALP) serum level changes post-neoadjuvant therapy, tumor cell necrosis rate, and 1- and 2-year disease-free survival (DFS) rates between the two groups were evaluated. Tumor responses were evaluated according to the response evaluation criteria in solid tumors (version 1.1). DFS was defined as the time from the surgery to the first occurrence of signs of recurrence or metastasis. The between-group rates of drug-related AEs and surgery-related complications were compared. Surgery-related complications were graded using the Clavien–Dindo grading system.

2.4 Statistical analysis

Quantitative variables are presented as numerical values (percentages), medians (ranges), or medians (interquartile range). Categorical data were compared using Fisher's exact test, and continuous variables were compared using Student's t-test. Progression-free survival was estimated using the Kaplan–Meier method. The univariate Cox proportional hazards model was used to analyze the relationship between clinicopathological parameters and DFS. Statistical analyses were performed using SPSS (version 21.0; IBM, Armonk, NY, USA). All statistical analyses were two-sided, and a P-value <0.05 was considered statistically significant.

3 Results

3.1 Patient characteristics

A total of 42 osteosarcoma patients met the eligibility criteria for this study, with 19 and 23 patients included in the apatinib + AP and MAP groups, respectively. The median follow-up period was 28 (9–50) and 22 (9–42) months for the apatinib + AP and MAP groups, respectively.

The baseline characteristics of the patients are presented in [Table 1](#). All patients in both groups were younger than 30 years of age. The median ages of the patients at diagnosis were 18.0 (13.0–21.0) and 18.0 (14.5–20.5) years in the apatinib + AP and MAP groups, respectively. All patients in both groups were Enneking Stage II at the time of treatment initiation. The primary lesions were most commonly located in the long bones of the extremities. The diameters of the primary lesions in most patients in the two groups were >10 cm. The pre-neoadjuvant chemotherapy ALP serum level was >200 U/L in more than half of the patients in both groups. There were no significant differences between the groups in baseline characteristics ($P>0.05$, [Table 1](#)).

Four patients in the apatinib + AP group experienced recurrence or metastasis during the maintenance treatment with apatinib, leading to the discontinuation of apatinib treatment. The remaining 15 patients successfully completed a 1-year maintenance treatment.

3.2 Clinical effectiveness

Preoperative evaluation of the efficacy of neoadjuvant therapy revealed that 78.95% (15/19) and 73.91% (17/23) of patients in the apatinib + AP and MAP groups, respectively, experienced a reduction in ALP serum levels after neoadjuvant therapy ([Table 2](#)).

Although a decrease in ALP level was observed in a higher percentage of patients in the apatinib + AP group than in the MAP group, there were no significant differences between the two groups with respect to the DCR (84.21% vs. 78.26%, $P=0.852$; [Table 2](#)), tumor cell necrosis rate $\geq 90\%$ (78.95% vs. 69.57%, $P=0.726$; [Table 2](#)), 1-year DFS rate (78.9% vs. 59.4%, $P=0.165$; [Table 2](#), [Figure 1](#)), and the 2-year DFS rate (61.5% vs. 44.5%, $P=0.283$; [Table 2](#), [Figure 1](#)).

TABLE 1 Patient characteristics by treatment group.

Characteristics	Apatinib + AP group (n = 19)	MAP group (n = 23)	P-value
Sex			1.000
Male	10 (52.63%)	13 (56.52%)	
Female	9 (47.37%)	10 (43.48%)	
Median age (years)	17.26 $\pm$ 5.17	17.57 $\pm$ 5.13	0.851
ECOG PS			0.763
0	11 (57.89%)	12 (52.17%)	
1	8 (42.11%)	11 (47.83%)	
Enneking stage grade			1.000
IIA	8 (42.11%)	11 (47.83%)	
IIB	11 (57.89%)	12 (52.17%)	
Primary site			0.978
Femur	6 (31.58%)	8 (34.78%)	
Axial skeleton	2 (10.53%)	3 (13.04%)	
Tibia	5 (26.32%)	6 (26.09%)	
Humerus	3 (15.79%)	4 (17.39%)	
Fibula	1 (5.26%)	0 (0.00%)	
Radial	1 (5.26%)	0 (0.00%)	
Other	1 (5.26%)	2 (8.70%)	
Histologic subtypes			1.000
Conventional	16 (84.21%)	20 (85.96%)	
Small cell	2 (10.53%)	1 (4.35%)	
Telangiectatic	1 (5.26%)	2 (8.70%)	
Tumor size			0.748
Small (<10 cm)	7 (36.84%)	7 (30.43%)	
Large (≥ 10 cm)	12 (63.16%)	16 (69.57%)	
Pre-neoadjuvant chemotherapy ALP serum level (U/L)			1.000
<200	9 (47.37%)	11 (47.83%)	
≥ 200	10 (52.63%)	12 (52.17%)	

Data are presented as counts (percentages) or means $\pm$ standard deviations.

AP, doxorubicin-cisplatin chemotherapy; MAP, methotrexate-doxorubicin-cisplatin chemotherapy; ECOG PS, Eastern Cooperative Oncology Group performance status; ALP, alkaline phosphatase.

3.3 Toxicity and complications

Patients in the apatinib + AP group experienced more AEs than those in the MAP group. Some AEs were significantly more common in the apatinib + AP group than in the MAP group ($P<0.05$), and these included oral mucositis (grades 3 and 4) (52.6% vs. 17.4%, respectively, $P=0.023$), limb edema (47.4% vs. 17.4%, respectively, $P=0.049$), hand-foot syndrome (31.6% vs. 0%,

TABLE 2 Clinical outcomes of the two groups.

Characteristics	Apatinib + AP group (n = 19)	MAP group (n = 23)	P-value
Changes in ALP serum level post neoadjuvant therapy			1.000
Decreased	15 (78.95%)	17 (73.91%)	
Not decreased	4 (21.05%)	6 (26.09%)	
Response evaluated before surgery (RECIST)			0.852
PR	1 (5.26%)	1 (4.35%)	
SD	15 (78.95%)	17 (73.91%)	
PD	3 (15.79%)	5 (21.74%)	
Type of surgery			1.000
Limb salvage	17 (89.47%)	20 (86.96%)	
Amputation	2 (10.53%)	3 (13.04%)	
R0 resection			1.000
Yes	19 (100%)	23 (100%)	
No	0	0	
Tumor cell necrosis rate (%)			0.726
<90	4 (21.05%)	7 (30.43%)	
≥90	15 (78.95%)	16 (69.57%)	
M-DFS (months)	NA	16	0.183
1-year DFS rate (%)	78.9 (0.626–0.996)	59.4 (0.420–0.841)	0.165
2-year DFS rate (%)	61.5 (0.426–0.888)	44.5 (0.277–0.718)	0.283

Data are presented as numbers (percentages) or means ± standard deviations.

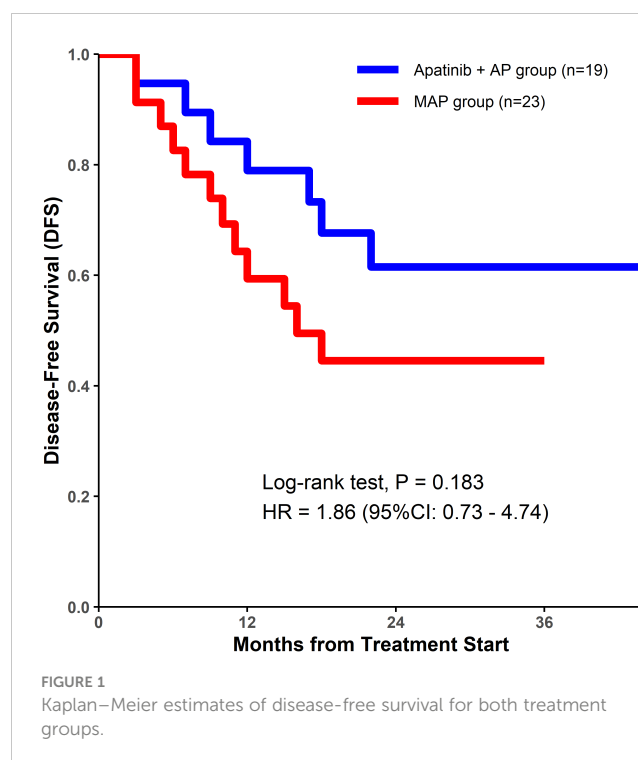
AP, doxorubicin-cisplatin chemotherapy; MAP, methotrexate-doxorubicin-cisplatin chemotherapy; ALP, alkaline phosphatase; RECIST, response evaluation criteria in solid tumors (version 1.1); PR, partial response; SD, stable disease; PD, progressive disease; M-DFS, median disease-free survival.

respectively, $P=0.005$), proteinuria (26.3% vs. 0%, respectively, $P=0.014$), hypertension (21.1% vs. 0%, respectively, $P=0.035$), and hypothyroidism (21.1% vs. 0%, respectively, $P=0.035$; Table 3).

Postoperative complications in each group are shown in Table 4. A grade IV complication (cardiac failure) occurred in one patient in the MAP group (Table 4). There was no statistically significant difference in the incidence of postoperative complications between the two groups ($P>0.05$, Table 4). No drug- and surgery-related deaths occurred.

3.4 Univariate Cox regression analysis

Univariate Cox regression analysis was performed to determine the relationship between DFS and the clinical characteristics of the patients in this study. In the apatinib + AP



group, patients with decreased ALP serum levels after neoadjuvant therapy, $\geq 90\%$ tumor cell necrosis rate, and disease control after neoadjuvant therapy had significantly longer DFS ($P<0.05$, Figure 2). In the MAP group, patients with a primary tumor located in the axial skeleton, $\geq 90\%$ tumor cell necrosis rate, and disease control after neoadjuvant therapy had significantly longer DFS ($P<0.05$, Figure 3).

4 Discussion

This study is the first to report the safety and effectiveness of apatinib plus AP for the neoadjuvant treatment of patients with osteosarcoma. Based on the different treatments received, patients were divided into the apatinib + AP and MAP groups. AEs were more prevalent in patients treated with apatinib plus AP than in those treated with MAP. The 1- and 2-year DFS rates in the apatinib + AP group were higher than those in the MAP group, but the difference was not significant.

Perioperative chemotherapy has repeatedly been shown to be indispensable as a standard of care for non-metastatic osteosarcoma (3, 7). However, chemotherapy drugs used remain controversial (8). The history of neoadjuvant chemotherapy in osteosarcoma is a balance between efficacy and toxicity, and researchers have tried to improve efficacy by increasing the dose or number of different drugs used as much as possible while maintaining a tolerable level of toxicity (3, 19). Currently, AP and MAP chemotherapies are generally recognized as efficacious, but they have a high toxicity rate, and their cure rate should be further improved (8). Improvements in neoadjuvant therapy for osteosarcoma will continue as new drugs are being developed (20–22).

TABLE 3 Neoadjuvant therapy-related adverse effects per treatment groups.

Characteristics	Apatinib + AP group (n = 19)		MAP group (n = 23)		P-value	
	All grades	Grade >2	All grades	Grade >2	All grades	Grade >2
Any toxicity	19 (100%)	19 (100%)	23 (100%)	21 (91.3%)	1.000	0.492
Leucopenia	19 (100%)	18 (94.7%)	23 (100%)	19 (82.6%)	1.000	0.356
Anaemia	18 (94.7%)	13 (68.4%)	21 (91.3%)	13 (56.5%)	1.000	0.530
Alopecia	18 (94.7%)	0 (0%)	21 (91.3%)	0 (0%)	1.000	1.000
Thrombocytopenia	17 (89.5%)	12 (63.2%)	15 (65.2%)	10 (43.5%)	0.083	0.232
Nausea	16 (84.2%)	11 (57.9%)	19 (82.6%)	9 (39.1%)	1.000	0.352
Oral mucositis	16 (84.2%)	10 (52.6%)	13 (56.5%)	4 (17.4%)	0.093	0.023
Fatigue	15 (78.9%)	5 (26.3%)	17 (73.9%)	2 (8.7%)	1.000	0.214
Anorexia	14 (73.7%)	5 (26.3%)	12 (52.2%)	2 (8.7%)	0.208	0.214
Transaminase increase	14 (73.7%)	4 (21.1%)	11 (47.8%)	1 (4.3%)	0.120	0.158
Vomiting	13 (68.4%)	4 (21.1%)	14 (60.9%)	3 (13.0%)	0.750	0.682
Fever	12 (63.2%)	2 (10.5%)	9 (39.1%)	1 (4.3%)	0.215	0.581
Diarrhoea	11 (57.9%)	2 (10.5%)	11 (47.8%)	1 (4.3%)	0.551	0.581
Pain	11 (57.9%)	0 (0%)	10 (43.5%)	1 (4.3%)	0.536	1.000
Limb edema	9 (47.4%)	2 (10.5%)	4 (17.4%)	0 (0%)	0.049	0.199
Weight loss	7 (36.8%)	0 (0%)	8 (34.8%)	0 (0%)	1.000	1.000
Constipation	6 (31.6%)	1 (5.3%)	7 (30.4%)	0 (0%)	1.000	0.452
Hand-foot syndrome	6 (31.6%)	1 (5.3%)	0 (0%)	0 (0%)	0.005	0.452
Proteinuria	5 (26.3%)	1 (5.3%)	0 (0%)	0 (0%)	0.014	0.452
Hypertension	4 (21.1%)	0 (0%)	0 (0%)	0 (0%)	0.035	1.000
Hypothyroidism	4 (21.1%)	0 (0%)	0 (0%)	0 (0%)	0.035	1.000
Dysgeusia	3 (15.8%)	0 (0%)	2 (8.7%)	0 (0%)	0.644	1.000
Cough	3 (15.8%)	0 (0%)	5 (21.7%)	0 (0%)	0.709	1.000
Pneumothorax	2 (10.5%)	1 (5.3%)	0 (0%)	0 (0%)	0.199	0.452

Data are presented as counts (percentages).

AP, doxorubicin-cisplatin chemotherapy; MAP, methotrexate-doxorubicin-cisplatin chemotherapy.

Since it was launched, apatinib, a multi-target TKI, has been shown to have good efficacy in the treatment of advanced osteosarcoma that has failed multi-line therapy (23). Our previous study demonstrated that apatinib can reverse the resistance of osteosarcoma cells to doxorubicin (17). Different studies have also demonstrated inhibition of the targets of apatinib to be beneficial for the treatment of osteosarcoma (24). At present, multiple clinical trials of TKI combined with chemotherapy in the treatment of sarcoma have achieved benign results (25, 26). In the present study, the tumor necrosis rate and 1- and 2-year DFS rates in the apatinib +AP group were higher than those in the MAP group. This suggests that the addition of apatinib to neoadjuvant therapy for osteosarcoma could achieve better outcomes. This confirms the results of our previous study and similar studies (27, 28). However, the improvement in efficacy in the present study was not significant. We believe that the reason for this might be the limited number of patients. Therefore, it is

necessary to further assess the efficacy of apatinib as neoadjuvant treatment of osteosarcoma in a prospective randomized controlled clinical trial with a large sample size.

Several previous studies have demonstrated that apatinib is highly toxic. This has led to a reduction in the dose used in clinical practice for the treatment of various malignancies from 750 mg to 500 mg (29, 30). Studies on various tumor types have demonstrated that apatinib combined with chemotherapy can be severely toxic (30, 31). This is an important reason apatinib has not been tested as neoadjuvant therapy for osteosarcoma, despite the evidence for efficacy in advanced sarcomas. In the present study, to reduce the toxicity of apatinib when combined with chemotherapy, the AP regimen was used for the combined group, which was also recommended as first-line treatment by the National Comprehensive Cancer Network guidelines (32). Nevertheless, the results of this study show that AEs were more prevalent in the apatinib + AP group than in the MAP group. However, these

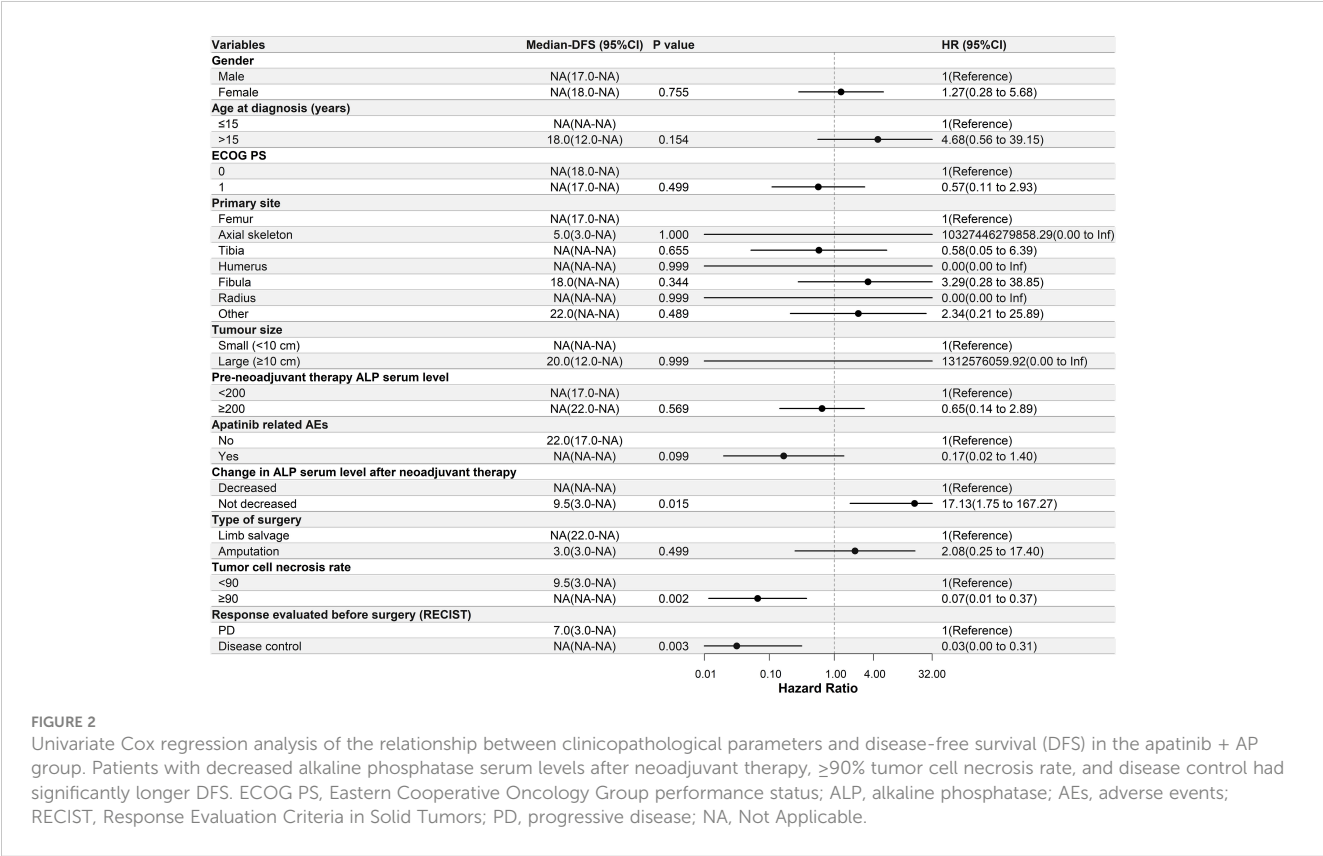
TABLE 4 Surgery-related complications per treatment group.

Complication	Apatinib +AP group (n = 19)	MAP group (n = 23)	P-value
Clavien-Dindo grading			0.912
Grade I	4	3	
Grade II	8	10	
Grade III	7	9	
Grade IV	0	1	
Grade V	0	0	
Wound infection	3 (15.8%)	4 (17.4%)	1.000
Pulmonary infection	2 (10.5%)	2 (8.7%)	1.000
Hemorrhage	2 (10.5%)	1 (4.3%)	0.581
Superficial wound dehiscence	7 (36.8%)	8 (34.8%)	1.000
Cardiac/respiratory failure	0	1 (3.85%)	1.000
The implant nonunion or fracture	5 (26.3%)	7 (30.4%)	1.000
Reoperation	7 (36.8%)	9 (39.1%)	1.000
Death	0	0	1.000

Data are presented as counts (percentages).
AP, doxorubicin-cisplatin chemotherapy; MAP, methotrexate-doxorubicin-cisplatin chemotherapy.

significantly increased adjuvant treatment-related AEs did not hinder the output of surgical treatment, let alone lead to adjuvant treatment-related death. This suggests that apatinib combined with AP in the neoadjuvant setting results in an acceptable level of toxicity. Here, we emphasize three points. First, in this study, the initial dose of apatinib was individualized according to the patient's BSA, and the dose of apatinib was dynamically adjusted according to the occurrence of AEs. This is an important point for clinical decision-making and study design. Second, patients older than 30 years of age were excluded from receiving the combined regimen, which was fully considered from the outset of the study. Older patients have been excluded from several studies of neoadjuvant treatment in osteosarcoma (33). Younger patients appear to be more tolerant of the AEs caused by apatinib combined with chemotherapy. In addition, it is worth noting that in this study, seven patients younger than 10 years of age did not experience AEs when receiving apatinib combined with neoadjuvant chemotherapy. This suggests that apatinib can be safely added to neoadjuvant therapy in children with osteosarcoma. Finally, the maintenance treatment with apatinib after the MAP regimen is also a worthwhile treatment option. This approach not only avoids the toxicity of chemotherapy plus apatinib but also preserves the benefits of these two treatment options. The maintenance with apatinib after MAP may even be better than the apatinib + AP regimen.

Researchers have attempted to find the best evaluation system for neoadjuvant therapy for osteosarcoma (34–36). We found that decreased ALP serum levels after neoadjuvant therapy, ≥90% tumor



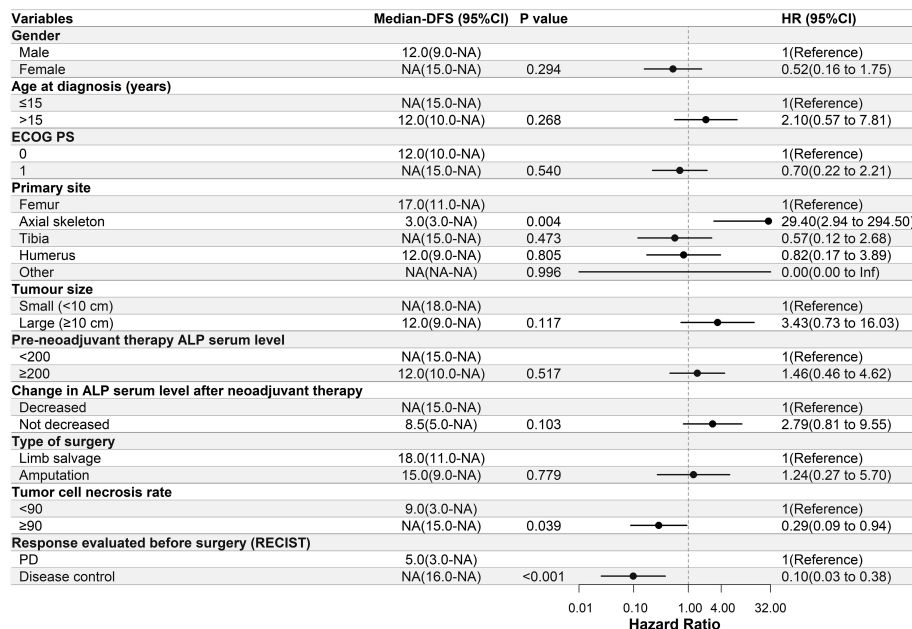


FIGURE 3

Univariate Cox regression analysis of the relationship between clinicopathological parameters and disease-free survival (DFS) in the methotrexate-doxorubicin-cisplatin group. Patients with a primary site located in the axial skeleton, ≥90% tumor cell necrosis rate, and disease control had significantly longer DFS. NA, Not Applicable. ECOG PS, Eastern Cooperative Oncology Group performance status; ALP, alkaline phosphatase; RECIST, Response Evaluation Criteria in Solid Tumors; PD, progressive disease; NA, Not Applicable.

cell necrosis rate, and disease control were significantly associated with longer DFS in the present study. This is similar to the results of other studies (12). However, it is unclear which method is the most suitable for efficacy evaluation and prediction of survival rates in patients treated with apatinib combined with AP neoadjuvant therapy. Answering this question requires further prospective studies and long-term follow-ups.

This study had some limitations, including its retrospective nature, small sample size, and short follow-up period. All these factors make it difficult to analyze the differences in outcomes and complications. Prospective registered clinical trials are required to continue investigating the effectiveness of the apatinib + AP regimen. Moreover, further research on the efficacy of apatinib in postoperative maintenance therapy for osteosarcoma is warranted.

5 Conclusions

The results of this study suggest that apatinib + AP may be a promising candidate for neoadjuvant therapy for osteosarcoma, warranting further validation in prospective randomized controlled clinical trials with long-term follow-up.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethics Committee of Henan Cancer Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Combining nanoparticle albumin-bound paclitaxel with camrelizumab in advanced soft tissue sarcoma: activity, safety, and future perspectives

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Background: It is still uncertain whether Nanoparticle albumin-bound paclitaxel (nab-paclitaxel) and programmed cell death protein 1 (PD-1) inhibitor have synergistic effects on metastatic soft tissue sarcomas (STSs). The purpose of this study was to evaluate the safety and activity of nab-paclitaxel plus camrelizumab (a PD-1 inhibitor) in patients with advanced STS who had previously failed chemotherapy.

Methods: In this single-center, open-label, single-arm phase II clinical trial, patients with advanced (unresectable or metastatic) STS who had previously failed chemotherapy received up to six cycles of nab-paclitaxel plus camrelizumab, whereas camrelizumab treatment was continued for up to 1 year. The median progression-free survival (PFS), objective response rate (ORR) and safety were collected and evaluated.

Results: This trial included 40 patients (28 men and 12 women). The overall ORR was 22.5%, and the median PFS was 1.65 months (95% confidence interval [CI], 1.3–2.0 months). Patients with epithelioid sarcoma demonstrated a longer PFS compared with those with other histological subtypes (2.3 months vs. 1.5 months, respectively); however, this difference was not significant. Patients who had received only one line of previous chemotherapy had a significantly longer PFS compared with those who had undergone two or more lines of previous chemotherapy (2.8 months vs. 1.3 months, respectively, $p = 0.046$). In terms of safety, the toxicity of this combination therapy is mild and no serious adverse events have occurred.

Conclusion: Nab-paclitaxel plus camrelizumab exhibited modest activity and mild toxicity in treating epithelioid sarcoma, angiosarcoma, and fibrosarcoma. The overall effectiveness of this treatment regimen for advanced STS is relatively low. Further research on combining nab-paclitaxel with effective drugs, including chemotherapy and targeted agents, for these specific STS subtypes is needed.

KEYWORDS

nab-paclitaxel, camrelizumab, PD-1 inhibitor, sarcoma, immunochemotherapy

1 Introduction

Soft tissue sarcomas (STSs) are malignancies with a low incidence rate (approximately four per 100000 people) and high heterogeneity (>70 subtypes) (Yang et al., 2019; WHO Classification of Tumours, 2020; Buja et al., 2023). Moreover, approximately 50% of STS cases eventually progress to late stages. Traditionally, the main treatment method for advanced STS is chemotherapy, with first-line and second-line chemotherapy regimens including doxorubicin and docetaxel plus gemcitabine (Tian and Yao, 2023; von Mehren et al., 2020; George, 2019). However, the objective response rate (ORR) for this regimen is approximately 20%, and the median overall survival for patients with advanced STS is approximately 12 months (Tian and Yao, 2023). Therefore, effective treatment strategies are needed.

Nanoparticle albumin-bound paclitaxel (nab-paclitaxel) is a anticancer drug of taxane family (Yared and Tkaczuk, 2012; Kudlowitz and Muggia, 2014). It is a nano-sized paclitaxel, and has higher water solubility and bioavailability, lower toxicity, and improved antitumor efficacy compared with the two main taxanes (traditional paclitaxel and docetaxel) (Tian and Yao, 2022a; Mercatali et al., 2022). Nab-paclitaxel has been used to treat many types of cancer. In addition, recent reports have shown that it is effective in treating STSs (Tian et al., 2022a; Tian and Yao, 2022a).

Programmed cell death protein 1 (PD-1) inhibitors are the most widely used immunotherapy drugs in anticancer therapy, and they also have been used as novel antitumor therapies in the treatment and research of STS (Saerens et al., 2021). Despite recent evidence suggesting the low efficacy of PD-1 inhibitor monotherapy in STSs, there are promising reports of its effectiveness in some histological subtypes of sarcoma (Baldi et al., 2022; Kerrison et al., 2022). In addition, in order to improve the efficacy of PD-1 inhibitor, combination chemotherapy has been proven to be a promising method for treating malignancies (including STS) (Tian and Yao, 2022b).

Nab-paclitaxel plus PD-1 inhibitor has achieved promising results in the treatment of various types of cancer (Li et al., 2021; An et al., 2023; Sonoda et al., 2023; Yin et al., 2023; Zhang et al., 2023). However, clinical trials evaluating the efficacy and safety of this combination for STS treatment have not been reported. We have carry out a single-center, open-label, single-arm phase II clinical trial that used nab-paclitaxel plus camrelizumab (a PD-1 inhibitor) as a second-line treatment for metastatic or locally unresectable STS. We report the results of this trial here, and hoped to provide references for the treatment and clinical research for patients with STS.

2 Materials and methods

2.1 Patients

In this trial, the effects of nab-paclitaxel plus camrelizumab as a second or subsequent line of therapy for advanced STS were assessed. All patients received nab-paclitaxel plus camrelizumab between January 2022 and March 2023. The main eligibility criteria included: 1) age ≥ 18 years and <70 years, 2) histologically proven STS [include undifferentiated pleomorphic sarcoma (UPS), leiomyosarcoma, angiosarcoma, synovial

sarcoma, clear cell sarcoma, epithelioid sarcoma, fibrosarcoma, malignant peripheral nerve sheath tumor (MPNST) and undifferentiated or poorly differentiated liposarcoma], 3) an Eastern Cooperative Oncology Group (ECOG) performance status of 0–1, 4) locally unresectable or multiple metastatic disease, 5) failure of previous chemotherapy, 6) acceptable hematological, renal and hepatic functions, 7) measurable lesions according to the response evaluation criteria in solid tumors (RECIST; version 1.1).

This study was approved by the Ethics Committee of the Henan Cancer Hospital and registered at ClinicalTrials.gov (NCT05189483). Written informed consent was obtained from all patients. This trial was performed in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki.

2.2 Treatment protocol

All patients received 260 mg/m² of nab-paclitaxel (Hengrui Pharmaceutical, Lianyungang, China), via a 30-min intravenous

TABLE 1 Clinical characteristics of patients in this study.

Characteristics	No. (%) (n = 40)
Gender	
Female	12 (30.0%)
Male	28 (70.0%)
Age	49.28 $\pm$ 14.17
ECOG PS	
0	20 (50.0%)
1	20 (50.0%)
Histology	
UPS	10 (25.0%)
Epithelioid sarcoma	8 (20.0%)
Fibrosarcoma	7 (17.5%)
Angiosarcoma	5 (12.5%)
Myxofibrosarcoma	3 (7.5%)
MPNST	2 (5%)
Leiomyosarcoma	2 (5%)
Synovial sarcoma	2 (5%)
Dedifferentiated liposarcoma	1 (2.5%)
Primary site	
Extremities	26 (65.0%)
Trunk	13 (32.5%)
Head	1 (2.5%)
Stage	
IV	35 (87.5%)
Locally unresectable	5 (12.5%)
Metastatic site	
lungs	32 (80.0%)
other	3 (7.5%)
Lines of previous systemic therapy	
1	11 (27.5%)
2	15 (37.5%)
3	14 (35.5%)

Abbreviations: ECOG PS, eastern cooperative oncology group performance status; MPNST, malignant peripheral nerve sheath tumor; UPS, undifferentiated pleomorphic sarcoma.

infusion on day 1, and repeated every 3 weeks for up to six cycles or until the occurrence of progressive disease (PD) or unacceptable adverse events (AEs). All patients received 200 mg of camrelizumab (Hengrui Pharmaceutical, Lianyungang, China) via a 30-min intravenous infusion on day 1, and repeated every 3 weeks for up to 1 year unless there was PD or unacceptable AEs. Treatment could be delayed for a maximum of 2 weeks in the case of grade 3 or 4 AEs.

2.3 Evaluations

Patient demographics and characteristics were recorded. The RECIST (version 1.1) was used to assessed Activity. During the first 16 cycles, tumor imaging assessments were conducted every two cycles or immediately after obtaining a clear evidence of PD; Afterwards, tumor imaging assessments will be conducted every four cycles. Safety was evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.0). The main indicators included AEs and immune-related AEs (irAEs). The safe follow-up period for the subjects starts from the last dose and follows up every 30 days until 90 days after the last dose.

2.4 Statistical analysis

Perform all statistical analysis using SPSS (version 21.0; SPSS Inc., Chicago, Illinois, United States of America). The corresponding figures were drawn using Graph Prism 5.0 (GraphPad Software, San Diego, CA, United States of America). Two-tailed tests were performed at a significance level of $\alpha = 0.05$, with $p < 0.05$ indicative of statistical significance. Subgroup comparisons of count date were performed using the chi-square test or Fisher's exact test. The relationship between the variables and survival was assessed using Kaplan-Meier curves and the log-rank test's subgroup differences in survival were assessed. The follow-up period was extended to 30 September 2023.

3 Results

3.1 Patient characteristics

From January 2022 to March 2023, a total of 40 patients with unresectable or metastatic STS were enrolled in this study, with 28 men and 12 women. The average age of the patients was 49.28 ± 14.17 years (Table 1). All patients had an ECOG performance status of 0 or 1. The histological subtypes included UPS (n = 10), epithelioid sarcoma (n = 8), fibrosarcoma (n = 7), angiosarcoma (n = 5), myxofibrosarcoma (n = 3), MPNST (n = 2), leiomyosarcoma (n = 2), synovial sarcoma (n = 2), and differentiated liposarcoma (n = 1). The primary tumor sites were predominantly the limbs, followed by the trunk. Most patients experienced lung metastases, with a small number (12.5%) of them being locally unresectable. All patients had previously received at least one line of chemotherapy (Table 1).

3.2 Activity of treatment

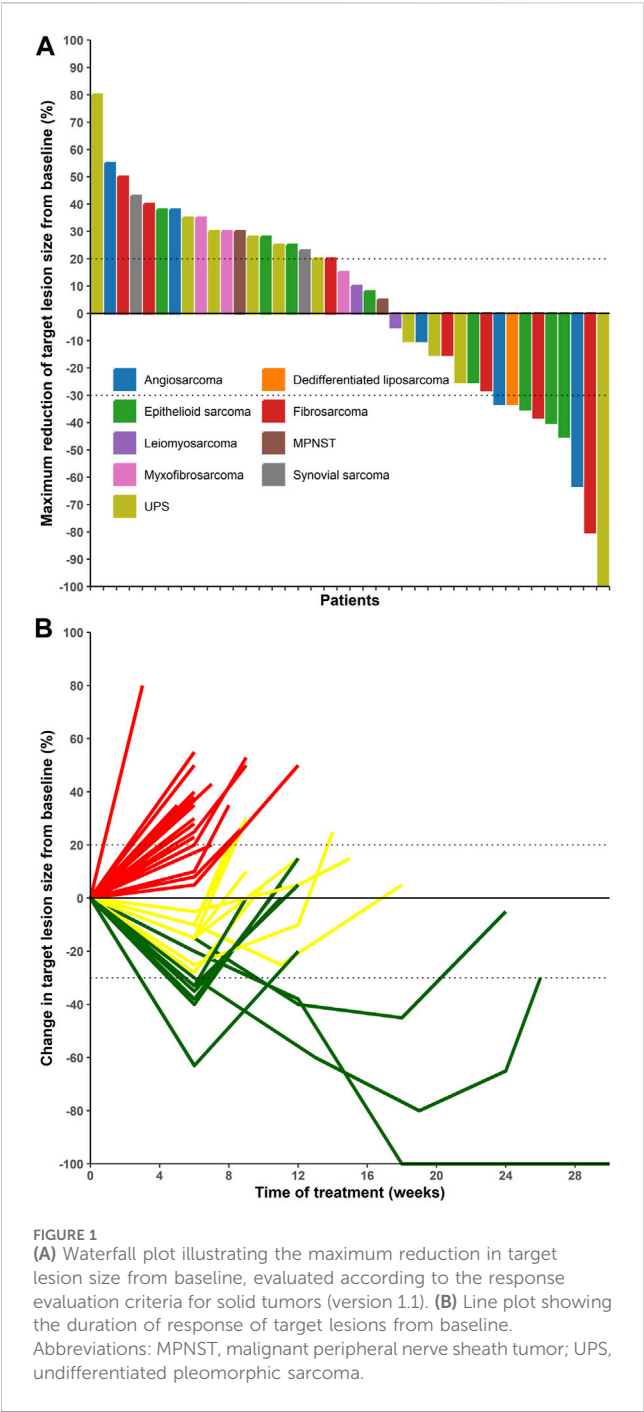
Among the 40 patients, 1 case of CR (UPS) and 8 cases of PR (3 epithelioid sarcomas, two fibrosarcomas, two angiosarcomas, and one dedifferentiated liposarcoma) were identified (Table 2; Figure 1). The ORR, DCR, median PFS, and 4-month PFS rates were 22.5%, 50%, 1.65 months, and 7.5%, respectively (Table 3 and Figure 1).

Patients with epithelioid sarcoma had a longer PFS than those with other histological subtypes (2.3 months vs. 1.5 months, respectively); however, this difference was not significant (Figure 2). Patients who had received only one line of previous chemotherapy had a significantly longer PFS compared with those who had undergone two or more lines of previous chemotherapy (2.8 months vs. 1.3 months, respectively, $p = 0.046$) (Figure 2).

TABLE 2 Efficacy of various histological subtypes.

Histology	Number of patients			
	CR	PR	SD	PD
UPS (n = 10)	1	0	3	6
Epithelioid sarcoma (n = 8)	0	3	2	3
Fibrosarcoma (n = 7)	0	2	2	3
Angiosarcoma (n = 5)	0	2	1	2
Myxofibrosarcoma (n = 3)	0	0	1	2
MPNST (n = 2)	0	0	1	1
Leiomyosarcoma (n = 2)	0	0	1	1
Synovial sarcoma (n = 1)	0	0	0	2
Dedifferentiated liposarcoma (n = 1)	0	1	0	0
Total	1	8	11	20

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; MPNST, malignant peripheral nerve sheath tumor; UPS, undifferentiated pleomorphic sarcoma.



3.3 Toxicity and safety

In general, nab-paclitaxel plus camdelizumab was well-tolerated (Table 4). Most AEs were associated with nab-paclitaxel treatment. The most common grade 1–2 AEs were alopecia (89.3%, 25/28), lymphocytopenia (75.0%, 30/40), leukopenia (55.0%, 22/40), anemia (32.5%, 13/40), and nausea (22.5%, 9/40). The most common grade 3 AEs were lymphocytopenia (22.5%; 9/40) and leucopenia (15.0%, 6/40). No grade 4 AEs were observed. IrAEs were mild and were of only two types: hyperthyroidism (15%, 6/40) and rash (5.0%, 2/40). No patient needed to reduce the dosage of nab-paclitaxel or PD-1 inhibitor due to AEs, and there were no treatment-related deaths.

TABLE 3 Efficacy of all patients to the treatment.

Characteristics	Data
ORR (%)	22.50 (95% CI: 10.84–38.45)
DCR (%)	50.00 (95% CI: 33.80–66.20)
Median-PFS (months)	1.65 (95%CI: 1.30–2.00)
4-month PFS rate (%)	7.50 (95%CI: 1.94–18.24)
6-month PFS rate (%)	2.50 (95%CI: 0.20–11.27)

Abbreviations: ORR, objective response rate; DCR, disease control rate; PFS, progression-free survival; CI, confidence interval.

4 Discussion

The study aimed to assess the activity and safety of nab-paclitaxel in combination with a PD-1 inhibitor, camrelizumab, as a second or subsequent line of therapy for advanced STS. Our results revealed noteworthy findings regarding this treatment approach.

Evidence suggests that chemotherapy can enhance the anti-tumor effects of PD-1 inhibitor by reducing the number of tumor cells, promoting immunogenic tumor cell death, consuming immunosuppressive cells, increasing the number and activity of anti-tumor immune effector cells, and enhancing the secretion of cytokines that promote immune cell proliferation (Principe et al., 2022; Zhu et al., 2022). Nab-paclitaxel is an immunogenic cell death inducer that has been shown to enhance the efficacy of PD-1 inhibitors by regulating various immune functions (Li et al., 2021; Yoneshima et al., 2021). Currently, chemotherapy combined with PD-1 inhibitor has been approved for the treatment of gastroesophageal, lung and breast cancers (Tian and Yao, 2022b; Principe et al., 2022).

In this study, although an ORR of 22.5%, comparable to that achieved with doxorubicin plus PD-1 inhibitors, was achieved (Pollack et al., 2020; Livingston et al., 2021; Tian et al., 2022b), the median PFS was only 1.65 months. This indicates that the treatment only elicited therapeutic effects for a brief initial period, and that there is no synergistic effect. Notably, a CR was observed in a patient with UPS. This response may be due to the well-documented sensitivity of UPS to PD-1 inhibitors, as indicated in previous studies (Moreno Tellez et al., 2022). The efficacy in the other histological subtypes can be attributed to nab-paclitaxel. Although the overall treatment effect is not satisfactory, this regimen exhibited therapeutic effects against epithelioid sarcoma, angiosarcoma, and fibrosarcoma, consistent with previous studies (Tian et al., 2022a). Owing to the observed short PFS for these STS histological subtypes, the use of nab-paclitaxel alone or in combination with a PD-1 inhibitor is not recommended for advanced STS treatment. Instead, consideration should be given to exploring combinations involving nab-paclitaxel with other effective drugs, such as chemotherapeutic and targeted drugs, for these subtypes.

In terms of safety, our findings revealed a relatively low incidence of AE, with rare occurrences of grade 3–4 AEs. This indicates that the combination of nab-paclitaxel and a PD-1 inhibitor is safe, consistent with previous studies (Hao et al., 2023), and notably better than first-line chemotherapy for STS (Gronchi et al., 2017; Seddon et al., 2017). This safety profile can greatly enhance patient satisfaction and quality of life during treatment. Given the lower incidence of AEs, considering the addition of other drugs, such as targeted drugs or chemotherapy drugs, to the protocol used in this study may improve treatment effectiveness.

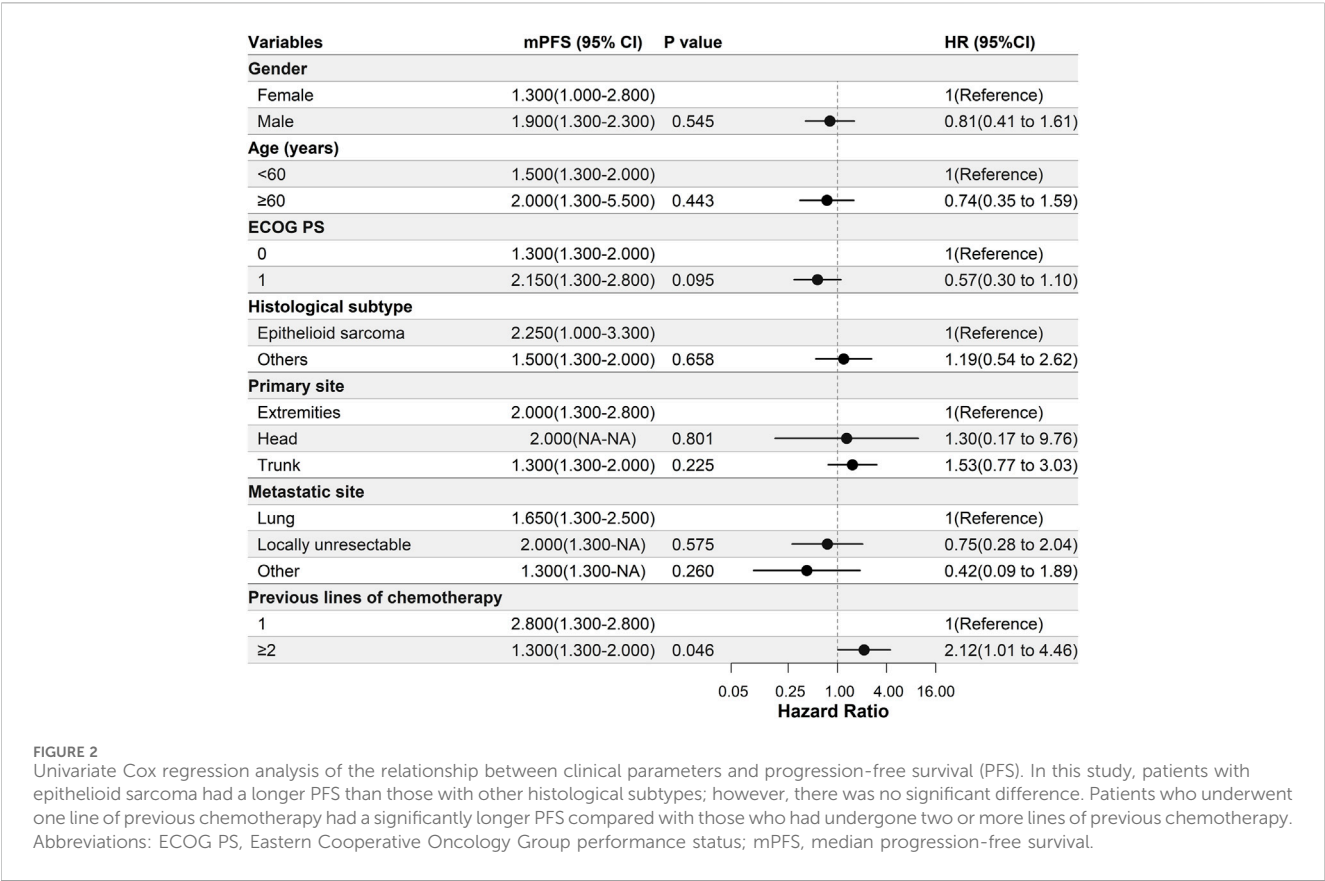


TABLE 4 Adverse events.

Adverse events	All grades	Grade 3–4
Alopecia	90.0% (36/40)	
Lymphocytopenia	75.0% (30/40)	22.5% (9/40)
Leucopenia	55.0% (22/40)	15.0% (6/40)
Anemia	32.5% (13/40)	2.5% (1/40)
Nausea	22.5% (9/40)	
Numbness of limbs	20.0% (8/40)	2.5% (1/40)
Pain	20.0% (8/40)	2.5% (1/40)
Thrombocytopenia	17.5% (7/40)	2.5% (1/40)
Fatigue	17.5% (7/40)	
Transaminase increase	17.5% (7/40)	
Anorexia	15.0% (6/40)	
Hypothyroidism	12.5% (5/40)	2.5% (1/40)
Diarrhea	12.5% (5/40)	
Fever	7.5% (3/40)	
Rash	5.0% (2/40)	

Our study has certain limitations, including the absence of a control group, a relatively small number of patients, and a short follow-up period. Despite these limitations, this study demonstrated the limited efficacy of nab-paclitaxel plus

camrelizumab in treating STSs. Nonetheless, nab-paclitaxel shows promise in treating epithelioid sarcoma, angiosarcoma, and fibrosarcoma. Therefore, further research investigating the use of nab-paclitaxel in combination with other effective drugs, such as chemotherapy and targeted drugs, for these specific subtypes of STSs is warranted.

5 Conclusion

Nab-paclitaxel plus camrelizumab exhibited modest activity and mild toxicity in treating epithelioid sarcoma, angiosarcoma, and fibrosarcoma. The overall effectiveness of this treatment regimen for advanced STS is relatively low. Further research on combining nab-paclitaxel with effective drugs, including chemotherapy and targeted agents, for these STS subtypes is needed.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the Henan Cancer Hospital. The studies were

conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

ZT: Conceptualization, Investigation, Methodology, Writing–original draft, Writing–review and editing. YF: Conceptualization, Methodology, Software, Writing–review and editing. YaY: Data curation, Formal Analysis, Methodology, Software, Writing–review and editing. XL: Investigation, Writing–original draft. GQ: Investigation, Writing–review and editing. YoY: Methodology, Resources, Writing–review and editing. XW: Investigation, Resources, Writing–review and editing. JW: Investigation, Resources, Writing–review and editing. PZ: Investigation, Resources, Writing–review and editing. WY: Conceptualization, Data curation, Investigation, Methodology, Resources, Software, Writing–review and editing.

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Conflict of interest

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Nomenclature

PD-1	programmed cell death protein 1
STS	soft tissue sarcoma
UPS	undifferentiated pleomorphic sarcoma
MPNST	malignant peripheral nerve sheath tumor
ECOG	Eastern Cooperative Oncology Group
RECIST	response evaluation criteria in solid tumors
AEs	adverse events



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Surgery combined with anlotinib for local control of patients with resectable extremity desmoid fibromatosis: a retrospective study

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Background: Desmoid fibromatosis (DF) is a pathological intermediate fibroblastoma that is difficult to control locally due to its invasive nature, especially in the extremities. Although anlotinib demonstrated efficacy in treating DF with tolerable safety, the impact of surgical intervention in conjunction with anlotinib administration on local control in patients with extremity DF remains undetermined.

Methods: We conducted a retrospective examination of the clinical medical documentation belonging to patients with resectable DF of the extremities who were treated with surgery between January 2010 and June 2022. The patients were divided into two cohorts: surgery alone cohort and surgery combined with anlotinib group (surgery plus anlotinib cohort), crossover to surgery plus anlotinib cohort was admissible for patients in the surgery alone cohort who experienced disease recurrence postoperatively. Clinical data such as basic information, tumor location, anlotinib toxicity, time to recurrence, surgical complications, follow-up time, visual analogue scale (VAS) score and Musculoskeletal Tumor Society (MSTS) score at the last follow-up were collected.

Results: In total, 48 consecutive patients (19 males and 29 females) with resectable DF of the extremities, including 25 patients in the surgery alone cohort, 23 patients in the surgery plus anlotinib cohort, and 10 patients who were transferred from the surgery alone cohort to the surgery plus anlotinib cohort. The VAS score at the last follow-up was 5 (IQR, 3–6) in the surgery alone cohort and 2 (IQR, 1–3) in the surgery plus anlotinib cohort, respectively; the MSTS score at the last follow-up was 19 (IQR, 16.5–24) in the surgery alone cohort and 27 (IQR, 25–28) in the surgery plus anlotinib cohort, respectively; these characteristics were statistically different between the two cohorts. The 3-year recurrence-free survival (RFS) of the surgery alone cohort and the surgery plus anlotinib cohort were 37.7% and 72.6%, respectively, and the difference was statistically significant ($p = 0.022$).

Conclusion: Surgery combined with anlotinib appears to be effective in controlling local recurrence in patients with resectable DF of the extremities, and the side effects were acceptable.

KEYWORDS

desmoid fibromatosis, surgery, anlotinib, local recurrence, side effects

1 Introduction

Desmoid fibromatosis (DF) is a pathologically intermediate fibroblastic tumor that is difficult to control locally due to its infiltrative nature. DF consists of spindle-shaped cells embedded within a dense collagenous matrix abundantly interspersed with prominent blood vessels, which often demonstrating a locally infiltrative proliferation pattern. DF possesses an annual incidence of 5–6 cases per million inhabitants, which may be underestimated due to the stealthiness and spontaneous regression of the disease (Kasper et al., 2015; Kasper et al., 2017a; Penel et al., 2021). DF possesses the potential to appear anywhere throughout the body, mainly in the extremities, intra-abdominal, and abdominal wall. On the contrary, extremity DF generally predicts an increased risk of recurrence and a poor prognosis because tumors are often adjacent to vascular nerves (Wirth et al., 2018; Mandel et al., 2022; Lehnhardt et al., 2023).

DF are predominantly sporadic, and approximately 90% of DF are associated with mutations in exon 3 of the somatic b-catenin gene (CTNNB1). However, although CTNNB1 plays a role in the pathogenesis of DF, the prognostic value of CTNNB1 mutations has yet to be elucidated thus far (Penel et al., 2022). Ten percent of DF are associated with germline adenomatous polyposis mutations and familial adenomatous polyposis (Norkowski et al., 2020; Riedel and Agulnik, 2022). In addition, it has been posited that estrogenic hormones may be implicated in the pathogenic genesis of DF, such that the incidence of DF is highest in women during or after pregnancy (Trautmann et al., 2020; Riedel and Agulnik, 2022).

Two decades earlier, surgical resection with negative margins was deemed the archetypal intervention for patients with DF. However, due to the high local recurrence and complications after surgery, a transition to a more conservative approach has been newly promulgated. An international guideline for the management of DF has recently been introduced, which takes into account the patient's perspective. Active surveillance with scheduled magnetic resonance imaging (MRI) is first-line treatment for DF. The anatomic location of the tumor should be considered before any therapeutic intervention is identified and risk-benefit assessments should be performed, weighing adverse effects against lasting sequelae (Shido et al., 2009; Prodinger et al., 2013; Dürr et al., 2020; Sobczuk et al., 2021).

For progressive DF in the extremities, medical treatment is recommended following active surveillance. Medical treatment includes surgery, radiation therapy, low-dose or conventional chemotherapy, non-steroidal anti-inflammatory drugs (NSAIDs), anti-hormone therapy, and tyrosine kinase inhibitors (TKIs), but there is no standard treatment regimen (Prendergast et al., 2022; Tsukamoto et al., 2023). To date, there is no evidence to confirm the efficacy of NSAIDs and anti-hormone therapy for patients with DF according to the Desmoid Working Group (Kasper et al., 2015; Kasper et al., 2017a). TKIs, including anlotinib, sorafenib, imatinib, and pazopanib, have been evaluated as new non-chemotherapeutic

systemic therapies in patients with unresectable, recurrent, or progressive DF and have yielded some promising clinical results (Gounder et al., 2011; Kasper et al., 2017b; Agresta et al., 2018; Zheng et al., 2020). Anlotinib is a novel multi-targeted TKI that selectively inhibited platelet-derived growth factor receptor, vascular endothelial growth factor receptor-1, -2, -3, and hepatic cytokine receptor (Shen et al., 2018). However, the effect of surgical treatment combined with anlotinib on local control in patients with extremity DF remains undetermined; therefore, we retrospectively compared the clinical efficacy of surgery alone and surgery combined with anlotinib. To evaluate the local control rate of surgical treatment combined with anlotinib.

2 Patients and methods

2.1 Study population and design

We conducted a retrospective examination of the clinical medical documentation belonging to patients with resectable DF of the extremities who were treated with surgery between January 2010 and June 2022 at our center. The criteria requisite for inclusion in the study were delineated as follows: 1) The diagnosis of DF was pathologically confirmed in the Department of Pathology of West China Hospital; 2) tumor located in the extremity, including buttock; 3) patients had clinical symptoms, mainly including pain, functional limitation, and compression symptoms, which were not relieved after 6 months of observation; 4) the tumor could be surgically removed without damaging vital neurovascular bundle; 5) tumor resection was performed by Professor Hong Duan; and 6) postoperative follow-up time greater than 12 months. The exclusion criteria from the study were delineated as follows: 1) chemotherapy or radiation therapy had been used before treatment in our hospital; 2) the patient's general condition was poor and could not tolerate surgery; 3) the patient's follow-up data were insufficient.

The patients were divided into two cohorts: surgery alone cohort and surgery combined with anlotinib group (surgery plus anlotinib cohort), as shown in Figure 1. Before 2018, we treated DF mainly by surgery with or without chemoradiotherapy. The treatment strategy after 2018 was surgery combined with anlotinib if the patient had clinical symptoms that did not relieve after 6 months of observation. Crossover to the surgery plus anlotinib cohort was admissible for patients in the surgery alone cohort who experienced disease recurrence postoperatively.

Resectable tumor was delineated as: marginal or extensive resection of the tumor was considered feasible without injury to the vital neurovascular bundle, or would engender tolerable morbidity subsequent to extensive or marginal resection. Whenever possible, the aim was to obtain a negative resection margin, unless the tumor was adjacent to the neurovascular bundle. For cases where negative

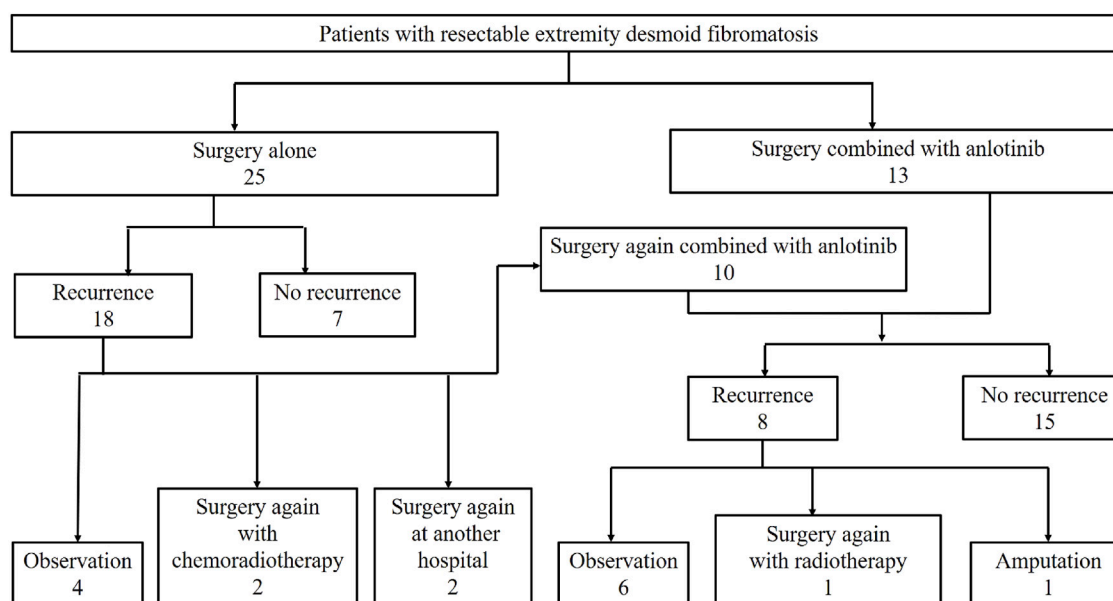


FIGURE 1
Flow chart of patient treatment strategy and results.

margins were difficult to obtain, marginal resection was chosen. The treatment strategy and the final decision on whether to operate or otherwise were discussed at a multidisciplinary oncology meeting. All patients taking anlotinib provided informed consent for anlotinib therapy. This study was presented to and approved by the Institutional Review Board of Sichuan University West China Hospital (No 2022793).

2.2 Clinical data collection of patients

Routine clinical and imaging examinations were performed at monthly outpatient follow-up after surgery, and MRI examinations were performed at the third, sixth and 12th month, and then once a year thereafter. During follow-up, local tumor recurrence was found, and the patient was asked to return to the hospital for continued treatment in Hong Duan's treatment group. If the patient found abnormalities (mass, pain, functional limitations, etc.), the frequency of MRI examinations increased. Recurrence was assessed by MRI. The subsequent clinical information was assembled: age, gender, status of disease, therapeutic history, tumor size, tumor location, date of surgery, toxicity of anlotinib, time to recurrence, operative complications, length of follow-up, visual analogue scale (VAS) score and Musculoskeletal Tumor Society (MSTS) score at the last follow-up, date of death if available. The size of the DF was defined as the maximum diameter on MRI prior to surgery or anlotinib therapy.

2.3 The use of anlotinib

For patients in the surgery plus anlotinib cohort, the starting dose of anlotinib was 8 mg once a day for 2 weeks of treatment followed by the cessation of treatment for 1 week. Preoperative and postoperative

medication should be used for at least 4 courses, respectively. Postoperative medication should be prolonged to 1 year as far as possible, and the medication regimen and specific time of postoperative withdrawal should be comprehensively determined according to whether the patient had recurrence and adverse reactions. The dose was reduced to 6 mg if the patient experienced intolerable or uncontrolled pharmaceutical-induced toxicity. If a patient had relapsed following surgery combined with anlotinib and was observed with progressive disease or clinical symptoms, the dose will be increased to 10 mg (Eisenhauer et al., 2009); if a patient developed refractory adverse reactions during the subsequent two cycles of 10 mg, the drug would be permanently discontinued and other treatment approaches would be employed.

2.4 Clinical evaluation

Recurrence-free survival (RFS) constituted the primary outcome measure and was defined as the time interval from the date of surgical intervention to the date of tumor recurrence or patient death due to the tumor or the last follow-up. Clinical efficacy was mainly evaluated by pain relief and functional activity, quantified by the VAS score and the MSTS score, respectively. Pharmaceutical-associated adverse effects were categorized and stratified according to the Common Terminology Criteria for Adverse Events (version 4.0) (Basch et al., 2021).

2.5 Data analysis

Differences between two cohorts of patients were evaluated using the Fisher's exact test or chi-square or Mann-Whitney U test. Descriptive statistics included median, interquartile range

TABLE 1 The particulars of the individuals included in this study at baseline.

Variables	Total (n = 48, %)	Number of patients (n, %)		p-value
		Surgery alone (n = 25)	Surgery with anlotinib (n = 23)	
Age at diagnosis (years)				0.269
Median	25	32	22	
IQR	19–38.8	19.5–42.5	18.0–33.0	
Sex				0.769
Male	19 (39.6)	9 (36.0)	10 (43.5)	
Female	29 (60.4)	16 (64.0)	13 (56.5)	
Tumor location				0.361
Gluteal region	12 (25.0)	5 (20.0)	7 (30.4)	
Thigh	10 (20.8)	5 (20.0)	5 (21.7)	
Scapular region	10 (20.8)	5 (20.0)	5 (21.7)	
Popliteal region	4 (8.3)	2 (8.0)	2 (8.7)	
Forearm	4 (8.3)	3 (12.0)	1 (4.3)	
Foot	4 (8.3)	3 (12.0)	1 (4.3)	
calf	3 (6.3)	2 (8.0)	1 (4.3)	
Axillary region	1 (2.1)	0 (0)	1 (4.3)	
Tumor size (cm)				0.635
Median	8.2	9.3	7.8	
IQR	5.8–11.3	5.8–13.2	5.9–11.2	
Number of previous tumor surgery				0.330
Median	2	2	1	
IQR	1–2	1–2	1–2	
Year of surgery				<0.001
2010–2017	13 (27.1)	13 (52.0)	0 (0)	
2018–2023	35 (72.9)	12 (48.0)	23 (100)	
Status of disease				0.303
Primary	8 (16.7)	3 (12.0)	5 (21.7)	
Recurrent	40 (83.3)	22 (88.0)	18 (78.3)	

IQR, interquartile range.

(IQR), counts, and percentages. Kaplan-Meier survival analysis and log-rank test were used to compare the RFS and survival curves between the two cohorts, respectively. A *p*-value <0.05 was considered statistically significant. Statistical analysis was performed using IBM SPSS version 21.0 (IBM Corp.).

3 Results

3.1 Characteristics of the study population

From January 2010 to June 2022, 48 consecutive patients (19 males and 29 females) with resectable DF of the extremities were admitted,

including 25 patients in the surgery alone cohort, 23 patients in the surgery plus anlotinib cohort, and 10 patients who were transferred from the surgery alone cohort to the surgery plus anlotinib cohort (Figure 1). The median age was 25 years (IQR, 19–38.8); the median tumor size was 8.2 (IQR, 5.8–11.3); the median number of previous tumor surgery was 2 (IQR, 1–2); there were 8 primary tumors and 40 recurrent tumors; the most common tumor location was the gluteal region, followed by the thigh and scapula region; these characteristics were not statistically different between the two cohorts. 13 patients underwent surgery before 2018 and 35 patients underwent surgery after 2018, of which all patients in the surgery plus anlotinib cohort underwent surgery after 2018, with a statistically significant difference between the two cohorts (Table 1).

TABLE 2 Treatment characteristics and outcome of the patients included in this study.

Variables	Number of patients (n, %)		p-value
	Surgery alone (n = 25)	Surgery with anlotinib (n = 23)	
Follow up period (months)			0.117
Median	45	40	
IQR	28.5–66.5	27–50	
Recurrences at the last follow-up			0.019
no	7 (28.0)	15 (65.2)	
yes	18 (72.0)	8 (34.8)	
Interval between the surgery and recurrence			0.165
Median	17.5	24	
IQR	12.5–31	19.5–35	
VAS score at the last follow-up			<0.001
Median	5	2	
IQR	3–6	1–3	
MSTS score at the last follow-up			<0.001
Median	19	27	
IQR	16.5–24	25–28	
The number of postoperative anlotinib treatment courses			-
Median	-	26	
IQR	-	21.5–42	
Surgical complications			0.748
Infection	1	2	
Wound healing issues	3	4	
Temporary iatrogenic nerve damage	2	1	

IQR, interquartile range.

3.2 Treatment outcomes

The median follow-up time was 45 months (IQR, 28.5–66.5 months) in the surgery alone cohort and 40 months (IQR, 27–50 months) in the surgery plus anlotinib cohort; the median interval between surgery and recurrence was 17.5 months (IQR, 12.5–31 months) in the surgery alone cohort and 24 months (IQR, 19.5–35 months) in the surgery plus anlotinib cohort; these characteristics were not statistically different between the two cohorts (Table 2). The number of recurrences at the last follow-up was 18 and 8 in the surgery alone cohort and surgery plus anlotinib cohort, respectively, with a statistically significant difference between the two cohorts. Overall, none of the patients died from the disease. The VAS score at the last follow-up was 5 (IQR, 3–6) in the surgery alone cohort and 2 (IQR, 1–3) in the surgery plus anlotinib cohort, respectively; the MSTS score at the last follow-up was 19 (IQR, 16.5–24) in the surgery alone cohort and 27 (IQR, 25–28) in the surgery plus anlotinib cohort, respectively; these characteristics were statistically different between the two cohorts (Table 2).

The median RFS was 31 months and the 3-year RFS rate was 37.7% in the surgery alone cohort; the median RFS was 42 months and the 3-year RFS rate was 72.6% in the surgery plus anlotinib cohort. Significant difference in RFS was observed between the two cohorts ($p = 0.022$, Figure 2). The typical case of the surgery alone cohort was shown in Figure 3: A 20-year-old female patient with postoperative recurrence of DF in the right thigh was included in the surgery alone cohort. The patient still recurred 17 months after surgery with clinical symptoms. The patient was transferred to the surgery plus anlotinib cohort for continued treatment. After preoperative use of anlotinib, the tumor was resected again, the vascular nerve was preserved, and MRI showed no recurrence 14 months after surgery. A typical case of surgery plus anlotinib cohort was shown in Figure 4: A 21-year-old female was diagnosed with DF of the right buttock. The patient visited our hospital for the first time and underwent a needle biopsy at our hospital to confirm the diagnosis. The tumor was resected after preoperative use of anlotinib. MRI showed no tumor recurrence at 27, 39 and 49 months after the operation.

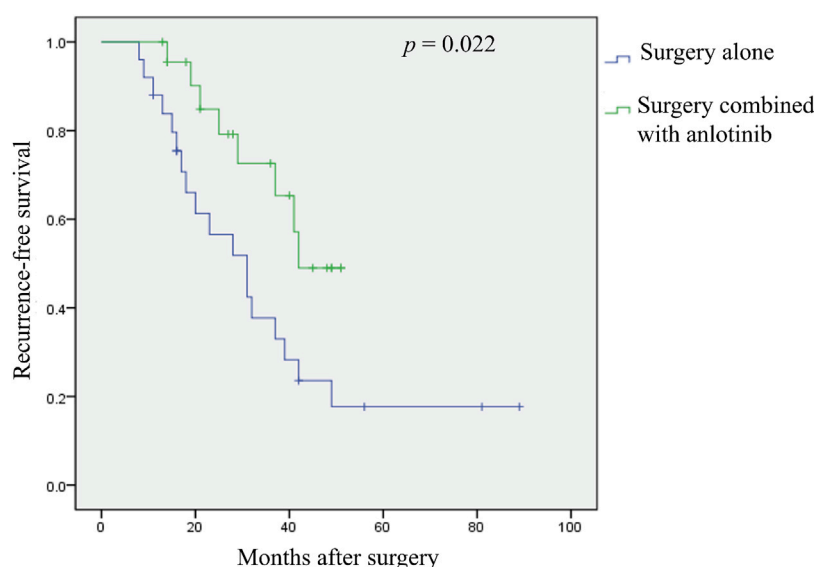


FIGURE 2
Kaplan-Meier analysis of recurrence-free survival was performed according to different treatment strategies.

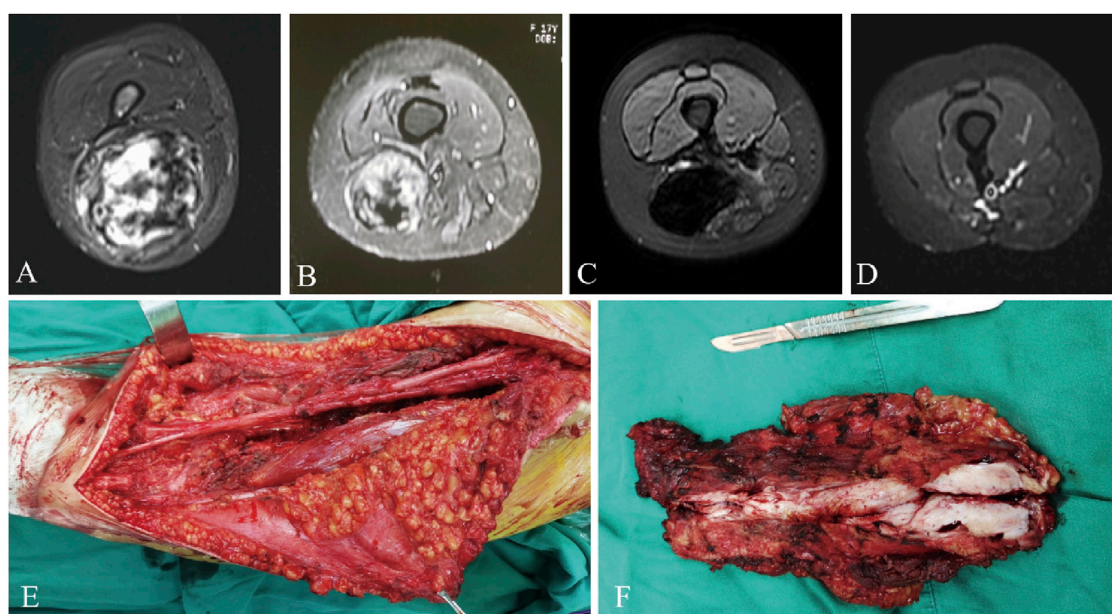


FIGURE 3
A 20-year-old female presented with postoperative recurrence of DF of right thigh (A), which still recurred 17 months after tumor resection in our hospital (B). MRI was re-examined after using anlotinib (C), and the tumor was resected again with preserved vascular and nerve during the operation (E, F), and MRI was re-performed 14 months after surgery (D). MRI, magnetic resonance imaging.

3.3 Complications and toxicity

Six patients in the surgery alone cohort had surgical complications, including 1 case of wound infection, 3 cases of wound healing problems, and 2 cases of temporary iatrogenic nerve injury; 7 patients in the surgery plus anlotinib cohort had complications, including 2 cases of wound infection, 4 cases of wound healing problems, and 1 case of temporary iatrogenic nerve

injury. There was no statistical difference in complications between the two cohorts. Wound infections were resolved by debridement, wound healing problems by prolonging healing time, and temporary iatrogenic nerve injuries were all recovered within 6 months by the use of trophic nerve drugs (Table 2). Major adverse events included hand-foot-skin syndrome ($n = 12$, 52.2%), hypertension ($n = 10$, 43.5%), fatigue ($n = 10$, 43.5%), paresthesia ($n = 8$, 34.8%), vomiting ($n = 7$, 30.4%), general or local pain ($n = 7$, 30.4%), proteinuria ($n =$

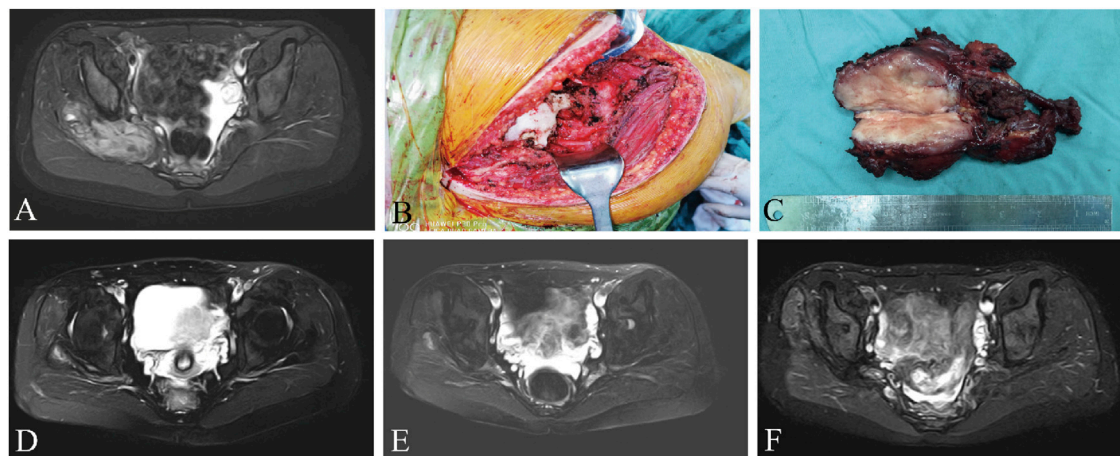


FIGURE 4
A 21-year-old female presented with DF of the right buttock, preoperative MRI (A), surgical resection of the tumor (B, C), and reexamination of MRI (D–F) after 27, 39, and 49 months after surgery, respectively. MRI, magnetic resonance imaging.

TABLE 3 Adverse events of anlotinib treatment.

Adverse events	n (%)	Grade 1	Grade 2	Grade 3
Hand-foot-skin syndrome	12 (52.2)	6	5	1
Hypertension	10 (43.5)	6	4	1
Fatigue	10 (43.5)	3	7	0
Parosmia	8 (34.8)	6	2	0
Vomiting	7 (30.4)	2	5	0
General or local pain	7 (30.4)	3	4	0
Proteinuria	5 (21.7)	5	0	0
Oral pain	4 (17.4)	2	2	0
Hemorrhage	3 (13.0)	2	1	0
Dizziness headache	2 (8.7)	1	1	0

5, 21.7%), oral pain ($n = 4$, 17.4%), hemorrhage ($n = 3$, 13.0%), dizziness headache ($n = 2$, 8.7%). Parosmia was present in 8 female patients, accounting for 61.5% of female patients. These adverse events were generally grade 1 to 2, and only two patients had grade 3 adverse events (hypertension and hand-foot-skin syndrome), which were well controlled with symptomatic treatment or reduction in drug dose. None of the patients experienced grade 4 adverse events or discontinued the anlotinib because of side effects of the drug, which were considered tolerable by the patients (Table 3).

4 Discussion

The present retrospective study described and analyzed the data from a series of patients with resectable extremity DF. DF of the extremities was usually located adjacent to neurovascular structures (as shown in Figure 4), and there was a greater risk of surgically

injured vascular nerves compared to other areas, and more attention should be paid to such tumors. Therefore, patients with DF of the extremities were specifically included in this study. The primary objective of this study was to evaluate the tumor local recurrence rate of surgery combined with anlotinib in treating resectable extremity DF, and simultaneously, to evaluate the side effects of anlotinib.

The clinical management of DF remains challenging, and surgical resection of the tumor has previously been the standard primary treatment modality; however, in recent years, a shift to a more conservative management model has been introduced. A recent consensus reached by the DF Working Group suggests that aggressive treatment is recommended only in case of persistent progression, given the benign character and only local aggressiveness of the disease (2020). Clinical symptoms are incompletely associated with DF progression; some stable DF may be accompanied by clinical symptoms, while some progressive DF may have no clinical symptoms (Gronchi et al., 2014). Surgery may be considered if expected surgical morbidities are limited (Penel et al., 2021). Therefore, we focused on patients with clinical symptoms (mainly including pain, functional limitation, and compression symptoms), as an inclusion criterion to assess the efficacy and toxicity of surgery combined with anlotinib in the treatment of resectable extremity DF.

Many studies have shown that 23%–77% of tumors still have local recurrence after wide surgical resection (Pritchard et al., 1996; Vora et al., 2021; Mandel et al., 2022; Prendergast et al., 2022). The location of the tumor seems to have a major impact on local recurrence, with DF located in the extremities having recurrence rates of even as high as 80% (Salas et al., 2011; Lehnhardt et al., 2023). Similar to our findings, the postoperative local recurrence rate reached 72.0% in the surgery group alone at the last follow-up. With the use of combination therapies, including low-dose or conventional chemotherapy, NSAIDs, and TKIs, local recurrence rates have improved over the past few decades and have reportedly dropped to 17%–30% (Merchant et al., 1999; Shkalim Zemer et al., 2017; Wirth et al., 2018; Gronchi and Jones, 2019; Mikhael et al., 2022; Riedel and Agulnik, 2022; Tsukamoto et al., 2023). Although

the RFS rate at the last follow-up in the surgery plus anlotinib cohort of this study was 34.8%, which was slightly higher than that reported in the literatures, there was a significant decrease compared to 72.0% in the surgery alone cohort. Meanwhile, the 3-year RFS rate was 72.6% in the surgery plus anlotinib cohort and 37.7% in the surgery alone cohort, and Kaplan-Meier analysis demonstrated that the RFS rate within the surgery plus anlotinib cohort exceeded that of the surgery alone cohort, with statistical significance ($p = 0.022$). The results showed that surgery combined with anlotinib could significantly reduce the local recurrence rate compared with surgery alone in the treatment of DF. While the local recurrence rate was effectively controlled, the MSTS score of patients in the surgery plus anlotinib cohort was significantly increased and the VAS score was significantly decreased compared with the surgery alone cohort, indicating that the clinical symptoms of patients in the surgery alone cohort were significantly improved.

Resection margins of the tumor are less important than maintaining function for the patient and do not have a significant impact on local recurrence (Wirth et al., 2018; Dürr et al., 2020). Unless the tumor was adjacent to the neurovascular bundle, the surgical procedure should aim for extensive resection. For cases with severe complications due to adjacent critical structures or after extensive resection, marginal resection was selected (as shown in Figures 3, 4). In a retrospective study of 426 patients diagnosed with DF, surgical margins (R2 v R0/R1) were found to have a significant impact on progression-free survival (PFS), but R0 v R1 did not (Salas et al., 2011). All surgical patients included in our study achieved R0 or R1 resection, and marginal resection of tumors adjacent to vascular nerves could be regarded as R1 resection, and those who could not reach R1 resection were considered unresectable lesions. Therefore, we did not include surgical margin classification as a study object in this study. Because the patients were compared between two different periods, although tumor resection was performed by the same surgeon in both cohorts, the surgical technique improved over time, which may also be one of the reasons for the decrease in local recurrence rate.

Recently, a phase III, randomized, placebo-controlled trial explored the efficacy of niraparic acid, a γ -secretase inhibitor, in adult patients with progressive DT (Gounder et al., 2023). The investigators randomly assigned 142 patients with desmoid tumors to receive either niraparic acid or placebo, and PFS was the primary endpoint. The study showed a significant PFS benefit with niraparic acid compared with placebo (hazard ratio for death or disease progression, 0.29; $p < 0.001$). The proportion of patients with objective response was significantly higher in the niraparic acid group than in the placebo group (41% vs. 8%; $p < 0.001$); the proportion of patients with complete response was 7% and 0%, respectively. Ninety-five percent of common adverse events with niraparic acid were grade 1 or 2, including diarrhea (84% of patients), nausea (54%), and fatigue (51%). Because of this study, niraparic acid was approved by the US Food and Drug Administration for the treatment of desmoid tumors. TKIs, as one of the systemic therapies, are effective in the treatment of DF, with 6-month PFS ranging from 65% to 96%, and the adverse event rates of grades 3 and 4 ranged from 0% to 15% and 0%–3%, respectively (Gounder et al., 2011; Kasper et al., 2017b; Agresta et al., 2018; Zheng et al., 2020). Imatinib was the first TKI used to

treat DF, with a disease control rate of 78%–92% (Penel et al., 2011; Kasper et al., 2017b). In a double-blind phase III trial investigated by Gounder MM et al. (Gounder et al., 2018), 87 patients with DF received either sorafenib or matching placebo. With a median surveillance of 27 months, the 2-year PFS rate reached 81% and the median time to objective response was 9.6 months, both superior to placebo group. This clinical trial found that sorafenib significantly prolonged PFS with mild to moderate side effects, mainly including rash, fatigue, and hypertension events. Anlotinib is a novel multi-target TKI that inhibits tumor proliferation and angiogenesis with disease control rates and toxicity similar to sorafenib (Gounder et al., 2018; Shen et al., 2018; Tsukamoto et al., 2023). Zheng et al. (2020) retrospectively investigated the clinical data of 21 patients with extremity DF treated with anlotinib. 38.1% of the patients had partial response, 47.6% had stable disease, disease control rate up to 86.0%, and no patients had complete response. The results of the study showed that anlotinib was effective in DF with acceptable safety (mainly mild to moderate side effects) and significantly slowed disease progression (Zheng et al., 2020). In our study, the side effects of anlotinib were mainly mild to moderate and resolved by adjusting the drug and/or symptomatic treatment, and no drug withdrawal due to side effects occurred. Safety and side effects were controllable and similar to those reported in the literature. Furthermore, we used a lower dose (starting at 8 mg/day) compared to the dose used for soft tissue sarcomas (Chi et al., 2018), which theoretically would have lower side effects. In summary, escalation from less side effects to stepped therapy with more toxic agents is recommended for DF that requires medical treatment.

Additionally, we acknowledge that our study has several limitations. First, this study was retrospective and at risk of selection bias and recall bias. Second, owing to the limited number of patients in the study, there was a risk of Type II error. Third, there were few and incomplete data of patients who underwent surgery combined with radiotherapy or chemotherapy in our hospital, and no efficacy comparison was performed, but according to the results in the literature as a reference basis. Fourth, the follow-up period of this study was relatively short, and the duration of anlotinib administration was uncertain, and some side effects of long-term efficacy could not be observed. At the same time, the follow-up time was different between the two cohorts, which may also cause bias, and further follow-up observation was required. Finally, the study excluded patients with abdominal or trunk lesions, and the sample size became limited with selection bias. Therefore, future studies including patients with abdominal or trunk lesions are warranted. These limitations should be taken into account when analyzing our findings.

5 Conclusion

In summary, in this retrospective study, surgery combined with anlotinib appears to be effective in controlling local recurrence in patients with resectable DF of the extremities, and side effects were acceptable. Additionally, the level of evidence in this study is observational and retrospective and that prospective randomized clinical trials with adequate power are needed to validate the

therapeutic efficacy of surgery combined with anlotinib in resectable DF of the extremities.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethical committee of West China Hospital, Sichuan University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because patient consent was waived due to the fact that a retrospective study was conducted using data extracted from medical records.

Author contributions

DY: Conceptualization, Data curation, Investigation, Methodology, Software, Validation, Writing—original draft. YL: Data curation, Investigation, Methodology, Writing—original draft. XF: Visualization, Writing—original draft. FW: Data curation, Visualization, Writing—review and editing. SL: Data curation, Software, Visualization, Writing—review and editing. LT:

Data curation, Software, Writing—review and editing. FK: Data curation, Writing—review and editing. YG: Investigation, Writing—review and editing. CG: Investigation, Writing—review and editing. WZ: Supervision, Validation, Writing—review and editing. HD: Conceptualization, Supervision, Validation, Writing—review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Radiological evaluation of response in patients with locally advanced/metastatic soft tissue sarcoma treated with trabectedin

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Background: Trabectedin is an antineoplastic drug approved for patients (pts) with advanced soft tissue sarcomas (STS). Interestingly, the radiological evaluation of response during trabectedin therapy is peculiar.

Methods: The aim of this single-center retrospective study is to analyze the concordance of response assessment according to RECIST compared with Choi criteria in patients with STS treated with trabectedin between 2009 and 2020 at Regina Elena National Cancer Institute in Rome.

Results: We present the preliminary data collected in the last 2 months (mos) on 37 pts who received the diagnosis between 2015 and 2020, with a median age of 52.5 years (range 32–78). The median number of trabectedin cycles administered was four (range 2–50) for a median follow up of 5.83 months (range 1–60). Histological subtypes of STS were five (13.5%) leiomyosarcoma, 14 (37.8%) liposarcoma, nine (24.3%) undifferentiated pleomorphic sarcoma, three (8.1%) synovial sarcoma, and six (16.2%) other rare histological subtypes. Eight pts (21.6%) received trabectedin in the first line setting, 21 (56.8%) in the second line, and seven (18.9%) received it in subsequent lines. One pt received trabectedin as neoadjuvant therapy in a clinical trial (ISG-STS 1001). Median progression-free survival was 3.6 months (CI95% 2.7–4.6); median overall survival was 34.3 months (CI95% 0–75.4). The radiological responses were evaluated with both RECIST and Choi criteria; responses matched in 33 pts (89.2%) but not in four (10.8%). The best responses obtained according to RECIST criteria were two (5.4%) partial response (PR), 13 (35.1%) stable disease (SD), and 22 (59.5%) progressive disease (PD). Instead, two (5.4%), 13 (35.1%), and 22 (59.5%) pts obtained PR, SD, and PD respectively, according to Choi criteria. Cohen's kappa coefficient of concordance was 0.792 (p -value <0.002). A specialized radiologist performed all imaging examinations using a dedicated workstation in the same center.

Conclusion: In this first analysis, the concordance between RECIST and Choi assessments demonstrates no statistically significant difference. Responses did not match for four pts. We are expanding the analysis to all pts included in the original cohort to confirm or deny these initial results.

KEYWORDS

sarcoma, soft tissue, trabectedin, response assessment, real-life

1 Introduction

Soft tissue sarcomas (STS) are rare tumors that arise from anatomical structures of mesenchymal origin. Their global incidence is around 3–5 cases/per 100,000 people/year, and they represent 1% of adult cancers (Sbaraglia et al., 2021). More than 50 different histological subtypes with specific biological characteristics and distinct behaviors are grouped under the term “STS”, and histological evaluation before any therapeutic step is mandatory to define the most correct therapeutic strategy. Magnetic resonance imaging (MRI) represents the main imaging modality for diagnosis and follow-up, especially for STS of the extremities, the pelvis, and the trunk. Computed tomography (CT) is another radiologic technique useful for staging and evaluation of response during active treatments and in follow up. PET is a second-choice test mainly used to better characterize CT or MRI findings or to identify bone metastases (Gronchi et al., 2021). Surgery is the cornerstone treatment for localized STS disease, followed by postoperative radiotherapy in high-grade (G2-3) lesions. Systemic treatment is based mainly on chemotherapy that can be offered in peri-operative settings (neo-adjuvant or adjuvant) for high-risk patients and in metastatic disease and anthracyclines alone or in combination with ifosfamide, and are the first line of reference for most chemosensitive histotypes (Gronchi et al., 2021; Blay et al., 2022). The second line is a limited number of chemotherapeutic agents proven to be active in STS. A greater understanding of the different chemosensitivities for each histological subtype with respect to different drugs has led to a histotype-tailored approach (Scurr, 2011). Among the drugs usually employed as second-line treatments, trabectedin was approved in 2007 by the European Medicines Agency (EMA) for use in patients with advanced STS after the failure of previous chemotherapies, including anthracyclines, or for patients not eligible for this latter treatment. Trabectedin is an anticancer drug; chemically it is a tetrahydroisoquinoline discovered in 1969 and is obtained from *Ecteinascidia turbinata*, a Caribbean Sea ascidian (Trabectedin: Ecteinascidin 743, 2006). This drug has proven to be particularly active in leiomyosarcomas and liposarcomas (especially myxoid liposarcomas), although responses were also obtained in other rarer histotypes (van Kesteren et al., 2003; Allavena et al., 2005; Vincenzi et al., 2010).

Trabectedin exhibits a complex mechanism of action by affecting key processes of cell biology at both the level of tumor cells and the tumor microenvironment. Unlike other alkylating agents that act on the major groove of DNA, trabectedin binds to the minor groove and interferes with DNA repair mechanisms, altering the transcription

regulation of induced genes. This molecule also acts on the tumor microenvironment by modulating pro-tumor inflammatory phenomena through the induction of apoptosis of tumor-infiltrating macrophages (TAM) with associated reduced angiogenesis (D’Incalci and Jimeno, 2003; Germano et al., 2013).

The radiological evaluation of response during trabectedin chemotherapy is of particular interest. Preclinical studies have shown that trabectedin is effective in modulating the transcription of oncogenic fusion proteins, and clinically meaningful results were observed in sarcomas associated with translocations (e.g., myxoid liposarcoma and synovial sarcoma) (Scurr, 2011; Palmerini et al., 2022). Early in clinical development, trabectedin demonstrated relevant antitumor activity against myxoid-round cell liposarcoma (MRC-L-sarcoma). This high activity seems to be related to trabectedin’s ability to counteract the biological activity of the chimeric FUS-DDIT3 oncoprotein, a defining characteristic of this disease (Scurr, 2011; Palmerini et al., 2022). In myxoid liposarcoma, which is a type of sarcoma associated with specific translocation DDIT3-FUS or DDIT3-EWSR1, trabectedin proved to be particularly active, and a change in tumor density has often been observed, associated or not with a subsequent reduction in tumor diameters (Taieb et al., 2015; Baheti et al., 2017). Synovial sarcoma is characterized by the presence of the SS18-SSX fusion gene. Trabectedin can disrupt the transcriptional activity of the SS18-SSX fusion oncoprotein, inhibiting its role in cell proliferation and survival (Fiore et al., 2021).

Consequently, new “functional” imaging techniques have been proposed to assess treatment response that are capable of detecting tissue changes earlier before a change in size, such as MR perfusion (for qualitative-quantitative study vascularization and capillary permeability), MRI diffusion (to more accurately identify changes in cell density by quantifying the mobility of the water molecules present), and PET-TC (for the functional evaluation of the pathological tissues thanks to the variation of cellular metabolism) (Marcus et al., 2009; Baheti et al., 2017; Fanciullo et al., 2022). The Choi and the RECIST criteria are both used to assess tumor response to treatment, but they have different approaches. Choi criteria incorporates size and tumor attenuation (density) changes, while RECIST focuses solely on size changes (Choi et al., 2007; Eisenhauer et al., 2009).

The aim of this single-center retrospective observational cohort study is to evaluate patients with various histotypes of STS treated with trabectedin, comparing the traditional morphological criteria of response (response evaluation criteria in solid Tumors—RECIST) with “functional” criteria (Choi criteria) (Taieb et al., 2015).

2 Patients and methods

Eligible patients were adults (age ≥ 18 years) with various histotypes of STS undergoing treatment with trabectedin after a confirmed local relapse or metastatic disease. Other main inclusion criteria were: Eastern Cooperative Oncology Group performance status (ECOG PS) ≤ 2 ; normal bone marrow, liver and kidney function; availability of CT for the assessments under study; availability of clinical follow-up. The study has been conducted under the principles of “Good Clinical Practice” required by the regulatory authorities and the main European and national regulations. The data, material and documentation related to the study were collected, stored, and processed following the provisions of the relevant legislation/regulations in a manner that guarantees its confidentiality. The study was conducted in accordance with the Declaration of Helsinki and current legislation in this regard and has been approved by the local ethics committee. Written informed consent from the participants was not required following national legislation and institutional requirements.

2.1 Study design and endpoints of the study

This is a single-center retrospective observational cohort study on patients with STS who are undergoing treatment with trabectedin at the Regina Elena National Cancer Institute in Rome (European Reference Network for Rare Adult Solid Cancers—EURACAN—referral center) over the reference time 2015–2020. The aim of this study was to evaluate radiological best response as assessed by CT scan in patients with unselected histotypes of STS treated with trabectedin, comparing the traditional morphological criteria of response (RECIST) with “functional” radiological evaluation criteria (Choi criteria). As per clinical practice, re-evaluation with CT was performed every three courses of treatment or at any time when disease progression was clinically suspected. Response assessment to decide continuation (disease response or stabilization) or discontinuation (disease progression) of trabectedin therapy was performed according to RECIST criteria.

2.2 Statistical analyses

From 2015 to 2020, the data relating to all the patients who meet the envisaged requirements were analyzed and processed. Descriptive statistics were calculated for all variables of interest. Categorical variables were reported through absolute frequencies and relative percentage values, while continuous variables will be reported through medians and ranges. All associations among the categorical variables considered were evaluated by Pearson’s chi-square test or Fisher’s exact test. DFS and OS curves were evaluated by the Kaplan–Meier method and the Mantel–Haenszel log-rank test, which were employed to compare survival between groups. Hazard ratio (HR) and odds ratio (OR) estimates, which allow quantification of the relative

TABLE 1 General demographic and clinical characteristics in treated patients.

Treated pts, n (%)	37	100%
Median age, years (range)	52.5 (32–78)	
Gender, M/F	21/16	
Histological subtypes, n (%)	37	100%
• Liposarcoma	14	38%
• Undifferentiated pleomorphic sarcoma	9	24%
• Leiomyosarcoma	5	14%
• Synovial sarcoma	3	8%
• Other	6	16%
Sarcoma primitive lesion, n (%)	37	100%
• Extremities	23	62%
• Retroperitoneal	8	22%
• Trunk	6	16%
Stage of disease at diagnosis, n (%)	37	100%
• Locally advanced	10	27%
• Metastatic	27	73%
Previous treatments, n (%)		
• Surgery	34	92%
• Radiotherapy	5	14%
• Chemotherapy	32	86%
Median number of previous metastatic systemic treatments, n (range)	3 (1–5)	
Starting dose of trabectedin, n (%)	37	100%
• 1.3 mg m2	12	32%
• 1.5 mg/mq	1	3%
• n.a.	24	65%
Median duration of treatment with trabectedin, months (range)	5.8 (1–60)	
Median number of trabectedin cycles, n (range)	4 (1–60)	
Line of therapy with trabectedin, n (%)	37	100%
• First line	8	22%
• Second line	22	59%
• Subsequent lines	7	19%

Abbreviation: n.a., not applicable. The bold value indicates the total number of patients for each main section.

effect of each predictor on the outcome considered, and the corresponding 95% confidence intervals were calculated using the Cox regression model with proportional hazards and the logistic regression model. A p -value ≤ 0.05 was considered statistically significant.

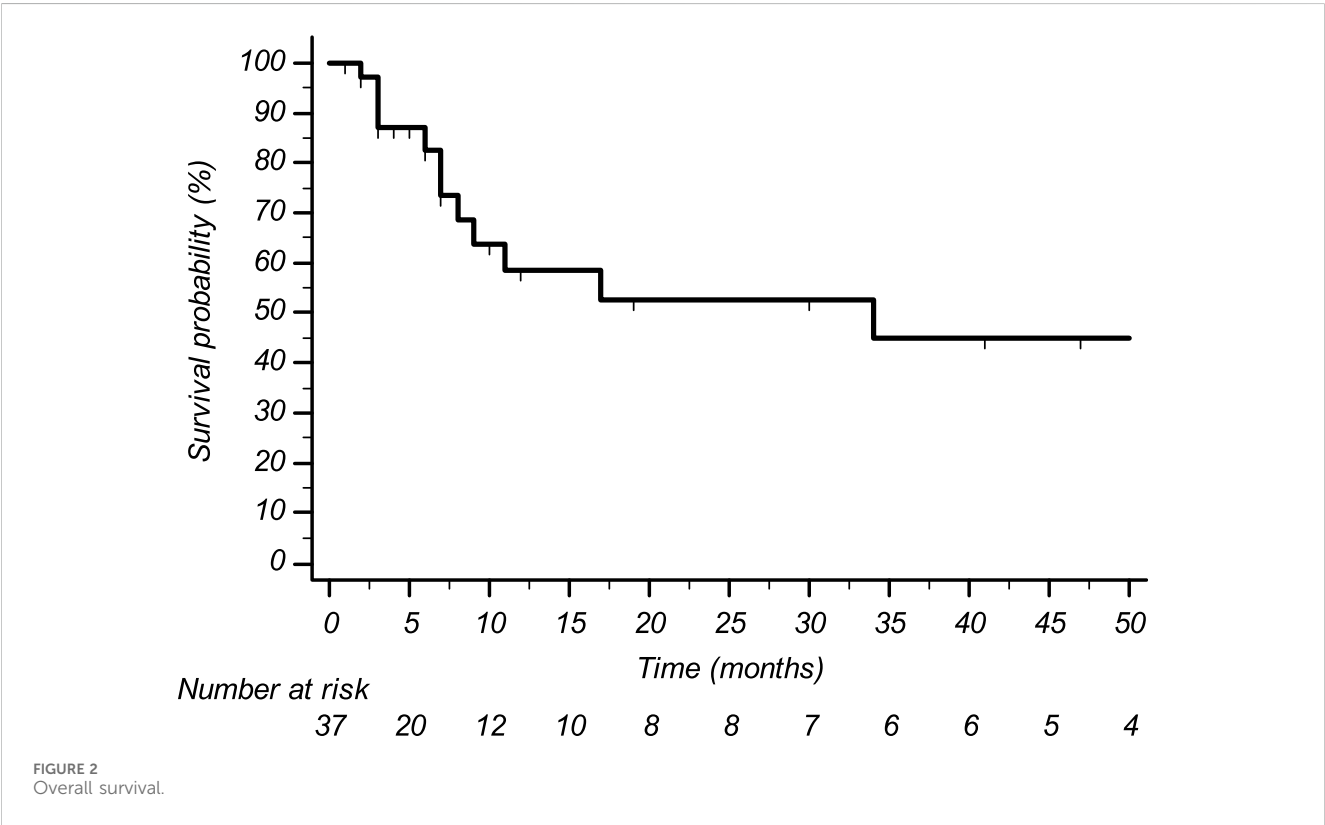
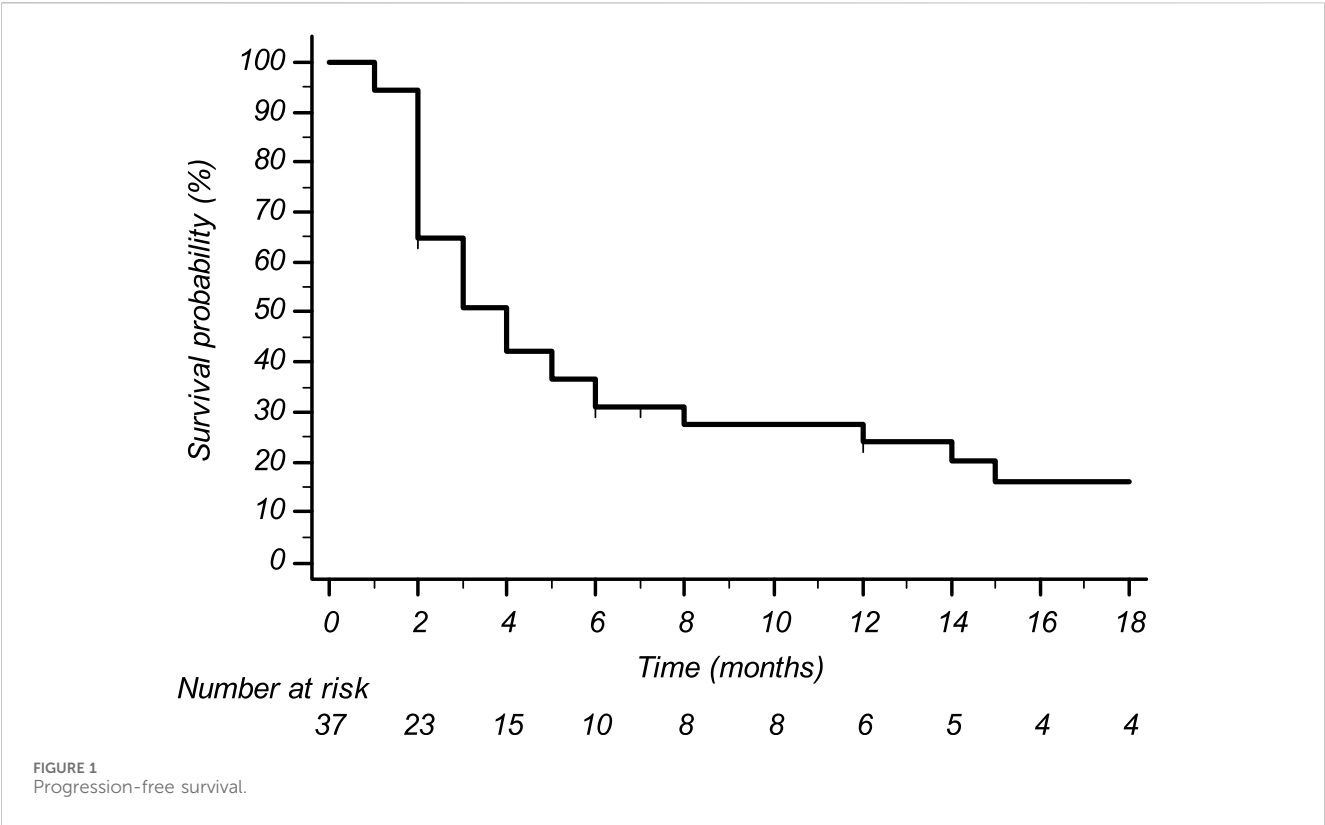


TABLE 2 Best response according to physician evaluation RECIST, Choi N, %.

All treated pts, N = 37	RECIST criteria	Choi criteria
Partial response (PR)	2 (5.4%)	2 (5.4%)
Stable disease (SD)	13 (35.1%)	13 (35.1%)
Progressive disease (PD)	22 (59.5%)	22 (59.5%)

3 Results

We present data collected on 37 patients (pts) who received the diagnosis over 2015–2020, with a median age of 52.5 years (range 32–78) (Table 1).

Histological subtypes of STS were five (13.5%) leiomyosarcoma, 14 (37.8%) liposarcoma, nine (24.3%) undifferentiated pleomorphic sarcoma, three (8.1%) synovial sarcoma, and six (16.2%) other histological subtypes. Eight pts (21.6%) received trabectedin in the first-line setting (five had previously undergone treatment with anthracyclines in the adjuvant or neoadjuvant setting; three had contraindication to anthracyclines due to cardiac comorbidities), 22 pts (59.5%) in the second line (of whom 20 were treated with anthracyclines +/- ifosfamide in the neoadjuvant, adjuvant, or first-line setting, and two were treated with anthracyclines +/- ifosfamide in the neoadjuvant or adjuvant setting and subsequently received gemcitabine-docetaxel), and seven pts (18.9%) in subsequent lines. The median number of administered trabectedin cycles was four (range 2–50) with a median treatment duration of 5.8 months (range 1–60). Median progression-free survival was 3.6 months (CI95% 2.7–4.6) (Figure 1); median overall survival was 34.3 months (CI95% 0–75.4) (Figure 2).

A specialized radiologist performed all the imaging examinations using a dedicated workstation in the same center. The radiological responses were evaluated with both RECIST and, retrospectively, Choi criteria. The best responses obtained according to RECIST criteria were two (5.4%) partial response (PR) represented by a pleomorphic liposarcoma (PLPS) and an undifferentiated pleomorphic sarcoma (UPS), 13 (35.1%) stable disease (SD), and 22 (59.5%) progressive disease (PD). Two (5.4%), 13 (35.1%), and 22 (59.5%) pts obtained PR, SD and PD respectively according to CHOI criteria (Table 2).

In 33 pts (89.2%), the responses assessed according to RECIST and Choi criteria matched, whereas four pts (10.8%) did not match. Two pts were considered in SD according to RECIST 1.1 and PD with Choi criteria; two others with PD according to RECIST 1.1 were classified as SD with Choi criteria (Table 3). In pts 1 and 4, PD according to CHOI criteria was represented by an increase in the vascularized intralesional component, while dimensional stability was observed as per RECIST criteria. In pts 2 and 3, PD is attributed to an increase in the size of the target lesions, while SD was observed according to CHOI criteria due to intralesional remodeling and an increase in tissue density.

Cohen's kappa coefficient of concordance was 0.792 (p -value <0.002). The first pt affected by liposarcoma showed SD according to RECIST criteria and PD according to CHOI criteria. She discontinued trabectedin treatment and is reported as lost to follow-up. Pt 2, diagnosed with leiomyosarcoma, underwent

TABLE 3 Patients with RECIST and Choi criteria dissociated responses.

Patients, N = 4	Histological subtypes	RECIST criteria	Choi criteria
Patient 1	Pleomorphic liposarcoma	SD	PD
Patient 2	Leiomyosarcoma	PD	SD
Patient 3	Alveolar sarcoma	PD	SD
Patient 4	Myxoid liposarcoma	SD	PD

trabectedin treatment in the second line and subsequently, following RECIST-defined disease progression, received three additional lines of therapy with modest benefit (gemcitabine-docetaxel, dacarbazine, and ifosfamide with PD after three, five and two cycles of treatment, respectively). Pt 3, with alveolar sarcoma, initiated trabectedin treatment in the sixth line and maintained disease stability for 21 months. Later, the pt underwent another and final line of treatment with off-label bevacizumab, with rapid disease progression after 3 months. Pt 4 underwent surgery after showing disease stability according to RECIST criteria, followed by a disease-free interval of 2 years. The treatment with trabectedin was overall well-tolerated. The most frequently reported toxicities were neutropenia and transient transaminase increase according to the literature. All pts received steroid pre- and post-medication as per recommended dosage.

4 Discussion

The RECIST 1.1 guidelines (Eisenhauer et al., 2009) represent the system mainly used for the assessment of disease status based on changes in tumor size. In selected cases, such as during treatment with tyrosine kinase inhibitors, different assessment methods could be useful because both changes in volume and density may better represent drug activity instead of classical two dimensional evaluation (Schuetze, 2005). Treatment-related changes in STS, especially assessment of trabectedin response, have been shown to be closely related to altered tumor composition and density; thus therapeutic benefit without tumor shrinkage appears to be relevant in STS (Schuetze, 2005). This novel response pattern was first described by Choi et al. (2007), defining it in the setting of patients with gastrointestinal stromal tumor (GIST) treated with imatinib. They described criteria based on both dimensional and density changes in GIST treated with the TKI imatinib, arguing that RECIST criteria significantly underestimate tumor response. Specifically, variations in tumor mass dimensions may not accurately reflect tumor activity; changes in tumor density represent an additional measure of treatment response, which can be objectively assessed and measured based on radiological images (Choi et al., 2007). In a retrospective study, Taieb et al. (2015) suggested that Choi's criteria can help identify cases of false progression (tumor progression according to RECIST but PR or SD according to CHOI criteria), demonstrating a longer OS in those patients compared to cases where progression is confirmed by both RECIST and Choi criteria. In this patient setting, the correct definition of disease progression is therefore crucial, considering the decisions in therapeutic strategies and

the impact on disease outcomes (Taieb et al., 2015). Dependent on histology and treatment, different changes can be distinguished after therapy such as necrotic cells, granulation tissue, fibrosis, and calcifications (Lucas et al., 2008). Edema and intratumoral hemorrhage may show radiological changes in terms of increase in size, despite an excellent histologic response. In this case, a stability or progression of disease according to RECIST criteria could underestimate a histopathology (Lucas et al., 2008). Different radiological techniques such as MRI, CT, and 18F-FDG PET could detect these changes in order to formulate a more appropriate definition of radiological responses (Gennaro et al., 2021).

In our real life analysis, the overall disease control rate was 40%, consistent with recent retrospective analyses (Palmerini et al., 2021). The lower objective response rate (only 5%) with a median PFS of 3.6 months could be explained by both the small sample size of our patient population and the previous lines of treatment received by about 20% of patients on their third and greater line of systemic therapy. Radiological evaluation with Choi criteria changed the response in four cases. One of the two patients in SD according to Choi criteria had alveolar soft part sarcoma. A Choi criteria evaluation would have allowed the patient to be maintained on therapy, especially considering the limited therapeutic options in this histotype and in a patient with highly pretreated disease. Additionally, alveolar soft part sarcoma has proven to be a histotype that is responsive to trabectedin as per Taieb et al. (2015). The other patient, after disease progression according to RECIST criteria, exhibited a brief response to subsequent treatments (short PFS). Conversely, an assessment of treatment response according to Choi functional criteria would have allowed the patient to continue trabectedin treatment. The first patient was an elderly woman affected by bulky abdominal disease with bone involvement, and she is reported as lost to follow-up; therefore, it is challenging to understand whether the early detection of progression with Choi criteria may have impacted the prognosis. The patient diagnosed with myxoid liposarcoma underwent surgery after disease stability according to RECIST criteria and experienced a recurrence of the disease 2 years after the trabectedin treatment. It is plausible that the limited progression detected by Choi criteria could have provided a positive impact on the prognosis of this pt in the absence of further systemic treatments.

The differences between the two assessment strategies did not show statistical significance, as Cohen's coefficient of agreement kappa was 0.792 (p -value <0.002). Use of the standard evaluation executed with both RECIST and Choi criteria remains a challenge as it requires a specialist radiologist for this patient setting. Indeed, it is internationally recognized that the rarity of sarcomas and the variety of histotypes imply that a multidisciplinary approach, including a radiological evaluation of the response by a dedicated and experienced radiologist, in a referral center remains the most effective way to impact the prognosis of these malignancies (Gronchi et al., 2021).

This study represents a first analysis, and secondary objectives are underway for identifying, in patients affected by STS treated with trabectedin, predictive or prognostic parameters according to objective response, progression-free survival (PFS), and overall survival (OS). There are some limitations due to the limited number of cases and, consequently, less power in the comparison

of radiological tumor response assessments. Furthermore, the study had a retrospective design. A larger cohort and a prospective multicenter study would be necessary to achieve more consistent results.

5 Conclusion

A functional assessment combined with changes in tumor size is crucial in patients with advanced STS treated with trabectedin in order to prevent an early treatment interruption that may deprive the patient of a therapeutic option. In this first analysis, the concordance between RECIST and Choi assessments demonstrates no statistically significant difference. Responses did not match for four patients. The goal is to define a consistent and unbiased evaluation of the efficacy of both local and systemic therapies through imaging to find more personalized therapeutic approaches.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Regina Elena Ethical committee. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

SC: Writing—original draft. CO: Writing—review and editing. SV: Writing—review and editing. AT: Data curation, Formal Analysis, Writing—review and editing. AC: Data curation, Formal Analysis, Writing—review and editing. FR: Investigation, Methodology, Writing—review and editing. MM: Investigation, Methodology, Writing—review and editing. MC: Investigation, Methodology, Writing—review and editing. GB: Investigation, Methodology, Validation, Writing—review and editing. MR: Investigation, Methodology, Validation, Writing—review and editing. VA: Investigation, Methodology, Validation, Writing—review and editing. IS: Data curation, Formal Analysis, Writing—review and editing. RB: Project administration, Supervision, Writing—review and editing. VF: Project administration, Supervision, Writing—review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Immunotherapy for lung adenocarcinoma patients with bone metastases: who really needs it

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Background: Lung adenocarcinoma patients are often found to have developed bone metastases at the time of initial diagnosis. With the continuous development of technology, we have successfully entered the era of immunotherapy. This study aimed to determine the efficacy of immunotherapy in lung adenocarcinoma patients with bone metastases (LABM) through a multicenter retrospective analysis and to develop a novel tool to identify the population that could benefit most from immunotherapy.

Methods: To assess the impact of immunotherapy on LABM in terms of overall survival, we used analytical tools such as Kaplan-Meier analysis, Log-ranch test, and propensity score matching (PSM) method. A predictive model for constructing overall survival was constructed using Cox regression modeling. Based on this, we developed a risk classification system depicting Kaplan-Meier curves for subgroup analysis to determine the optimal beneficiary population for immunotherapy in different risk subgroups.

Results: A total of 20073 eligible patients were enrolled in this study, of whom 8010 did not receive immunotherapy, while 12063 patients received immunotherapy. After 1:1 PSM, 15848 patients were successfully coordinated, yielding a balanced cohort. Kaplan-Meier survival curves showed significantly enhanced overall survival ($P < 0.001$) in patients who received immunotherapy compared to those who did not. The results of Cox regression analyses showed that age, race, sex, primary site, immunotherapy, surgery, chemotherapy, brain metastasis, liver metastasis, lung metastasis, and marital status were independent prognostic factors. The area under the curve for all three cohorts was close to 0.7, indicating that the model was well-discriminating. The calibration curves further proved that the model had a high predictive accuracy. Decision curve analysis demonstrated that the model could achieve a high net clinical benefit. The risk classification system developed based on the model successfully screened the best beneficiary population for immunotherapy.

Conclusion: This study provides convincing evidence that immunotherapy provides a significant survival advantage for LABM. Secondly, the clinical tools constructed in this study can help clinicians identify the optimal population to benefit from immunotherapy in LABM, thus enabling precise treatment and avoiding the waste of medical resources and over-treatment of patients.

KEYWORDS

immunotherapy, lung cancer, bone metastases, SEER, prognosis

Introduction

Lung cancer is the most common malignant tumor in the world, with approximately 1.8 million new cases of lung cancer diagnosed worldwide, including 1.6 million deaths (1). Over 85% of lung cancer patients are diagnosed with non-small cell lung cancer, with lung adenocarcinoma being the most common histologic type (2). Early symptoms of lung cancer are atypical, which makes early diagnosis particularly difficult. When patients develop more obvious clinical symptoms such as hemoptysis, chest pain, and chest tightness, the disease may have progressed to an advanced stage, often accompanied by distant metastases (3). Among them, bone is the most common site of metastasis in lung cancer patients, and about 30%-40% of patients have bone metastasis (4). Bone metastasis not only has a significant impact on the quality of life of patients but also significantly reduces the survival time of patients. Among patients with bone metastasis of lung cancer, 50.3% are adenocarcinoma, and the most common sites of bone metastasis are the spine and trunk bone (5). Therefore, we may need to pay more attention to lung adenocarcinoma patients with bone metastases (LABM).

Surgery is considered an effective treatment for early-stage lung cancer, but it is usually not considered the preferred treatment option for patients with advanced lung cancer. This is because even if surgery is chosen, postoperative recurrence and distant metastasis remain unavoidable (6). Since the main treatment goals of these patients are to relieve pain, improve their quality of life, and prolong survival, systemic therapy is usually chosen. In this regard, the role of immunotherapy in lung cancer patients has received increasing attention, especially with the significant advances in the use of immune checkpoint inhibitors (7). Immunotherapy works by enhancing or modifying the patient's immune system so that it can recognize and attack cancer cells (8). Compared to traditional treatments (e.g., radiotherapy, chemotherapy), immunotherapy is more specific in its targeting, providing long-term control and fewer side effects (9). In lung cancer patients with bone

metastases, immunotherapy may work by modulating the tumor microenvironment and activating immune cells (10). Previous studies have shown that immunotherapy significantly prolongs progression-free survival and overall survival (OS) in patients with non-small cell lung cancer, bringing hope to patients with advanced lung cancer (11–13). In 2015, immunotherapy was officially approved as a standard treatment option for patients with advanced non-small cell lung cancer (11). Since bones have a unique immune microenvironment different from other organs, the efficacy of immunotherapy may be compromised for lung cancer patients with bone metastases (14). Currently, few studies have been conducted to report the efficacy of immunotherapy in LABM, and most of them are studies with limited sample sizes. Therefore, the exact mechanism and efficacy of immunotherapy in LABM need to be further investigated.

Although immunotherapy has been reported to provide survival benefits for lung cancer patients with bone metastases, it is undeniable that not all lung cancer patients with bone metastases benefit from it. Therefore, this study aimed to retrospectively analyze data from LABM from a multicenter medical institution to verify the efficacy of immunotherapy with LABM. At the same time, a practical mortality risk classification system was developed on this basis, which was further validated with patient data from external medical institutions. By using the mortality risk classification system, we can identify the largest beneficiary population of immunotherapy among LABM, which provides the basis for personalized and precise treatment for patients.

Methods

Patient cohort

Data for this study were obtained in part from the Surveillance, Epidemiology, and End Results (SEER) database, which encompasses cancer-related demographic data from 17 U.S. cancer registries covering approximately 30% of the U.S. population. The database provides comprehensive information on patient demographics, tumor characteristics, diagnosis, initial treatment regimen, and vital status updates. The SEER database strictly maintains patient confidentiality and does not disclose

Abbreviations: LABM, Lung adenocarcinoma patients with bone metastases; SEER, Surveillance, Epidemiology, and End Results; OS, Overall survival; ROC, Receiver operating characteristic curves; AUC, Area under the curves.

personally identifiable information. Therefore, relevant analyses of SEER data are not subject to medical ethical review or the need to obtain informed consent from participants. The study also collected clinicopathologic data from external medical institutions (Wenzhou, China). In 2015, immunotherapy-related drugs were officially approved for the treatment of patients with advanced lung cancer. This approval not only means the resolution of the treatment stalemate for advanced lung cancer patients but also heralds the official entry of lung cancer treatment into the immunization era. To assess the effectiveness of immunotherapy, we specifically targeted patients diagnosed with bone metastases from lung adenocarcinoma in 2015–2020, consistent with the approval of immunotherapy as a primary treatment modality in 2015. In contrast, patients with bone metastases from lung adenocarcinoma diagnosed between 2010–2015 were the comparison cohort for the study. Inclusion criteria were as follows: 1. Lung cancer was the only primary tumor; 2. Lung adenocarcinoma was diagnosed by histological examination. Exclusion criteria were: 1. concomitant multiple primary tumors; 2. incomplete information on relevant tumor characteristics; and 3. incomplete information on treatment and follow-up. Variables such as age, race, sex, primary tumor site, laterality, surgery (no or yes), radiotherapy (no or yes), chemotherapy (no or yes), immunotherapy (no or yes), lung metastasis (no or yes), liver metastasis (no or yes), brain metastasis (no or yes), and marital status were included in this study. The histological type was categorized into adenocarcinoma, squamous cell carcinoma, small cell lung cancer, and others based on pathologic findings. The optimal age threshold under OS was determined to be 74 using X-tile software, so age was categorized as <74 years and ≥74 years. The primary endpoint in this study was OS, which was defined as the date from diagnosis to death or last follow-up.

Statistical analysis

Selection bias inevitably permeated this study due to inconsistencies in the baseline characteristics of patients in the group receiving immunotherapy and those in the group not receiving immunotherapy. To reduce the impact of differences in baseline characteristics on OS, this study used a 1:1 propensity score matching (PSM) method, setting a caliper width of 0.01 to harmonize between patients in the group receiving immunotherapy and those in the group not receiving immunotherapy. Subsequently, Kaplan-Meier curve analysis and log-rank test were performed to measure the effect of immunotherapy on OS of LABM. Data from the total SEER cohort were randomly divided into a training cohort and an internal validation cohort in a 7:3 ratio using R software. The training cohort was used to develop the model, the internal validation cohort was used for internal validation of the model, and the collected external validation cohort performed external validation. Univariate and multivariate analyses were performed in the training cohort using Cox regression risk models. Variables significantly associated with survival in the univariate analysis were then included in the multivariate Cox analysis to exclude

confounding effects between variables. Clinical predictive models based on independent prognostic factors were constructed using R software and validated and evaluated in three cohorts. The area under the receiver operating characteristic curve was used to assess the discriminatory nature of the model, and the calibration curve was used to assess the predictive accuracy of the model. Decision curves were used to assess the predictive performance and clinical benefits of the models. A risk classification system was developed based on the clinical prediction model to successfully differentiate LABM at high, middle, and low risk of death. To determine the maximum beneficiary population of immunotherapy in each death risk subgroup, the study further conducted a subgroup analysis of each death risk group using Kaplan-Meier curve analysis and log-rank test. All statistical analyses in this study were performed using R software (version 4.3.3), where $p < 0.05$ was considered statistically significant.

Results

Demographic and clinicopathologic features

In this study, we performed a comprehensive analysis of the baseline demographic and clinical characteristics of the cohort involved (Table 1). A total of 14051 individuals were included in the training cohort, and there were no missing age or sex data in this group. Similarly, the internal validation cohort included 6022 subjects and showed no missing values for these demographics. Age distribution within the cohort showed no significant difference between the training cohort (73.5% <74 years and 26.5% ≥74 years) and the internal validation cohort (73.0% <74 years and 27.0% ≥74 years), with a P-value of 0.522. In terms of racial composition, the difference between the two cohorts was nonsignificant, with a majority of the cohort in both groups being white. Gender distribution analysis showed a similar pattern, with no significant differences found (P-value = 0.859). The primary site of the tumor did not differ between the two groups, with the majority of cases located in the upper lobes of the lungs (59.5% in the training cohort and 60.2% in the internal validation cohort). There were no statistically significant differences between groups for laterality, immunotherapy, surgery, radiotherapy, chemotherapy, brain metastasis, liver metastasis, lung metastasis, and marital status, with p-values ranging from 0.076 to 0.974. Together, these findings demonstrate a high degree of concordance between the training cohort and internal validation cohort in terms of demographic and clinical characteristics, providing a solid foundation for subsequent predictive modeling studies.

Selection of study cohort and propensity score matching

To reduce the effect of confounding variables, a 1:1 PSM strategy was used to produce a final matched cohort consisting of 12063 cases in the 'received immunotherapy' group and 8010 cases

TABLE 1 Demographic and clinicopathological characteristics of training cohort and internal validation cohort.

Characteristic	Cohort		P-value
	Training Cohort N = 14051	Internal validation Cohort N = 6022	
Age			0.522
<74	10323 (73.5%)	4398 (73.0%)	
≥74	3728 (26.5%)	1624 (27.0%)	
Race			0.091
Black	1468 (10.4%)	569 (9.4%)	
White	10629 (75.6%)	4593 (76.3%)	
Others	1954 (13.9%)	860 (14.3%)	
Sex			0.859
Male	7250 (51.6%)	3099 (51.5%)	
Female	6801 (48.4%)	2923 (48.5%)	
Primary site			0.487
Main bronchus	548 (3.9%)	237 (3.9%)	
Upper lobe	8354 (59.5%)	3627 (60.2%)	
Middle lobe	675 (4.8%)	304 (5.0%)	
Lower lobe	4474 (31.8%)	1854 (30.8%)	
Laterality			0.974
Left	6028 (42.9%)	2585 (42.9%)	
Right	8023 (57.1%)	3437 (57.1%)	
Immunotherapy			0.530
No	5587 (39.8%)	2423 (40.2%)	
Yes	8464 (60.2%)	3599 (59.8%)	
Surgery			0.558
No	13789 (98.1%)	5917 (98.3%)	
Yes	262 (1.9%)	105 (1.7%)	
Radiotherapy			0.076
No	5705 (40.6%)	2526 (41.9%)	
Yes	8346 (59.4%)	3496 (58.1%)	
Chemotherapy			0.526
No	4944 (35.2%)	2147 (35.7%)	
Yes	9107 (64.8%)	3875 (64.3%)	
Brain metastasis			0.845
No	9799 (69.7%)	4208 (69.9%)	
Yes	4252 (30.3%)	1814 (30.1%)	
Liver metastasis			0.164
No	10816 (77.0%)	4581 (76.1%)	
Yes	3235 (23.0%)	1441 (23.9%)	

(Continued)

TABLE 1 Continued

Characteristic	Cohort		P-value
	Training Cohort N = 14051	Internal validation Cohort N = 6022	
Lung metastasis			0.278
No	10090 (71.8%)	4279 (71.1%)	
Yes	3,961 (28.2%)	1743 (28.9%)	
Marital status			0.017
No	5804 (41.3%)	2597 (43.1%)	
Yes	8247 (58.7%)	3425 (56.9%)	

in the ‘did not receive immunotherapy’ group. Following this matching process, the two cohorts exhibited a high degree of concordance in baseline characteristics, as shown in [Table 2](#). This improved balance makes comparisons of treatment effects more reliable because the potential effects of confounders are minimized. The observed increase in p-values for previously significant covariates demonstrates the effectiveness of our matching technique in addressing potential confounders. This enhances the credibility of our findings and applies them to real clinical practice.

Immunotherapy and survival outcomes

Kaplan-Meier curves of the post-PSM cohort showed a significant improvement in OS in LABM who received immunotherapy compared to those who did not ([Figure 1](#), $P < 0.05$). These results suggest that immunotherapy can significantly improve the prognosis of LABM.

Construction and validation of the model

Univariate Cox regression analysis revealed that age, race, sex, primary tumor site, surgery, chemotherapy, immunotherapy, lung metastasis, liver metastasis, brain metastasis, and marital status were significantly associated with the prognosis of LABM ($P < 0.05$, [Table 3](#)). To remove the confounding effects among variables, the above-screened risk factors were further analyzed by multivariate Cox regression analysis. Multivariate Cox regression analysis showed that age, race, sex, primary tumor site, surgery, chemotherapy, immunotherapy, lung metastasis, liver metastasis, brain metastasis, and marital status were independent prognostic factors in LABM ($P < 0.05$, [Table 3](#)). We developed a clinical prediction model based on the screened independent prognostic factors to achieve accurate prediction of 1-, 2-, and 3-year OS in LABM. Each variable of a patient corresponds to a score value, and the corresponding score values are summed to obtain a total score, which gives the patient’s survival probability at 1, 2, and 3 years ([Figure 2](#)). The performance of the clinical prediction model was validated in both the training cohort and the internal validation

cohort as well as the external validation cohort. According to the ROC curve analysis, the time-dependent AUC values for 1, 2, and 3 years were 0.736, 0.720, and 0.719 for the training cohort, 0.733, 0.719, and 0.715 for the internal validation cohort, and 0.696, 0.683, and 0.677 for the external validation cohort, respectively (Figure 3). These results confirm that the model has good discriminatory power. The calibration curves for survival probability showed that the model had the best correlation between predictions and observations of OS in all three cohorts, further confirming that the model developed in this study reliably predicted patient survival (Figure 4). Decision curve analysis, on the other hand, demonstrated the significant positive net benefit of the model over a wide range of mortality risks, further proving the high clinical utility of the model for LABM (Figure 5).

TABLE 2 Baseline characteristics of patients before and after propensity score matching.

Characteristics	Unmatched			Matched		
	Yes, N = 12063	No, N = 8010	p-value	Yes, N = 7924	No, N = 7924	p-value
Age			<0.05			0.73
<74	8728 (72%)	5993 (75%)		5942 (75%)	5923 (75%)	
≥74	3335 (28%)	2017 (25%)		1982 (25%)	2001 (25%)	
Race			<0.05			0.51
Black	1221 (10%)	816 (10%)		770 (10%)	807 (10%)	
White	9018 (75%)	6,204 (77%)		6194 (78%)	6137 (77%)	
Others	1824 (15%)	990 (12%)		960 (12%)	980 (12%)	
Sex			<0.05			0.83
Male	6113 (51%)	4236 (53%)		4192 (53%)	4179 (53%)	
Female	5950 (49%)	3774 (47%)		3732 (47%)	3745 (47%)	
Primary site			<0.05			0.24
Main bronchus	455 (4%)	330 (4%)		280 (4%)	322 (4%)	
Upper lobe	7168 (59%)	4813 (60%)		4828 (61%)	4775 (60%)	
Middle lobe	558 (5%)	421 (5%)		372 (5%)	399 (5%)	
Lower lobe	3882 (32%)	2446 (31%)		2444 (31%)	2428 (31%)	
Laterality			0.560			0.48
Left	5156 (43%)	3457 (43%)		3376 (43%)	3420 (43%)	
Right	6907 (57%)	4553 (57%)		4548 (57%)	4504 (57%)	
Surgery			<0.05			0.65
No	11866 (98%)	7840 (98%)		7805 (98%)	7798 (98%)	
Yes	197 (2%)	170 (2%)		119 (2%)	126 (2%)	
Radiotherapy			<0.05			0.60
No	5039 (42%)	3192 (40%)		3131 (40%)	3163 (40%)	
Yes	7024 (58%)	4818 (60%)		4793 (60%)	4761 (60%)	
Chemotherapy			<0.05			0.86
No	4377 (36%)	2714 (34%)		2676 (34%)	2686 (34%)	
Yes	7686 (64%)	5296 (66%)		5248 (66%)	5238 (66%)	
Brain metastasis			<0.05			0.88
No	8232 (68%)	5775 (72%)		5692 (72%)	5700 (72%)	
Yes	3831 (32%)	2235 (28%)		2232 (28%)	2224 (28%)	
Liver metastasis			<0.05			0.70
No	9187 (76%)	6210 (78%)		6165 (78%)	6145 (78%)	

(Continued)

TABLE 2 Continued

Characteristics	Unmatched			Matched		
	Yes, N = 12063	No, N = 8010	p-value	Yes, N = 7924	No, N = 7924	p-value
Yes	2876 (24%)	1800 (22%)		1759 (22%)	1779 (22%)	
Lung metastasis			0.526			0.47
No	8655 (72%)	5714 (71%)		5715 (72%)	5674 (72%)	
Yes	3408 (28%)	2296 (29%)		2209 (28%)	2250 (28%)	
Marital status			0.250			0.82
No	5088 (42%)	3313 (41%)		3265 (41%)	3279 (41%)	
Yes	6975 (58%)	4697 (59%)		4659 (59%)	4645 (59%)	

Risk classification system

Based on the developed predictive model to calculate the total score for each patient in the training cohort, the optimal cut-off values of 724 and 770 were determined using X-tiles software. Thus,

all patients were categorized into low-risk risk group (scores < 724), middle-risk group (724–770), and high-risk risk group (scores > 770). Kaplan-Meier survival curves for each risk subgroup were further plotted and log-rank tests were performed. The results, as shown in Figure 6, showed that there was a significant difference in

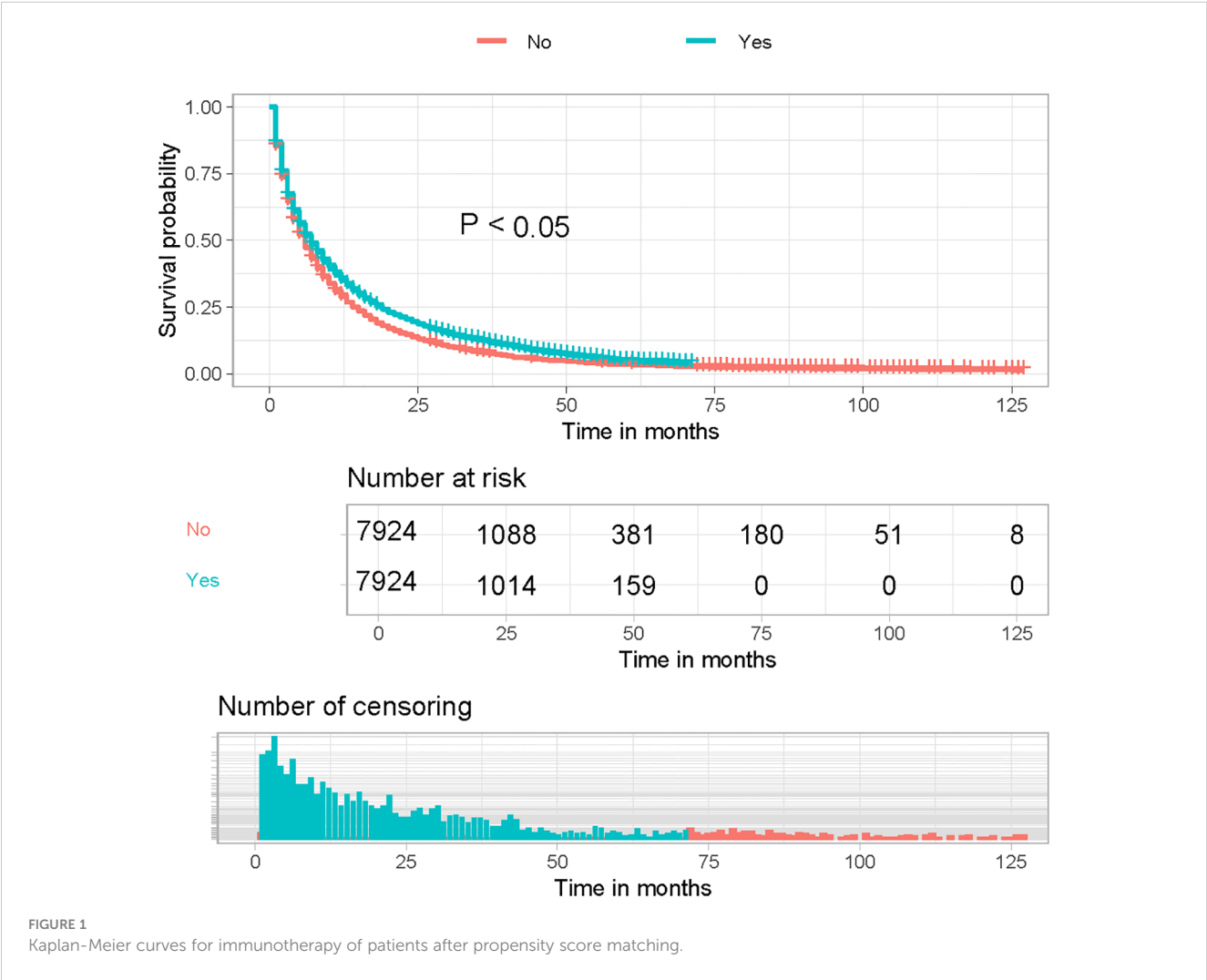


TABLE 3 Analysis of univariate and multivariate Cox regression in patients.

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age				
<74	Reference		Reference	
≥74	1.41 (1.35-1.46)	<0.05	1.25 (1.20-1.30)	<0.05
Sex				
Male	Reference		Reference	
Female	0.80 (0.77-0.83)	<0.05	0.78 (0.75-0.81)	<0.05
Race				
Black	Reference		Reference	
White	0.91 (0.86-0.96)	<0.05	0.94 (0.88-1.00)	<0.05
Others	0.59 (0.55-0.64)	<0.05	0.64 (0.59-0.69)	<0.05
Laterality				
Left	Reference			
Right	1.03 (1.00-1.07)	0.09		
Primary site				
Main bronchus	Reference		Reference	
Upper lobe	0.86 (0.79-0.95)	<0.05	0.90 (0.82-0.99)	<0.05
Middle lobe	0.82 (0.72-0.92)	<0.05	0.84 (0.74-0.95)	<0.05
Lower lobe	0.85 (0.77-0.94)	<0.05	0.91 (0.82-1.00)	<0.05
Immunotherapy				
No	Reference		Reference	
Yes	0.80 (0.77-0.83)	<0.05	0.76 (0.73-0.79)	<0.05
Radiotherapy				
No	Reference			
Yes	1.00 (0.97-1.04)	0.82		
Chemotherapy				
No	Reference		Reference	
Yes	0.38 (0.37-0.40)	<0.05	0.39 (0.38-0.41)	<0.05
Surgery				
No	Reference		Reference	
Yes	0.60 (0.53-0.69)	<0.05	0.58 (0.51-0.67)	<0.05
Brain metastasis				
No	Reference		Reference	
Yes	1.04 (1.00-1.08)	<0.05	1.15 (1.11-1.20)	<0.05
Liver metastasis				
No	Reference		Reference	
Yes	1.36 (1.31-1.42)	<0.05	1.43 (1.37-1.49)	<0.05

(Continued)

TABLE 3 Continued

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Lung metastasis				
No	Reference		Reference	
Yes	1.11 (1.06-1.15)	<0.05	1.09 (1.05-1.14)	<0.05
Marital status				
Unmarried	Reference		Reference	
Married	0.82 (0.79-0.85)	<0.05	0.90 (0.87-0.93)	<0.05

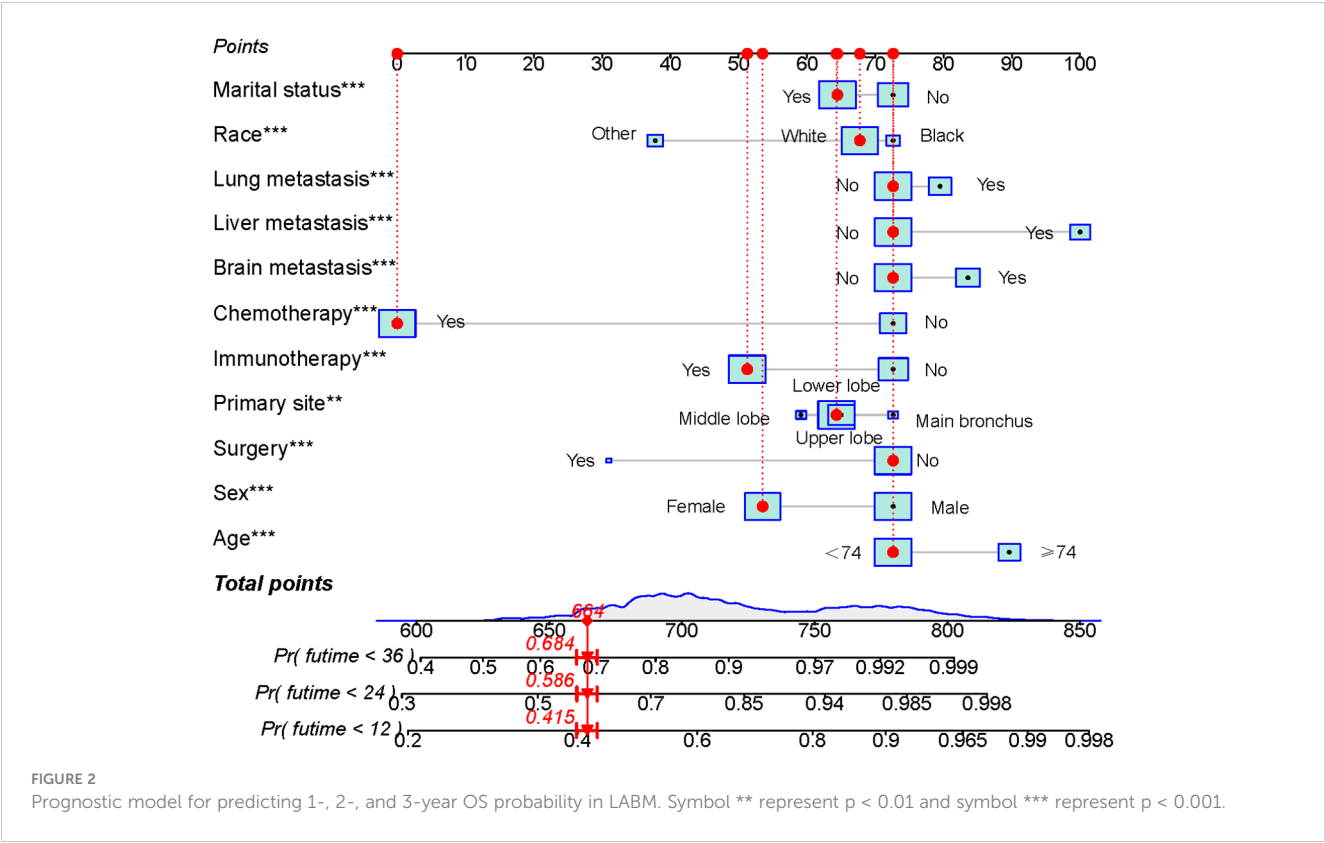
the prognosis of patients in different risk groups in the training and internal validation cohorts ($p<0.05$). The above results indicate that the risk classification system derived from the clinical prediction model has a high prognostic predictive value and can further distinguish the population with a better prognosis.

Determining the optimal beneficiary population for immunotherapy based on the risk classification system

The correlation of immunotherapy was analyzed by depicting Kaplan-Meier survival curves and performing log-rank tests for different risk subgroups differentiated by a risk classification system. As shown in [Figure 7](#), the OS of patients who received immunotherapy in the high-risk and middle-risk groups was not significantly different from that of patients who did not receive immunotherapy. In contrast, the OS of patients who received immunotherapy in the low-risk risk group was significantly better than that of patients who did not receive immunotherapy ($P < 0.05$). Therefore, we prefer to recommend immunotherapy to LABM who are distinguished as low-risk by the risk classification system because they can benefit most from immunotherapy.

Discussion

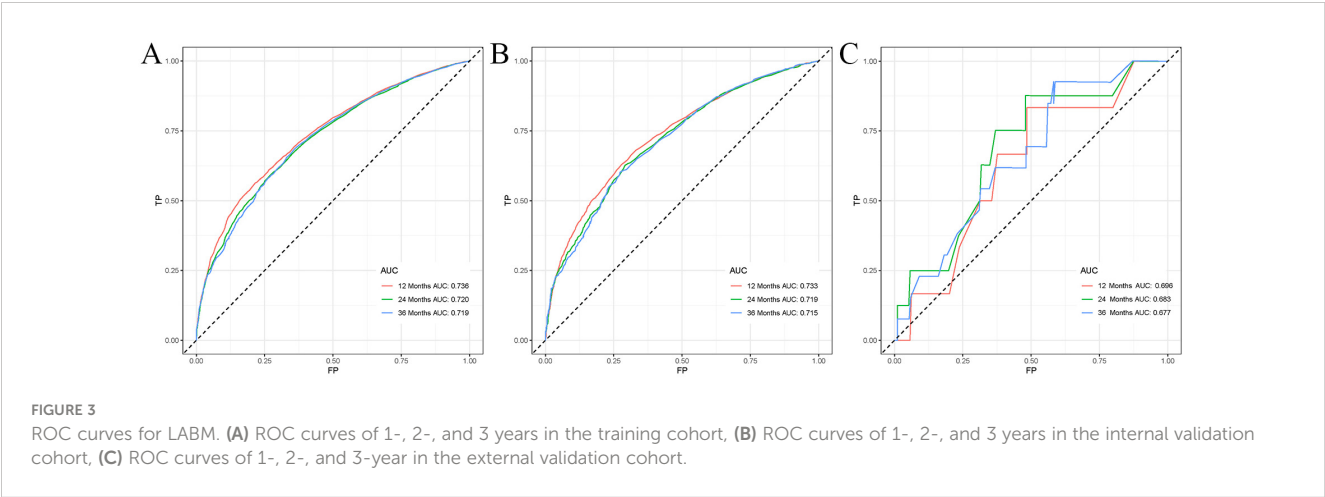
The treatment of lung cancer has fundamentally changed over the past two decades, especially with the intensive development of molecular pathology of lung cancer and the rise of immunotherapy. Pembrolizumab achieved a 5-year OS rate of 31.9% in the prestigious Keynote-024 trial, which was approved by the FDA as an effective first-line therapeutic option for patients with non-small cell lung cancer ([12](#)). However, it is important to recognize that not all patients will benefit from immunotherapy. Considering the poor prognosis of LABM, the therapeutic efficacy of immunotherapy in these patients is unknown, and there are fewer previous related reports. Therefore, this study retrospectively analyzed the data from the multicenter SEER database, firstly verified the significant prognostic improvement of immunotherapy in LABM by PSM,



and then constructed a model for predicting the survival of LABM. The performance of the model was also externally validated by collecting data from relevant patients from external medical institutions who met the inclusion-exclusion criteria. Finally, a risk classification system was constructed based on the predictive model, by which we successfully screened the population that could benefit most from immunotherapy among LABM. This provides strong evidence for rational allocation of medical resources and personalized and precise treatment.

Our study demonstrated that LABM who received immunotherapy had significantly longer OS than those who did not receive immunotherapy, similar to previous studies. However, the current study included a larger number of patients compared to

the previous study, and the patients were from multiple central medical institutions. Therefore, the results of this study are more compelling and representative. In this study, female patients were predictors of good prognosis, which is similar to previously reported results. Smoking rates are lower in women than in men, and therefore nonsmoking-related lung cancers (e.g., lung adenocarcinoma) are more prevalent in women (15). These types of lung cancers usually have milder biological behavior and are more sensitive to specific treatments (16). In addition, it has been proposed that sex hormones, particularly estrogen, may influence the biological behavior and response to treatment in lung cancer. Estrogen receptors are expressed in certain lung adenocarcinoma cells, which may influence how tumors grow and spread (17).



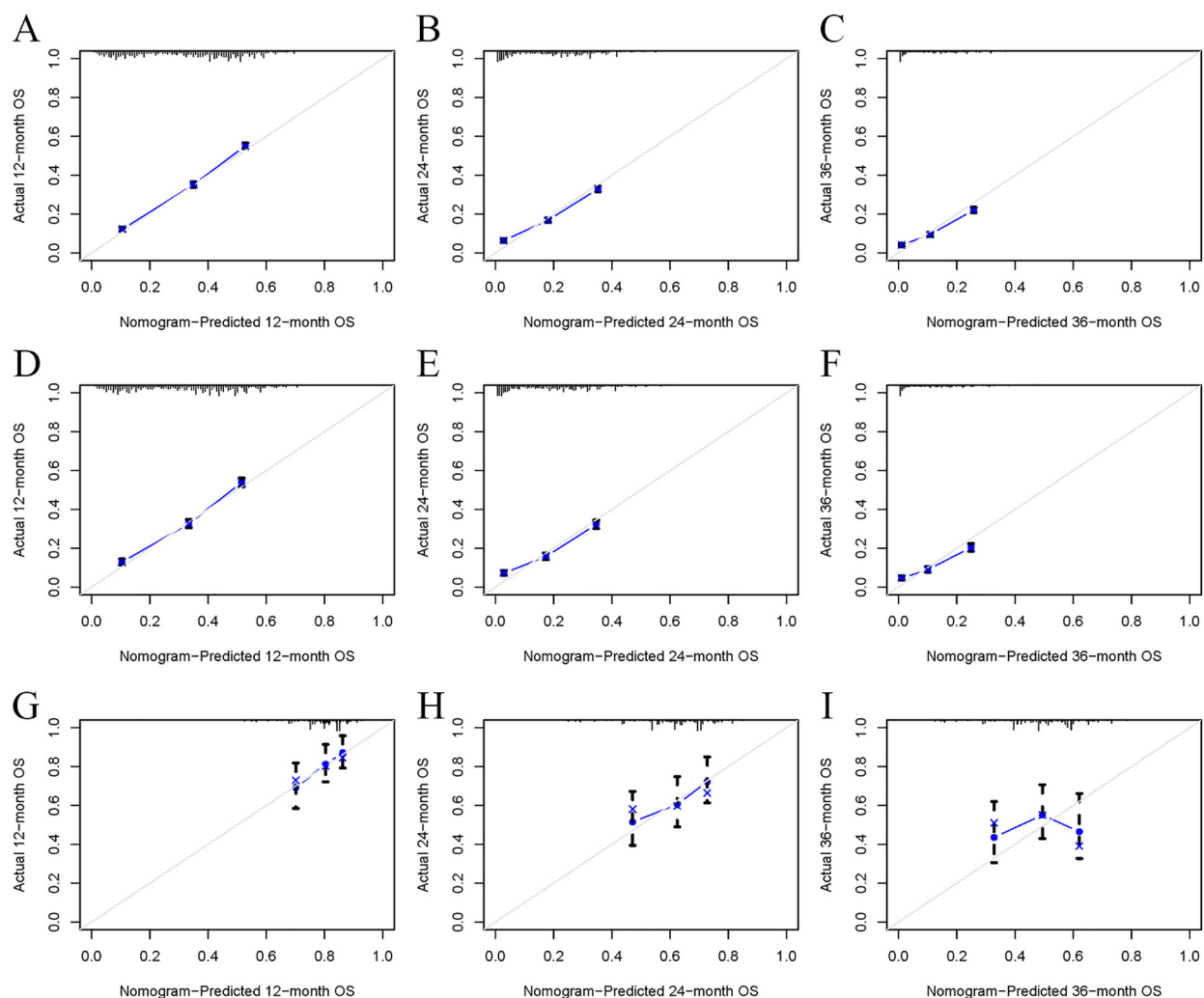


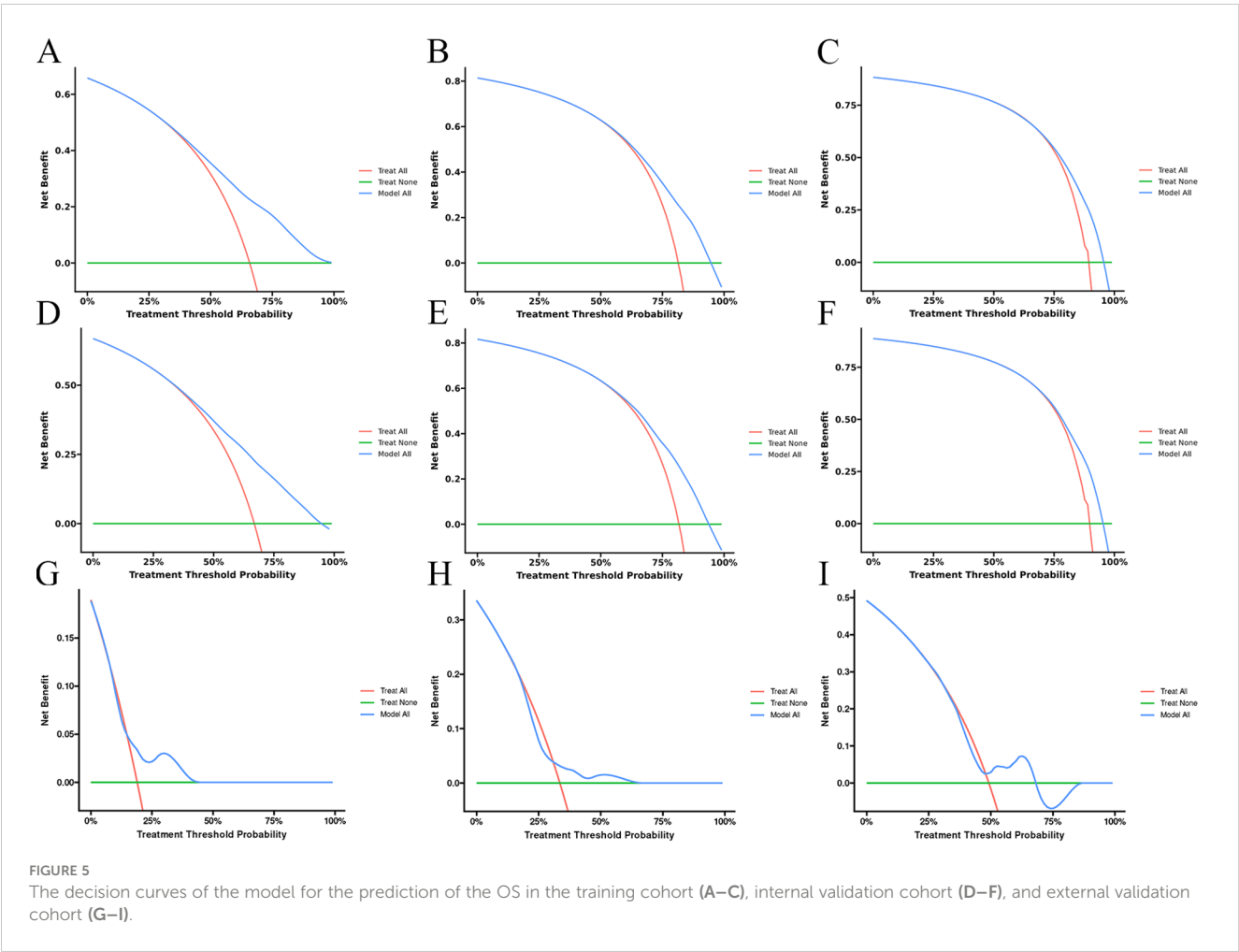
FIGURE 4

The calibration curves of the model for the prediction of the OS of patients in the training cohort (A–C), internal validation cohort (D–F), and external validation cohort (G–I).

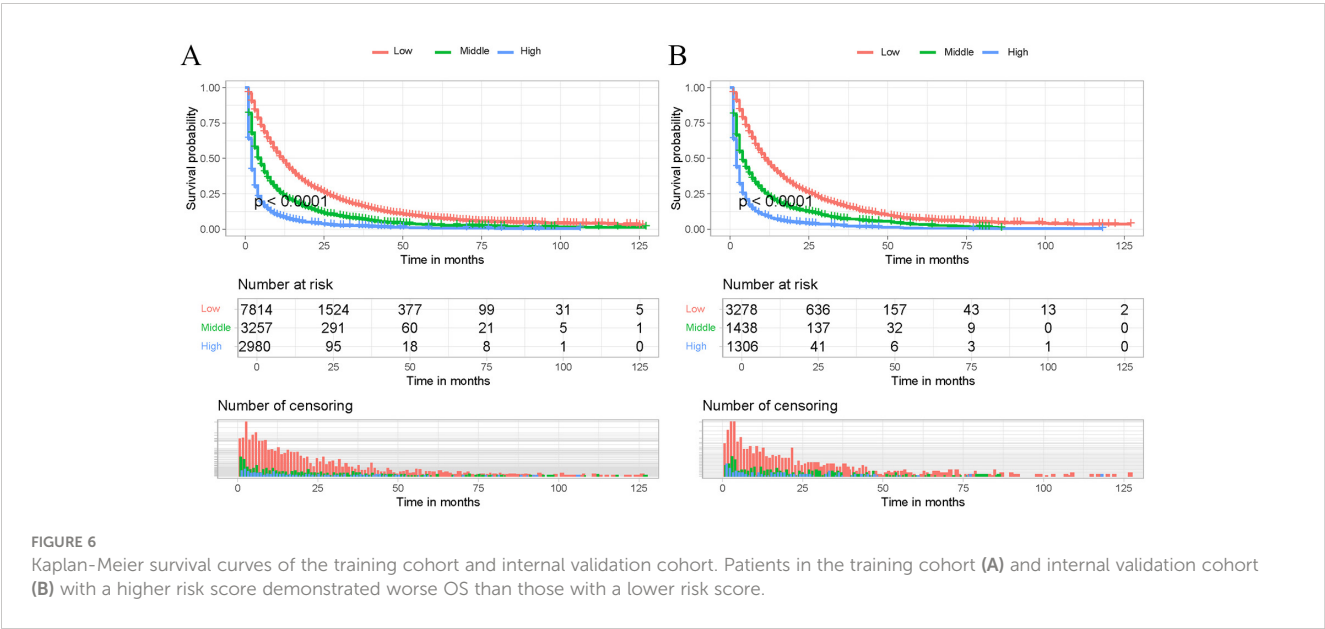
Gender has been reported to affect innate and acquired immune responses, as well as the expression and function of PD-L1 and PD-1 (18). Some studies suggest that women may be more resistant to immunotherapy because their tumor immunogenic response is weaker than men's (19). Some studies report that immunotherapy is more effective for men than women in non-small cell lung cancer (20). Bone metastases play a key role in modulating the immune response and influencing the response to immunotherapy (21, 22). Thus, among LABM female patients may have a better response to immunotherapy and thus have a better prognosis compared to male patients.

Bone metastases have traditionally been treated without cure, with patients receiving multidisciplinary treatment based on systemic therapy and optimal local therapy, including radiotherapy, targeted therapy, immunotherapy, and surgery (23). In these treatments, instead of targeting the primary site, surgery removes isolated bone metastases. The aim is to prevent and treat pathologic fractures and to reduce bone pain and spinal cord compression to improve the patient's quality of life (24). However, with advances in surgical techniques and

multidisciplinary approaches to care, adverse events associated with surgical death have decreased, and resection of the primary tumor has been reconsidered as part of the treatment of advanced lung adenocarcinoma (25). In recent years, several studies have shown that resection of the primary tumor improves the prognosis of stage IV non-small cell lung cancer (26, 27). Although there are no guidelines for re-recommending this therapy for patients with bone metastases from advanced non-small cell lung cancer, some evidence supports surgical treatment for them (28). This study suggests that surgery is an independent prognostic factor for LABM. In our study, we focused on a more specific type of lung cancer with bone metastases because it helps to make more precise individualized decisions and because lung adenocarcinoma has the highest incidence of bone metastases. We believe that removing the primary tumor slows tumor progression by reducing tumor load and decreasing the release of tumor cells into the bloodstream (29). Of course, not all LABM are suitable for surgical treatment by resection of the primary tumor, and more research is still needed in the future to further explore which LABM are more suitable for surgical treatment.



We found that chemotherapy is an independent prognostic risk factor for LABM. Chemotherapy drugs can shrink tumors and control their growth and spread by destroying rapidly dividing cancer cells (30). This can provide some relief of symptoms, pain, and other complications caused by the tumor in patients who have developed bone metastases. In some cases, chemotherapy may be the initial treatment option, especially if the patient has low levels of PD-L1 expression or no targetable gene mutations (31). Chemotherapy may be used as a first-line treatment to stabilize the disease and provide an opportunity for subsequent treatment. Confusingly, the study suggests



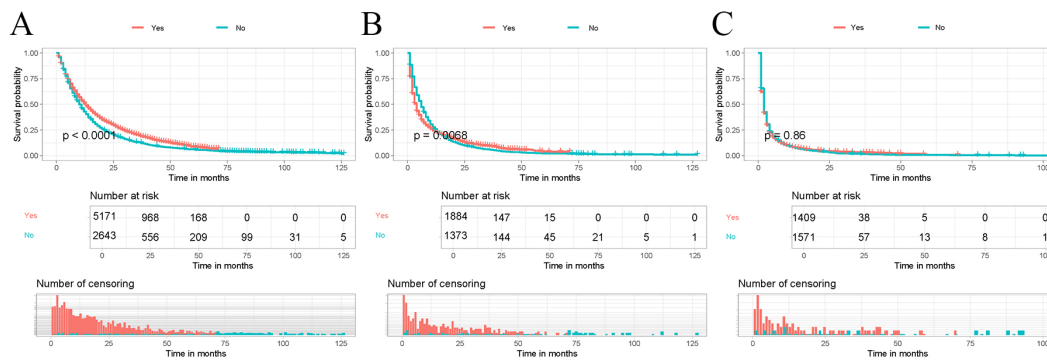


FIGURE 7

Kaplan-Meier curves were performed to analyze the OS of patients in different mortality risk groups by immunotherapy. (A) Kaplan-Meier curve for immunotherapy in the low-risk group; (B) Kaplan-Meier curve for immunotherapy in the middle-risk group; (C) Kaplan-Meier curve for immunotherapy in the high-risk group.

that radiotherapy is not a prognostic factor for LABM. Although radiotherapy is not usually a curative treatment, it can be effective in controlling the local spread of tumors. It offers a relatively gentle form of management for patients whose health or age precludes them from receiving more aggressive treatments such as chemotherapy or major surgery (32). We believe that for patients with bone metastases, radiotherapy is only a local treatment for relief of bone pain and prevention of pathologic fractures, and does not slow the progression of the tumor in a way that serves to prolong patient survival (33). Secondly, high doses and long courses of radiotherapy may cause severe myelosuppression, leading to a worse prognosis for the patient.

Although immunotherapies have shown significant efficacy in the treatment of lung cancer, to date, no large multicenter cohort study has analyzed their potential efficacy in LABM. It is important to emphasize that even though studies have shown that immunotherapy can improve survival in LABM, it has been shown that not all patients can benefit from it. Therefore, a risk classification system was further developed to better allocate healthcare resources and to enable precision treatment. By using the risk classification system to distinguish those who can benefit most from immunotherapy, we can achieve personalized and precise treatment, avoiding waste of resources and over-treatment of patients. However, the study has some limitations. First, as a multicenter retrospective study, selection bias is inevitable. Second, the patient-related information available in the SEER database is limited. For example, laboratory test results and relevant imaging results were missing. In the future, further large-scale prospective multicenter studies are needed to develop a more comprehensive and accurate risk classification system to differentiate the optimal beneficiary population for immunotherapy in LABM.

Conclusions

This study first demonstrated that immunotherapy can significantly improve the prognosis of LABM through a multicenter large-scale retrospective study. Second, a risk classification system was constructed to screen the best beneficiary population for immunotherapy in LABM. This tool can be extremely useful in the field of clinical decision-making,

laying the foundation for personalized and precise treatment, thus avoiding the waste of medical resources and over-treatment.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Author contributions

ZH: Conceptualization, Data curation, Formal Analysis, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. YT: Conceptualization, Data curation, Formal Analysis, Validation, Writing – original draft. LZ: Conceptualization, Data curation, Writing – original draft. BY: Conceptualization, Data curation, Methodology, Writing – original draft. KC: Data curation, Formal Analysis, Methodology, Writing – review & editing. PD: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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A narrative review on diagnosis and treatment of ifosfamide-induced encephalopathy, the perspective of a EURACAN reference center for sarcomas

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Ifosfamide (IFO) is a nitrogen derivative used at different doses, alone or in combination, in the treatment of various types of solid and hematologic cancers. It is a pro-drug activated by cytochrome P450 enzymatic system into ifosforamide mustard, the alkylating component that carries out the cytotoxic effect of the IFO. The most common toxicities of IFO are gastrointestinal, cutaneous, hematological, urological, and neurological. The neurotoxicity may occur in up to 30% of patients and can manifest with a wide spectrum of clinical presentations (lethargy, somnolence, confusion, hallucinations, irritability, excitement, disorientation, weakness, seizures, movement disorders, coma) and a variety of EEG abnormalities, and is known as IFO-induced encephalopathy (IIE). There is no definitive explanation of the mechanism underlying this phenomenon, even though metabolism of IFO, which leads to the formation of neurotoxic components, is probably at the basis of neurotoxicity according to many hypotheses. Consequently, the different factors involved in IFO metabolism (i.e., genetic polymorphism of CYP2B6, GSTM1, GSTP1, and GSTT1; concomitant administration of drugs that affect the cytochrome P450 enzyme system; drug formulation) could be responsible of IIE. IIE is usually reversible by interrupting the IFO infusion and starting intravenous hydration but in some cases further interventions are needed. The most used pharmacological treatment is methylene blue, whose efficacy both as a curative and a prophylactic treatment has been the object of many studies, with mixed results. Other interventions that showed efficacy are thiamine (tested also as a prophylactic drug), dexmedetomidine, and hemodialysis. Other pharmaceuticals have been tested in a preclinical setting showing some activity: trifluoperazine, morin, caffeic acid phenethyl ester (CAPE), and alpha lipoic acid (ALA). The aim of this review is to gather the current knowledge about the mechanisms underlying the IIE and the current therapeutic approach and the future perspectives.

KEYWORDS

ifosfamide, neurotoxicity, encephalopathy, sarcoma, treatment

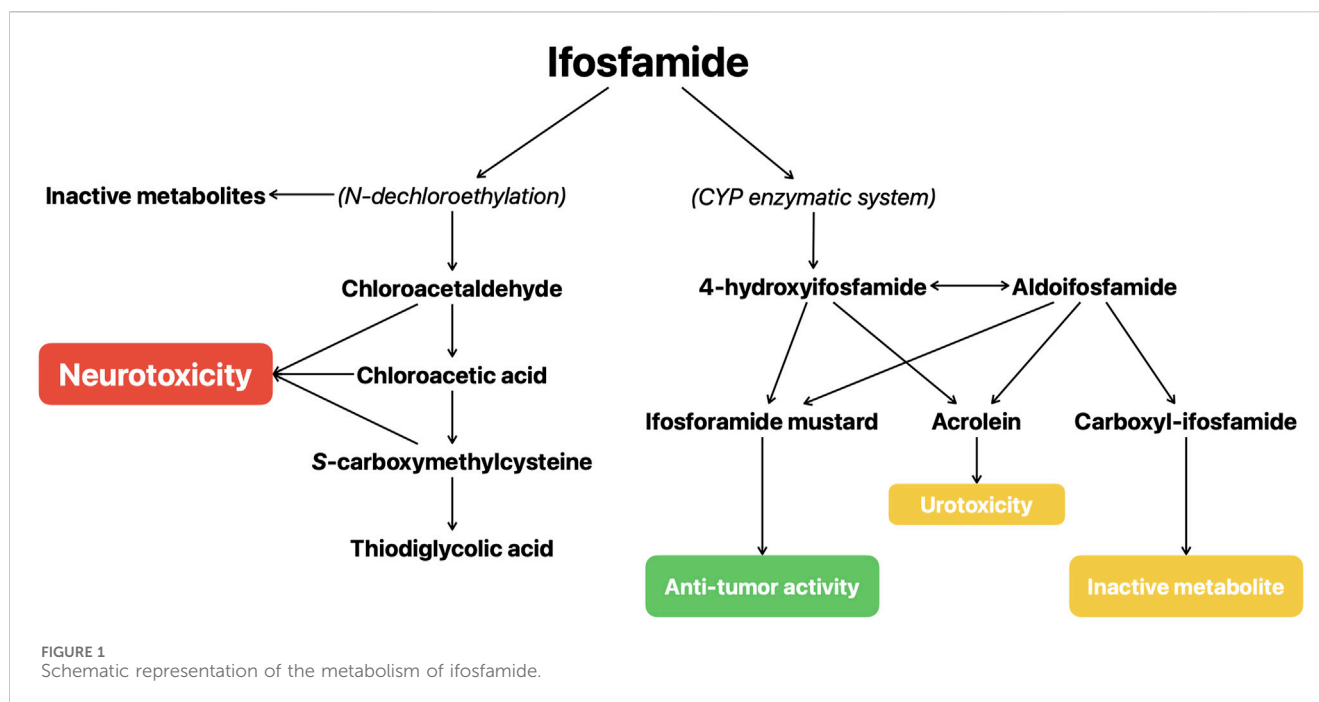
1 Introduction

Ifosfamide (IFO) is a chemotherapy medication used in the treatment of various types of cancer, including breast cancer, small cell lung cancer, testicular cancer, bladder cancer, non-small cell lung cancer, ovarian and cervical cancer, soft-tissue and bone sarcomas at different doses ranging from 50 mg/kg per day to 14 g/m² per cycle. It is a pro-drug converted by cytochrome P450 (CYP) enzymatic system into its active metabolite, ifosforamide mustard, a cytotoxic alkylating agent. A side product of this reaction is acrolein, considered responsible for the hemorrhagic cystitis, a dose-limiting urotoxicity of IFO, prevented thanks to the antidote mesna (Kerbusch et al., 2001). The introduction of mesna allowed the safe administration of higher doses of IFO, e.g., in regimens used in the treatment of sarcomas (Gronchi et al., 2021; Strauss et al., 2021). The other most common adverse events (AEs) of IFO are nausea and vomiting, alopecia, blood cells count decrease, and central nervous system (CNS) toxicity in the form of a metabolic encephalopathy, known as IFO-induced encephalopathy (IIE) (Fan et al., 2015). The term encephalopathy is a generic definition indicating a disease in which the functioning of the brain is modified by some agent or condition. It comprises different conditions affecting the brain that, among the others, can be associated with chemotherapeutic agents. They can cause encephalopathy through different mechanisms including direct neurotoxicity, oxidative stress, blood-brain barrier disruption, and metabolic disorders (Barbosa-Azevedo et al., 2024). The first reports of neurological symptoms in patients treated with IFO date back to the 1970s and the 1980s, after the introduction of mesna (van Dyk et al., 1972; Cantwell and Harris, 1985; Meanwell CA. et al., 1986; Meanwell C. et al., 1986). IIE is among the most clinically relevant AEs of IFO, it can have various clinical presentations ranging from somnolence, mild mental confusion, or depressive periods to a state of hallucinations or coma. More specifically, IIE symptoms may include impaired consciousness, lethargy, somnolence, confusion, hallucinations, delusions, irritability, excitement, anxiety, disorientation, weakness, seizures, movement disorders, extrapyramidal symptoms, tremors, and coma (Curtin et al., 1991; Cerny and Küpfer, 1992; DiMaggio et al., 1994; Anderson and Tandon, 1991; Danesh et al., 1989), with a reported incidence of 10%–15% (Szabatura et al., 2015; Tajino et al., 2010). IIE is a clinical diagnosis with both early and late onset, usually within 48 h and up to 6 days from the start of the IFO infusion. There is no standard scale to define the severity of this kind of encephalopathy. Nevertheless, to date, it can be graded according to the National Cancer Institute Common terminology criteria for adverse events (NCI CTCAE) version 5, based on the severity of symptoms and their impact on activities of daily living (ADL). Furthermore, the Glasgow coma scale (GCS) is a simple and well-known tool that can be used to monitor the clinical development of patients over time (Reith et al., 2016). Symptoms of IIE are usually temporary. However, in some cases, IIE can be persistent and, rarely, fatal (Kerbusch et al., 2001; Yeager and Basnet, 2020; Watkin et al., 1989; Chain et al., 2022; Ataseven et al., 2021). In this narrative review we will discuss the etiopathogenesis of the IIE and its management in the clinical setting.

2 Etiopathogenesis

The metabolism of the IFO seems to be central in the development of the IIE, as reported in a recent review by Idle and Beyoğlu (2023) focusing on the development and the metabolism of IFO. IFO is metabolized by the CYP enzymatic system, particularly by the CYP3A4 and CYP2B6, into the active metabolite (ifosforamide mustard) and various side products. IFO is converted to 4-hydroxyifosfamide (4-OH-IFO) from which ifosforamide mustard and acrolein are produced; 4-OH-IFO is in equilibrium with its tautomeric form, aldoifosfamide, that can be converted either into carboxyl-IFO (an inactive metabolite) and into acrolein and ifosforamide mustard. IFO can also undergo *N*-dechloroethylation reactions that lead to the formation of inactive metabolites and of chloroacetaldehyde (CAL), CAL could exert a neurotoxic effect through glutathione depletion, by influencing the mitochondrial terminal respiration chain, and through the formation of chloroacetic acid, a gluconeogenesis inhibitor, and *S*-carboxymethylcysteine, an agonist of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainite receptors (Kerbusch et al., 2001; Storme et al., 2009; Huang et al., 2000; Lerch et al., 2006; Chatton et al., 2001; Yip et al., 2017) (Figure 1). Cyclophosphamide is closely related to IFO but is not associated with neurotoxicity and, unlike IFO, only a small proportion of it is metabolized through *N*-dechloroethylation, causing IFO to be associated to a greater increase of CAL concentrations (Li et al., 2010).

The administration of drugs metabolized by CYP3A4 and CYP2B6 may be associated with the IIE onset. Aprepitant and its pro-drug fosaprepitant, two selective antagonists of brain neurokinin 1 (NK1) with an antiemetic effect, are CYP3A4 moderate inhibitors with also a possible inductive effect (Sarcev et al., 2008; Shadle et al., 2004). Data from previous studies and case reports are mixed, showing a positive correlation between the concomitant administration of IFO and aprepitant (or fosaprepitant) and IIE in some studies, not confirmed in others (Séjourné et al., 2014; Durand et al., 2007; Shindorf et al., 2013; Patel et al., 2017; Jordan et al., 2011; Xiong et al., 2019). The interaction between these drugs has been the object of a systematic review from Vazirian et al. (2022), that included one randomized clinical trial (RCT) and eight retrospective cohort studies reporting a positive trend not reaching statistical significance between IIE and the concomitant use of IFO and aprepitant or fosaprepitant. However, the populations of the studies were highly heterogeneous with possible confounding factors and the association between the IIE and the administration of the said treatments were not always statistically evaluated. The concomitant administration of other drugs influencing the activity of CYP3A4 and CYP2B6, such as opioids, benzodiazepines, corticosteroids, and metoclopramide, was also investigated in a retrospective study by Szabatura et al. (2015). The study included 200 patients treated with IFO, of which 29 experienced IIE. The reported results show no effect of CYP3A4 inhibitors and substrates on IIE, and a statistically significant association between IIE and both opioids (odds ratio 2.81) and CYP2B6 inhibitors (odds ratio 5.17), despite a previous



work suggested that the inhibition of the CYP2B6 pathway could be protective against IIE (Huang et al., 2000). Further data supporting the role of CYP2B6 derive from its genotyping performed by Duflot et al. on three pediatric patients experiencing IIE, reporting, in all 3 cases, the presence of loss-of-function variants (Duflot et al., 2018). An influence from variants of the genes of glutathione S-transferases (GST) was also hypothesized, albeit without a clear clinical significance (Zielińska et al., 2005).

Based on the neuropathological study of a patient who died due to IFO toxicity (including IIE) showing characteristics like those of Wernicke's encephalopathy, Buesa et al. suggested that IFO and/or its metabolites could impair the function of thiamine and alter the cerebral glucose metabolism resulting in neuronal cell death (Buesa et al., 2003).

Other possible factors increasing the risk of IIE are impaired renal function, that could be caused by pelvic disease or previous administration of cisplatin, reducing the clearance of IFO and its metabolites; low albumin levels; hepatic dysfunction and decreased bilirubin; acidosis; oral administration, shorter infusion time; central nervous system (CNS) metastases, previous CNS irradiation, pre-existent neurological disorders; electrolyte imbalance; both young and old age; female sex; obesity (Szabatura et al., 2015; Vazirian et al., 2022; Kettle et al., 2010; Lo et al., 2016; Howell et al., 2008). A recent review of the literature by Lee et al. (2020), summarizes the evidence on IIE risk factors, highlighting the insufficiency of data in this field, and the need for further research to establish the role of many suggested risk factors.

In a recent retrospective study on 172 sarcoma patients treated with IFO, Schmidt et al. showed a correlation between IIE and laboratory markers that can be associated with an inflammatory state, such as lower lymphocyte count, lower hemoglobin and calcium levels, elevated sodium, GGT and CRP levels, suggesting their potential utility in IIE prediction and diagnosis, being them routinely tested (Schmidt et al., 2022).

3 Neurological assessment

To date, there is no standardized approach for the assessment of IIE. Ideally, it should include both objective evidence of neurological deficits, and assessment of symptoms from a patient perspective, through neurological examination, neurophysiological parameters (EEG), patient-reported outcomes, and standardized evaluation scales. A pre-treatment neurological assessment could help to identify a pre-existing neurological dysfunction, that could increase the risk of neurotoxic adverse events from IFO. Likewise, an adequate assessment during the treatment is important to recognize the earliest signs of central nervous system toxicity, allowing a prompt intervention. Case reports describe EEG changes in patients with IIE (Müngen et al., 2022; Primavera et al., 2002; Feyissa and Tummala, 2014; Pavarana et al., 2005). The EEG alterations appeared during the acute phase of the encephalopathy, then gradually disappeared according to the clinical improvement of patients. A variety of abnormalities were recorded, comprising epileptiform discharges, background activity attenuation and slowing, and alterations consistent with non-convulsive status epilepticus (NCSE). Feyissa and Tummala (2014) evidenced that EEG could help identify patients with NCSE or those with epileptiform discharges who subsequently develop convulsive or non-convulsive seizures. In their study, the improvements in IIE symptoms after the interruption of the IFO infusion matched with the improved EEG changes upon repeated testing: resolution of electrographic seizures and epileptiform discharges and improvement in the background slowing. However, the results of a larger retrospective study by Gusdon et al. (2019) do not support this relation, instead suggesting that a marked background attenuation may be associated to poorer outcomes.

Conventional brain MRI could be useful to rule out other neurological conditions that may be responsible for the

symptoms. However, there are no specific neuro-radiological findings associated with IIE. Literature data from other pathologies, especially hematological malignancies treated with chimeric antigen receptor (CAR) T cells, could be useful in the management of IIE. In a proof-of-concept study, [Stoecklein et al. \(2023\)](#) assessed the dysconnectivity index (DCI), based on functional MRI (fMRI) and resting state functional MRI (rsfMRI), in a small group of patients with lymphoma and melanoma during immune effector cell-associated neurotoxicity syndrome (ICANS) showing that higher DCI scores were associated with higher ICANS grades.

In the context of CAR T therapy, the American Society for Transplantation and Cellular Therapy (ASTCT) developed a new grading system for the immune effector cells-mediated central neurotoxicity, to address the lack of objectivity in the CTCAE reporting system and to stop relying on the evaluation of ADL, which can be difficult to assess in hospitalized patients. Their grading system uses the Immune Effector Cell-Associated Encephalopathy (ICE) score, derived from the CARTOX-10 ([Neelapu et al., 2018](#)), and a granular evaluation of key symptoms and signs such as depressed level of consciousness, seizures, motor dysfunctions, and elevated intracranial pressure, aiming at objectively define the neurotoxicity ([Lee et al., 2019](#)).

4 Treatment

The management of IIE is essentially based on treatment discontinuation and hydration and there are no reference drugs with the specific indication. However, especially in case of severe toxicity, reversing agents are usually administered in clinical practice. The most used one is methylene blue (MB), administered intravenously at the dosage of 50 mg up to 6 times a day, whose effect is based on its activity as an electron acceptor, its ability to oxidate the excessive NADH formed through IFO metabolism and to inhibit the formation of CAA ([Kerbusch et al., 2001](#); [Patel, 2006](#); [Küpfer et al., 1996](#)). The rationale behind its efficacy in this setting was first showed by [Küpfer et al. \(1996\)](#) and then supported by case reports and reviews of the literature in the following years ([Patel, 2006](#)). [Pelgrims et al. \(2000\)](#) reported 12 patients with IIE, of whom eight were treated with MB infusion with full recovery after 24 h (4 patients), 48 and 72 h (2 patient, respectively); four patients did not receive MB and nevertheless recovered after 48 h. [Turner et al.](#) reported the cases of two patients treated with IFO and experiencing IIE, whose symptoms resolved after MB administration ([Turner et al., 2003](#)); [Abahssain et al. \(2021\)](#) reported four patients with IIE treated with MB, of which 3 showed a partial or total resolution of the neurological symptoms. They also conducted a review of the literature including 16 articles: 38 patients with IIE (65.5%) were treated with MB with a favorable response in 28 of them (75.6%). Despite its use in the clinical practice, there are no prospective randomized clinical trials evaluating its efficacy and safety in this setting and, whereas rare but potentially severe adverse reactions such as anaphylactic shock, Heinz body hemolytic anemia and serotonin syndrome have been reported, caution is needed ([Vanhinsbergh et al., 2018](#); [Sills and Zinkham, 1994](#); [Snyder et al., 2017](#); [Dewachter et al., 2005](#); [Dewachter et al., 2011](#);

[Nubret et al., 2011](#)). However, the use of MB in this setting can still be recommended based on the available data, considering the lack of established alternatives and the severity of the IIE.

Thiamine is another therapeutic option in the treatment of IIE, administered intravenously at the dosage of 100 mg every 4 h until symptoms resolution. The rationale for its use is based on the findings by [Buesa et al. \(2003\)](#) and on its favorable safety profile. Similarly to MB, data supporting the efficacy of thiamine in this setting comes from case reports. [Buesa et al. \(2003\)](#) reported the cases of 10 patients with IIE treated with thiamine with resolution of neurological symptoms such as low level of consciousness, confusion, hallucinations, anxiety, and asterixis. [Hamadani and Awan \(2006\)](#) reported three patients with IIE whose symptoms resolved after thiamine treatment within a mean time of 17 h. [Ataseven et al. \(2021\)](#) reported the case of a pediatric patient with IIE with severe clinical presentation treated with thiamine combined to MB whose neurological symptoms fully resolved. [Müngen et al. \(2022\)](#) reported a pediatric patient with severe symptoms from IIE successfully treated with thiamine to full recovery ([Table 1](#)).

Blood purification therapy has been shown to decrease IFO concentrations both *in vitro* and clinical studies ([Latcha et al., 2009](#); [Sauer et al., 1990](#); [Fiedler et al., 2001](#)). Furthermore, it can decrease the concentration of potentially neurotoxic IFO metabolites such as CAA ([Carlson et al., 1997](#)). Based on these data and on the fact that impaired renal function is a risk factor for the development of IIE, dialysis has been successfully used to treat IIE, particularly in patients with severe clinical presentation, not responsive to MB and thiamine and with concomitant nephrotoxicity ([Yeo and HaDuong, 2016](#); [Nishimura et al., 2014](#); [Cherry et al., 2013](#)).

4.1 Prophylaxis

Prophylaxis should be considered in patients with an episode of IIE who continued the treatment with IFO. The most used agents are, similarly to the therapeutic setting, MB, and thiamine. The efficacy of MB was first reported by [Küpfer et al. \(1996\)](#), and it is commonly used in the clinical practice at the dosage of 50 mg up to every 6 h administered intravenously from the day before the start of IFO infusion. Thiamine is also administered in this setting, at the dosage of 100 mg every 6 h, alone or in combination with MB. However, there is limited evidence supporting their routine utilization, merely based on case reports and some retrospective studies ([Buesa et al., 2003](#); [Pelgrims et al., 2000](#); [Turner et al., 2003](#); [Hamadani and Awan, 2006](#); [Kasper et al., 2004](#); [Gharaibeh et al., 2019](#)), contradicted by other retrospective studies that did not show any clinical benefit ([Lentz et al., 2020](#); [Richards et al., 2011](#)).

Low albumin concentration is a potential risk factor for IIE. Albumin infusion as a preventive treatment has been investigated in a retrospective study with negative results ([Kettle et al., 2010](#)).

4.2 Preclinical data with other agents

Data from preclinical studies support the efficacy of other agents in counteracting the neurotoxic effect of IFO. [Kiani et al. \(2020\)](#) evaluated the use of trifluoperazine (TFP) in protecting isolated rat neurons against the damage of IFO. TFP is a typical antipsychotic

TABLE 1 Reports on treatment of IIE with MB and thiamine.

	Author	Number of patients (pts)	Outcome	Notes
Methylene blue	Pelgrims et al.	12 pts with IIE 8 pts treated with MB	Received MB - 4 pts recovered within 24 h - 2 pts recovered within 48 h - 2 pts received within 72 h Did not receive MB - 4 pts recovered within 48 h	-
	Turner et al.	4 pts with IIE 2 pts treated with MB	Received MB: 2 pts recovered in 24 h Did not receive MB - 1 pt recovered within 24 h - 1 pt recovered (time unknown)	Symptoms included impaired consciousness, extrapyramidal symptoms, confusion, disorientation, nocturnal agitation, delusions, hallucinations, bizarre dreams, impaired sight Pts not treated with MB received it as prophylaxis before the subsequent cycle
	Abasshain et al.	4 pts with IIE treated with MB	3 pts recovered within 24 h; 1 pt died	Death due to malignancy progression
	Ataseven et al.	3 pts with IIE 1 pt treated with both MB and thiamine	Received MB and thiamine: 1 pt recovered in 2 weeks Did not receive MB and thiamine 2 pts recovered within 24 h	Pt treated with MB and thiamine was in a coma; patients not treated with MB and thiamine had seizures
Thiamine	Buesa et al.	10 pts with IIE treated with thiamine	10 pts recovered - 4 pts within 24 h - 4 pts within 48 h - 2 pts within 72 h	Symptoms included confusion, hallucinations, anxiety, asterixis, impaired consciousness
	Hamadani and Awan	3 pts with IIE treated with thiamine	2 pts recovered within 24 h, 1 pt within 36 h	Symptoms included confusion, disorientation, tremors, hallucinations, agitation, tremors; 1 pt was treated with MB before thiamine
	Ataseven et al.	3 pts with IIE 1 pt treated with both MB and thiamine	Received MB and thiamine: 1 pt recovered in 2 weeks Did not receive MB and thiamine: 2 pts recovered in 24 h	Pt treated with MB and thiamine was in a coma; patients not treated with MB and thiamine had seizures
	Müngen et al.	1 pt with IIE treated with thiamine	1 pt recovered within 24 h	Symptoms included impaired consciousness, agitation, disorientation, stupor

drug that can also acts as an inhibitor of calmodulin, preserving the cell against the deleterious effects of calcium overload. TFP pretreatment in isolated rat neurons exposed to IFO reduced its cytotoxic effect. Çelik et al. (2020) evaluated the use of morin, a compound with anti-oxidant, anti-inflammatory, neuroprotective, anti-carcinogenic and antidiabetic properties, as a chemoprotective agent. The administration of morin in IFO-treated male rats was associated with enhanced antioxidant system, decreased cholinergic markers and inflammatory mediators, reduced mitochondria-dependent apoptosis, and other surrogates of neuronal damage. Ozturk et al. (2014) investigated the effect of alpha lipoic acid (ALA) against the IFO-induced neurotoxicity in rats, the rationale being the antioxidative properties of ALA. Their results showed that ALA has a protective effect against the IFO-induced neurotoxicity preserving the redox state of the cells and interfering with the apoptosis, induced by IFO. Ginis et al. achieved similar results investigating the effect of caffeic acid phenetyl estere (CAPA), a compound with antioxidative properties and able to interfere with apoptosis, in IFO-treated rats (Ginis et al., 2016).

5 Discussion

IFO neurotoxicity is a significant concern in the clinical management of patients undergoing chemotherapy with IFO.

IIE can have various clinical presentations, ranging from transient somnolence to coma (Curtin et al., 1991; Cerny and Küpfer, 1992; DiMaggio et al., 1994; Anderson and Tandon, 1991; Danesh et al., 1989). Although its symptoms are usually mild and transient, IIE can have prolonged, severe, and sometimes fatal effects. The metabolism of IFO seems to be central in the etiopathogenesis of the neurotoxicity. Factors influencing its metabolism can however increase the risk of IIE, such as the concomitant administration of drugs influencing the activity of CYP3A4 and CYP2B6, impaired renal function, pelvic disease, previous administration of cisplatin, and low albumin levels (Vazirian et al., 2022; Duflot et al., 2018).

The management of IIE is based on treatment interruption and hydration and no drug has been proven to be effective. However, in the clinical practice, reversing agents as MB and thiamine are used in the more serious cases. Most of the data about their use in this setting comes from retrospective series and case reports and there are no prospective randomized clinical trials investigating their efficacy and safety. However, they still can be used to treat IIE based on the available data, considering the lack of alternatives and their favorable safety profiles. Both MB and thiamine are used in the clinical practice with a prophylactic intent, despite the low quality of the data about their efficacy, mostly derived from case reports (Buesa et al.,

2003; Küpfer et al., 1996). Finally, both clinical and preclinical data support the use of hemodialysis in this setting, mostly in patients unresponsive to MB and thiamine treatment, consistently with the role of IFO metabolism and impaired renal function in the onset of IIE (Fiedler et al., 2001). Other agents, such as TFP, morin, ALA, and CAPA, have shown potential effectiveness against the neurotoxic effects of IFO in murine models. However, these compounds have only been studied in a preclinical setting and are still distant from a clinical application (Kiani et al., 2020; Çelik et al., 2020; Ozturk et al., 2014; Ginis et al., 2016).

Electroencephalography can be useful in the clinical management of patients with IIE. Different abnormalities were associated with the neurotoxic effect of IFO, usually following the clinical course of the patients. Moreover, there is data suggesting a correlation between the severity of IIE and specific EEG patterns, although not concordant. Thanks to its widespread availability and its non-invasiveness, EEG can be a useful tool in early detection of IIE, monitoring the clinical course of patients, and early identification of cases that can evolve to a serious presentation (Feyissa and Tummala, 2014; Gusdon et al., 2019).

There are unmet needs that should be addressed in the future. The routine use of MB and thiamine in clinical practice is supported by case reports and retrospective data, lacking prospective controlled studies. This issue should be addressed to get more certain data about their efficacy and their safety, also considering the possibility of rare but serious adverse events of these drugs, such as anaphylactic shock, Heinz body hemolytic anemia and serotonin syndrome. The diagnosis of IIE is essentially based upon clinical evaluation and can be challenging due to its heterogeneous and nonspecific presentations. In this context, objective and standardized methods should be developed specifically for IIE, including biomarkers, cognitive assessments, neuroimaging, and EEG. They would be fundamental for a more accurate and earlier diagnosis, and for a better management, helping identify those patients with a poorer outcome. In a recent retrospective study from Schmidt et al. (2022), a correlation between routinely tested inflammatory markers and IIE was shown, suggesting their potential clinical role as predictive factors of neurotoxicity.

Tools such as EEG and fMRI could be helpful to select those patients at a higher risk of developing the IIE, for an early diagnosis, critical for the prompt interruption of the IFO infusion, to monitor the clinical course, and to identify patients with a poorer prognosis (Feyissa and Tummala, 2014; Gusdon et al., 2019; Stoecklein et al., 2023). Due to the lack of objective diagnostic tools, the diagnosis of IIE is essentially clinical, therefore it is crucial to educate the patients and caregivers about the symptoms with which it can manifest. Together with the variability of clinical presentations, this can cause inconsistencies in the documentation of the IIE hampering both the treatment of patients in clinical practice and the comparison of different cases for research purposes. The adoption, or the adaptation, of objective scales and assessment tools, such as the grading system proposed by the ASTCT in the CAR T mediated neurotoxicity (Lee et al., 2019), can be useful for a more accurate and earlier diagnosis, and to better monitor the evolution of symptoms, allowing a more consistent treatment approach, and enabling a reliable evaluation of outcomes across different centers.

As a national and European reference center in EURACAN for the treatment of sarcomas, it is our intention to conduct a prospective study aimed at identifying possible predisposing factors for the development of IIE and establish recommendations on the prevention of neurotoxicity in at-risk patients. In our clinical practice, before administration of high-dose IFO, we currently perform a baseline neurological evaluation and an EEG to rule out predisposing neurological pathologies, to try to quantify the risk of neurotoxicity and provide for a more intensive symptom monitoring. We manage IIE cases mostly with MB, aside from interruption of the infusion and supportive hydration. If treatment resumption is possible, we administer prophylactic MB before the subsequent IFO infusions.

In summary, IIE remains a relevant clinical matter, with a potential impact on the clinical course of patients treated with IFO chemotherapy. Unmet needs remain, both in the diagnostic workup and the treatment, that should be addressed by further studies testing the efficacy and safety of drugs already commonly used in this setting, and the accuracy of diagnostic tools, providing higher-quality data supporting the daily clinical practice.

Author contributions

AT: Conceptualization, Methodology, Writing–original draft, Writing–review and editing. SV: Conceptualization, Supervision, Writing–original draft, Writing–review and editing. CO: Conceptualization, Supervision, Writing–original draft, Writing–review and editing. SC: Conceptualization, Supervision, Writing–original draft, Writing–review and editing. MM: Writing–original draft, Writing–review and editing. GM: Writing–original draft, Writing–review and editing. FR: Writing–original draft, Writing–review and editing. WF: Writing–original draft, Writing–review and editing. MR: Writing–original draft, Writing–review and editing. VF: Supervision, Validation, Writing–original draft, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Challenges and hope: latest research trends in the clinical treatment and prognosis of liposarcoma

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Liposarcoma, as a complex disease, is characterized by intricate interactions between distinct histopathological subtypes and corresponding clinical outcomes, emphasizing the necessity of personalized approaches in diagnosis and treatment strategies. This malignant tumor originating from adipose tissue is classified into different subtypes with specific molecular markers, which not only distinguish them but also guide treatment directions. The main approach for treating liposarcoma is surgical resection, with the aim of complete excision and achieving clean margins (R0 resection) to minimize the risk of recurrence. This surgical principle emphasizes the critical need for precise preoperative planning, and in certain cases, the integration of neoadjuvant therapy may be needed to reduce the tumor to a surgically manageable size. In addition to surgery, systemic therapy plays a key role in the advanced stages of the disease, especially when resistance to traditional treatment arises. The emergence of novel systemic therapies, including chemotherapy, targeted therapy, and immunotherapy, has opened new avenues for treating this challenging malignancy. These systemic therapies are selected on the basis of the specific molecular features of the tumor, highlighting the importance of detailed molecular diagnostics. As our understanding of the molecular basis of liposarcoma deepens, integrating clinical and molecular features is crucial for optimizing treatment outcomes. This comprehensive approach, which combines surgical precision with systemic therapy innovations, will change the treatment landscape for patients with liposarcoma, advancing toward more personalized and effective treatment strategies.

KEYWORDS

liposarcoma, clinical and molecular characteristics, surgery, systemic treatment, prognosis

1 Introduction

Soft tissue sarcomas (STSs) account for 1% of adult malignancies and are a group of mesenchymal tumors comprising 179 histological subtypes (<https://seer.cancer.gov/statfacts/html/soft.html>) (Kallen and Hornick, 2021). Liposarcoma (LPS) accounts for 15%–20% of STSs and is a rare malignant tumor characterized by adipocyte differentiation (Gronchi et al., 2021). Approximately 41% of LPSs occur in the lower limbs, 36% in the retroperitoneum, 8% in the upper limbs, and 5% each in visceral organs and the trunk (De vita et al., 2016). According to the

5th edition of the WHO Classification of Soft Tissue Tumors released in 2020 (Schaefer and Gronchi, 2022), subtypes of LPS include atypical lipomatous tumor (ALT)/well-differentiated liposarcoma (WDLPS), dedifferentiated liposarcoma (DDLPS), myxoid liposarcoma (MLPS), pleomorphic liposarcoma (PLPS), and myxoid pleomorphic liposarcoma (MPLPS), with MPLPS being a new addition characterized by a nonspecific nuclear pattern, lacking the classic gene fusion of DDIT3 with FUS or EWSR1. Given that these subtypes have unique clinical, histological, biological, immunohistochemical, and molecular genetic features relevant to diagnosis, prognosis, and treatment sensitivity (Kallen and Hornick, 2021), individualized treatment methods should be formulated on the basis of the histological type (Haddox and Riedel, 2020). A series of novel antitumor drugs that target the specific molecular biology of LPS are actively being researched, offering hope for increasing treatment options for recurrent or unresectable LPS (Lee et al., 2018).

2 Clinical and molecular characteristics of each subtype of LPS

Each subtype of liposarcoma (LPS) has unique clinical and molecular characteristics, reflecting the diversity and complexity of this malignant tumor (Yao et al., 2020).

2.1 Cytogenetic characteristics of ALT/WDLPS and DDLPS

ALT/WDLPS is the most common subtype of liposarcoma, accounting for 40%–45%, and is commonly found in the limbs, buttocks, and deep soft tissues of the trunk, with 25% originating from the retroperitoneum. Its subtypes include lipomatous, sclerosing, inflammatory, and spindle cell variants (Sciot et al., 2020). They usually grow slowly, are prone to recurrence, and are resistant to radiotherapy and chemotherapy. Compared with WDLPS of the limbs, retroperitoneal WDLPS has a greater risk of dedifferentiation (Keung et al., 2018). DDLPS accounts for 15%–20% of cases, mostly in middle-aged and elderly people, with 75% occurring in the abdominal cavity and retroperitoneum (Gahvari and Parkes, 2020; Kilpatrick, 2021). DDLPS is characterized by increased aggressiveness and metastatic potential and is chemoresistant (Ghadimi et al., 2011). On a pathological level, it may exhibit homologous and heterologous dedifferentiation, with the majority being of high grade (Schaefer and Gronchi, 2022). DDLPS has a local recurrence rate of up to 40%, with a distant metastasis rate of 15%–30%, and the site of the lesion is an important prognostic factor. The molecular hallmark of WDLPS and DDLPS is amplification of chromosomal region 12q13-15 (Matthews et al., 2010), particularly amplification of the MDM2 and CDK4 genes (Aleixo et al., 2009), which drive tumor growth and dedifferentiation. Other key genes in this region, such as HMGA2, TSPAN31, FRS2, and GLI1, and new genes outside this area, such as DDR2, SDHC, and FGFR, also play significant roles in its pathogenesis (Pentimalli et al., 2003; Saâda-bouazid et al., 2015; Gao et al., 2013; Kanojia et al., 2015; Barretina et al., 2010). The signaling pathways of the FGFR/FRS2 and the PIK3R3/ERK/Nanog axis are closely linked to the development of DDLPS (Wang et al.,

2011; Zhang et al., 2013; Jing et al., 2018). While DDLPS shares common cytogenetic features, it presents more genomic abnormalities and complexity, exerting a greater impact on treatment response and prognosis. Mechanisms of liposarcoma dedifferentiation can be seen in Figure 1.

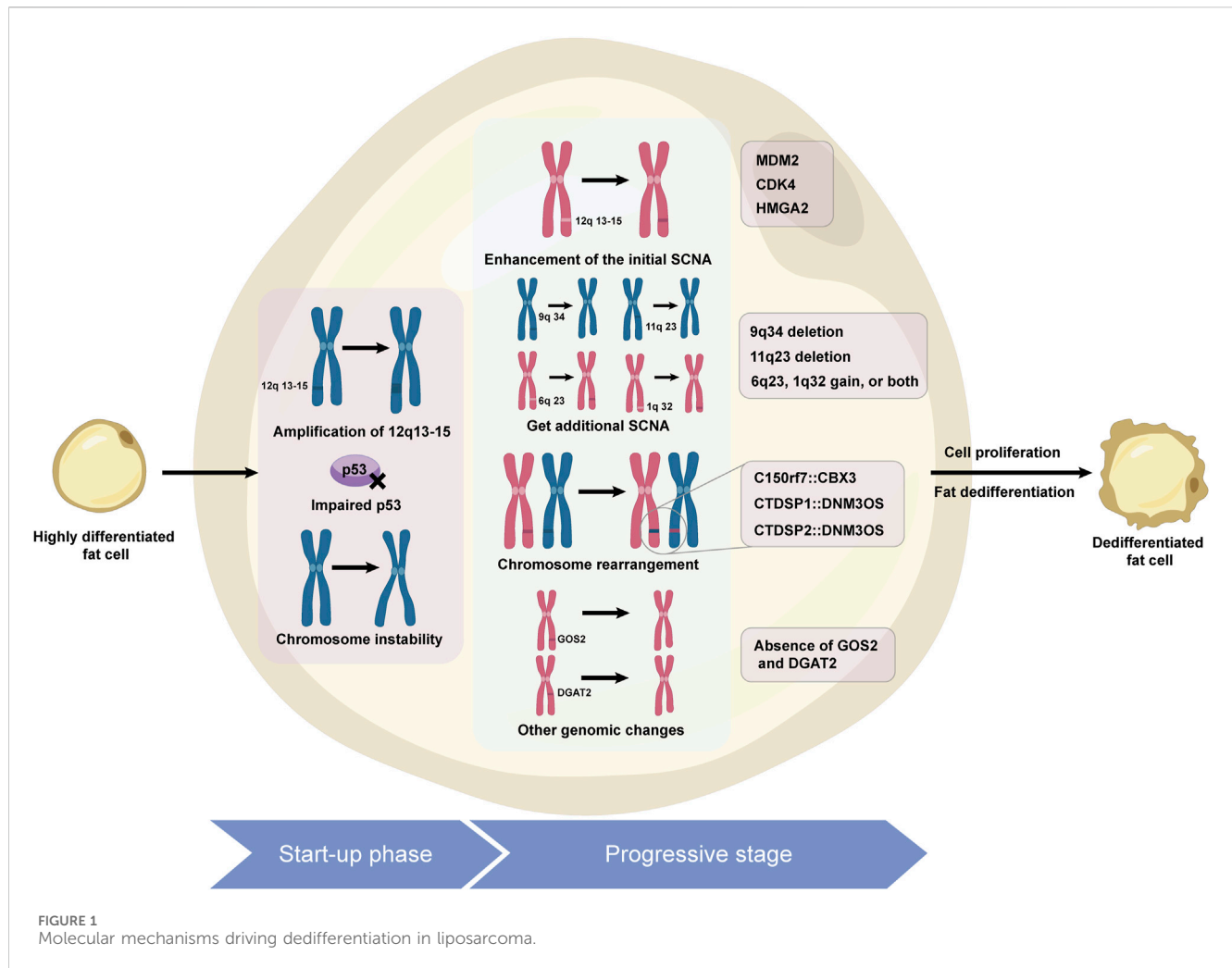
2.2 The genetic characteristics of MLPS

MLPS is the predominant form of LPS among children and adolescents, accounting for 20%–30% of cases, and is found mainly in the deep soft tissue of the limbs, particularly near the proximal thigh, with a rare occurrence in the retroperitoneum (approximately 2.3%) (Henze and Bauer, 2013). Approximately 12%–25% of patients are likely to experience local recurrence, and between 30% and 60% can metastasize (Schaefer and Gronchi, 2022). Pathology findings have shown that the presence of round cell components is associated with a poor prognosis (Schaefer and Gronchi, 2022; Setsu et al., 2016). In patients with MLS, dedifferentiation is rare (Ciongariu et al., 2023). More than 95% of MLS patients present with a t(12;16)(q13;p11) translocation, leading to the production of the FUS-DDIT3 fusion protein, impeding adipocyte terminal differentiation and facilitating tumor formation (Pérez-mancera et al., 2008; Powers et al., 2010; Xiao et al., 2018). Next-generation sequencing has identified new fusion genes and signaling pathway abnormalities, such as RET, FGFR2 (Künstlinger et al., 2015; Napolitano et al., 2021), PI3K/AKT/mTOR (Trautmann et al., 2019a; Berthold et al., 2022), Hippo/YAP1 dysregulation (Regina and Hettmer, 2019; Trautmann et al., 2019b), and TERT promoter mutations (Koelsche et al., 2014), revealing that FUS-CHOP activates the SRC/FAK/RHO/ROCK signaling axis, enhancing the invasive capacity of MLS cells (Tornin et al., 2018). Staaberg's team reported that a subgroup of MLS cells with CSC characteristics activate the JAK-STAT signaling pathway (Dolatatabadi et al., 2019), which could be a significant target for MLS treatment.

2.3 Genetic alterations in PLPS and MPLPS

PLPS, a subtype characterized by high invasiveness and poor prognosis, accounts for just 5%–10% of all LPSs, featuring elevated rates of local recurrence and metastasis (approximately 30%–50% each), with a 5-year survival rate of 60% (Schaefer and Gronchi, 2022). It affects mainly the deep soft tissues of the limbs, particularly the lower extremities. There is significant chemoresistance, which may be associated with P53 mutations. PLPS is characterized by pronounced chromosomal abnormalities, encompassing deletions and duplications (Conyers et al., 2011). Some studies indicate a correlation between RB1 mutations and PLPS (Libbrecht et al., 2021). MPLPS is a highly aggressive, rare tumor that predominantly occurs in the mediastinum of children and young adults and affects mainly females. It has complex chromosomal alterations and lacks FUS-DDIT3 gene fusion and MDM2/CDK4 gene amplification (Schaefer and Gronchi, 2022).

The diversity of the LPS subtypes in terms of clinical manifestations and molecular pathology underscores the need for a meticulous approach in diagnosis, treatment, and research to



accommodate the unique characteristics of each subtype. Advances in molecular genetics offer promising avenues for targeted treatments, emphasizing the importance of continued research to fully leverage these outcomes to improve patient care.

3 LPS treatment

3.1 Surgical intervention

The primary treatment for LPS is to perform R0 surgical resection as much as possible, avoiding unplanned resection (Qureshi et al., 2012). The survival duration of primary localized RPL is relatively short, with an overall 8-year survival rate ranging from 30% to 80% for different subtypes (Siew et al., 2022). All patients with resectable RPL should undergo initial extensive surgical resection for complete R0 excision (Delisle et al., 2022; Harati et al., 2017). Research has shown a significant association between OS, DFS, the LR rate, and LRFS in RPLPS patients and R0 resection (Paik et al., 2022). Compartment resection is the current standard procedure. Precision surgical principles require surgical stratification on the basis of the biological behavior of RPL. For radiotherapy and chemotherapy-resistant LPS, such as WDLPS/

DDLPS, surgical intervention remains the cornerstone of treatment. In case of surgical difficulties, neoadjuvant chemotherapy or radiotherapy may be considered to reduce the risk of recurrence. Given the high recurrence rate of RPL, measures such as radiotherapy and drug therapy may be considered, but controversy remains (Rust et al., 2022; D'ambrosio et al., 2022). Surgical intervention may have a certain effect on locally recurrent RPL, but the likelihood of long-term control decreases after each recurrence (Tseng et al., 2022). Research by Maria Anna Smolle et al. revealed that the survival rate of patients with primary localized limb MLPS who undergo metastatic liver resection after recurrence is higher than that of those who receive other treatments (Smolle et al., 2020). Studies suggest that simultaneous resection of the primary tumor and metastases in patients with LPS presenting with distant metastasis at diagnosis may prolong survival (Illuminati et al., 2010). Multiple studies have demonstrated that metastasectomy can increase survival rates (Chudgar et al., 2017; Marudanayagam et al., 2011). The Japanese JCO guidelines recommend resection of the primary lesion and metastases, but further research is needed on patient selection, considering factors such as patient condition, number of metastases, and status of the primary tumor. Studies (Tirotta et al., 2020) by Tirotta F et al. suggested that LRSM can result in prolonged patient survival, although factors such as

extrahepatic metastases, large metastatic lesions, chemotherapy resistance, and short DFI contribute to reduced survival rates.

3.2 Chemotherapeutic treatment

3.2.1 Neoadjuvant chemotherapy for high-risk patients with resectable LPS

For patients at very high risk or with early resection difficulties, neoadjuvant chemotherapy regimens resemble those of advanced treatment, frequently employing anthracycline-based agents. Meta-analyses demonstrated a 6% reduction in mortality risk with perioperative chemotherapy and an 11% reduction with the standard A + I regimen (Woll et al., 2012). Phase III trials have shown that the A + I regimen is superior to trabectedin (Gronchi et al., 2017; Gronchi et al., 2020). The results of phase II trials are promising (Tanaka et al., 2015; Tanaka et al., 2019). There is no evidence supporting the use of neoadjuvant chemotherapy for resectable RPS (Gronchi et al., 2021; Swallow et al., 2021). The TARPSWG study recommended the adoption of the A + I regimen for Grade 3 DDLPS patients (Tseng et al., 2021). The STRASS2 trial evaluated histology-tailored neoadjuvant chemotherapy, with the DDLPS regimen being doxorubicin + ifosfamide (Istl and Gronchi, 2022).

3.2.2 Systemic therapy for unresectable, advanced, or metastatic LPS

The efficacy of chemotherapy and overall survival rates vary depending on the LPS subtype (Schöffski, 2022). MLP exhibits high sensitivity to chemotherapy (Hindi and Haas, 2022); PLPS shows relative sensitivity to chemotherapy (Italiano et al., 2012); DDLPS demonstrates some response, whereas ALT/WDLPS are generally insensitive to chemotherapy. The median overall survival (mOS) for chemotherapy-sensitive subtypes in advanced stages is approximately 2 years (Abbas Manji et al., 2015). Presently, the first-line systemic therapy regimen is D + IFO (Crago and Dickson, 2016); however, there are inadequate specific research data on the various subtypes of LPS. Stacchiotti et al. reported that WD/DDLPS patients had response rates of 6.3% and 13% to first-line anthracycline and ifosfamide chemotherapy, respectively, whereas in the D + IFO group, the response rate was 22%. The response rate to first-line chemotherapy is significantly greater in MLPS patients than in WDLPS/DDLPS patients (48% vs. 11%) (Jones et al., 2005), and the overall survival with first-line chemotherapy is significantly longer in MPLS patients than in DDLPS/PLPS patients (Langmans et al., 2019).

Second-line and subsequent regimens include high-dose continuous infusion of ifosfamide, gemcitabine-based combination therapy (such as docetaxel and dacarbazine), and novel chemotherapy agents, including trabectedin, eribulin, and dacarbazine (Chamberlain et al., 2021). Second-line treatment with trabectedin significantly prolongs progression-free survival (PFS) in advanced LPS patients (Patel et al., 2019; Le cesne et al., 2021; De sande gonzález et al., 2020; Vincenzi et al., 2023). MLS is more sensitive to trabectedin (Assi et al., 2019). For unresectable/recurrent STS patients, the overall median PFS is 3.7 months, with a median PFS of 17.4 months for MLS patients and 3.7 months for DDLPS patients (Kobayashi et al., 2020). Trabectedin can be safely administered to elderly STS patients who are unsuitable for first-line

anthracycline therapy (Grosso et al., 2020). Eribulin affects tumor cells and the microenvironment by inhibiting microtubule growth and through various molecular mechanisms (De vita et al., 2021), with chemotherapy-induced peripheral neuropathy (CIPN) being the principal adverse effect. Phase III trials indicate that eribulin monotherapy is superior to dacarbazine monotherapy in patients with locally advanced, recurrent, or metastatic LPS (Frapolli et al., 2019). Novel treatment strategies, such as the combination of eribulin with lenvatinib and eribulin combined with gemcitabine, show promising efficacy (Chen et al., 2022; Kim et al., 2022). Future directions involve enhancing efficacy, mitigating toxicity, and identifying biomarkers to predict treatment response. Other novel agents, such as cabazitaxel, exhibit favorable activity in advanced DDLPS (Sanfilippo et al., 2022). Ascorbic acid and carfilzomib also demonstrate potential therapeutic effects (Schoenfeld et al., 2018; Jeitany et al., 2021).

3.2.3 Others

Research by Miao Chengli et al. revealed that performing HIPEC after surgery for retroperitoneal LPS can significantly reduce mortality and recurrence rates (Miao et al., 2022). Angeles et al. discovered that SN-38 induces apoptosis in DDLPS cells by increasing C/EBPα protein expression (Angeles et al., 2022).

3.3 Targeted therapy

3.3.1 Targeting MDM2

Currently, the MDM2 inhibitor DS-3032b shows potential efficacy in patients with WDLPS/DDLPS (Bauer et al., 2018), with comparative trials underway (Gounder et al., 2022a). The efficacy of AMG 232 is also under investigation (Gluck et al., 2020), and the MANTRA trial revealed that milademetan has failed as a second-line treatment for unresectable or metastatic DDLPS patients (Jones et al., 2023). Brigimadlin has demonstrated potential antitumor activity in DDLPS/WDLPS (Lorusso et al., 2023), with global phase II/III studies currently underway. Studies by Cissé MY et al. reported that MDM2-mediated serine metabolism control is a driving force in the growth of LPS (Cissé et al., 2020), whereas Seligson ND et al. suggested that targeting HDAC2 may be a potential strategy for modulating MDM2 expression in DDLPS (Seligson et al., 2019).

3.3.2 Targeting CDK4

In WD/DDLPS, the amplification rate of CDK4 is as high as 90%, making CDK4 another viable target (Assi et al., 2020). The CSCO guidelines recommend palbociclib as a second-line treatment, but practical application studies show poor outcomes (Nassif et al., 2022). The combination of palbociclib with recombinant methionase enhanced the efficacy of palbociclib (Higuchi et al., 2022). A phase III study of abemaciclib versus placebo is underway. MDM2 inhibitors combined with CDK4/6 inhibitors show manageable toxicity and good antitumor activity in advanced-stage patients (Abdul Razak et al., 2022).

3.3.3 Targeting vascular endothelial growth factor

Studies have shown that LPS contains more microvessels (Baneth et al., 2005) and is more sensitive to antiangiogenic

therapy. Anlotinib, as a second-line treatment for STS, is included in the CSCO guidelines. The ALTER0202 study showed significant efficacy, with a 12-week PFR of 63% and mPFS and mOS of 5.6 and 13 months, respectively (Chi et al., 2018). The ALTER-S006 study indicated that patients who were maintained on anlotinib after first-line chemotherapy had an mPFS of 12.5 months (Xu et al., 2023). Retrospective studies have shown that treatment with anlotinib in patients with metastatic or recurrent WDLPS/DDLPS resulted in an mPFS of 27.9 weeks, a 24-week PFR of 58.8%, and an OS of 56.6 weeks (Li et al., 2021).

3.3.4 Multitargeted tyrosine kinase inhibitors

Pazopanib is a second-line treatment option for STS recommended by the National Comprehensive Cancer Network (NCCN) guidelines (Cassinelli et al., 2022), but its efficacy as a monotherapy for LPS is limited. Phase II research revealed that pazopanib treatment resulted in a 12-week PFR of 68.3%, and the mPFS for DDLPS patients was 6.24 months (Samuels et al., 2017). A German phase II trial compared the efficacy of pazopanib combined with gemcitabine versus pazopanib alone in treating refractory LPS/LMS patients, noting an increase in toxicity with the combination treatment, which was manageable; however, phase III trials are needed to confirm its efficacy (Schmoll et al., 2021). Another phase II study evaluating preoperative pazopanib in high-risk STS patients reported no benefit (Ronellenfitsch et al., 2019). The SARC024 study indicated that regorafenib has poor efficacy in patients with advanced LPS (Riedel et al., 2020).

3.3.5 Additional potential targets

PARP-1 has emerged as a new therapeutic target for treating LPS (Bertucci et al., 2019). The TOMAS2 study from Italy revealed that the combination of trabectedin and the PARP inhibitor olaparib is effective in the treatment of LPS/LMS (D'Ambrosio et al., 2023). XPO1 represents another potential therapeutic avenue (Gounder et al., 2016), with selinexor demonstrating enhanced tumor responses in retroperitoneal DDLPS-PDXs (Thirasastr and Somaiah, 2022). The SEAL study revealed that the median PFS for advanced DDLPS patients treated with selinexor as second-line therapy was 2.8 months and that CALB1 could serve as a predictive biomarker (Gounder et al., 2022b). Selinexor treatment can reduce the pain rate in late-stage DDLPS patients, with a slower deterioration in quality of life (Gounder et al., 2021). Future endeavors should continue multidisciplinary research to explore novel drug targets and individualized treatment approaches.

3.4 Immunotherapy

Multiple clinical trials have explored immunotherapies for STSs, including ICIs, tumor vaccines, immune modulators, and TCR-T-cell therapy. Although STSs are considered “immunologically inert or cold” tumors, recent biomarker studies have shown significant immunoheterogeneity among different subtypes (Moreno tellez et al., 2022; Zhu et al., 2020). Biomarker-driven and tissue subtype-customized immunotherapy holds promise for improving the efficacy of immunotherapy (Roulleaux dugage et al., 2021). Immunotherapy combined with other treatment modalities, such as chemotherapy and radiotherapy, can transform “cold”

tumors into “hot” tumors (Rytlewski et al., 2021). Efficacy biomarkers such as TLSs, PD-L1 expression, and the TMB stratify patients to optimize efficacy, design improved clinical trials, and potentially enhance the effectiveness of immunotherapy (Nakata et al., 2021).

3.4.1 Monotherapy immunotherapy

Monotherapy with ICIs has not yet demonstrated definitive clinical benefits, but pembrolizumab has been shown to have antitumor effects in DDLPS-PDX models (Choi et al., 2020). In SARC028, the DCR for advanced STS patients was 18%, with an ORR of 40% for UPS and 20% for LPS (Tawbi et al., 2017; Burgess et al., 2019). In the Alliance A091401 trial, the overall response rate (ORR) of nivolumab monotherapy in metastatic STS patients was merely 5%.

3.4.2 Combination immunotherapy

Immunotherapy is continuously evolving in the field of LPS, with efforts focused on genomic analysis and research into the tumor immune microenvironment to identify additional combination treatment strategies, aiming to improve the effectiveness of immunotherapy in LPS patients.

3.4.2.1 Immunotherapy combined with chemotherapy

In patients with STS, the ORR of combination therapy with pembrolizumab and doxorubicin was 36.7%, with an mPFS and OS of 5.7 months and 17 months, respectively (Livingston et al., 2021). Among DDLPS patients, 1 patient achieved a complete response (CR), 1 patient achieved a partial response (PR), and 2 patients had stable disease (SD). In patients with L-type sarcoma treated with avapritinib combined with trabectedin, among 11 LPS patients, 7 patients achieved the best response of stable disease (SD), and 1 patient achieved disease stability for over 2 years (Wagner et al., 2022).

3.4.2.2 ICIs combined with antiangiogenic targeted therapy

Previous studies have shown that low-grade sarcomas typically exhibit a weak immune response and that antiangiogenic drugs can convert the immune microenvironment from “cold” to “hot,” increasing the sensitivity of the immune microenvironment to immunotherapy (De vita et al., 2016). J. Wu et al.'s retrospective study (Wu et al., 2023) investigated the treatment of L-type sarcomas with carfilzomib in combination with anlotinib and aidiublin. The ORR was 19.4%, and the DCR was 72.2%. Among nonsurgical patients, the mPFS values for LPS and LMS were 5.5 months and 6.2 months, respectively. Research by Zhou et al. revealed the satisfactory efficacy of pembrolizumab combined with anlotinib and paclitaxel in treating STS, with hematologic toxicity associated with paclitaxel being the primary adverse effect (Zhou et al., 2023).

3.4.2.3 Immunotherapy combined with small molecule inhibitors targeting epigenetics

Various subtypes of STSs exhibit defects in DNA damage repair and abnormalities in epigenetic regulation. Although epigenetic drugs can stimulate the immune system, increasing the immunogenicity of tumors, they may still suppress immune responses in the absence of immune checkpoint inhibitors

(Keenan et al., 2019; Nacev et al., 2020). Recent research (Starzer et al., 2021) suggests that the DNA methylation characteristics of tumors may serve as markers for the response to PD-1 ICI therapy in sarcomas. Que et al. reported HDAC gene amplification in patients with LPS, and the HDAC inhibitor chidamide increased PD-L1 expression, facilitating tumor regression (Que et al., 2021). Phase II trials have demonstrated that the combination of chidamide and trastuzumab is highly effective in treating STSs and has good tolerability, indicating promising therapeutic potential (Zhang et al., 2023). Ongoing clinical trials of tazemetostat combined with durvalumab for the treatment of STSs (NCT04705818) may offer new hope for patients.

3.4.2.4 Dual immunotherapy

In a phase II trial conducted by MD Anderson (NCT02815995), the efficacy of the PD-L1 monoclonal antibody durvalumab and the CTLA-4 monoclonal antibody tremelimumab in refractory advanced STSs was evaluated (Somaiah et al., 2022). The ORR was 12%, with a 12-week PFS rate of 49%, a median PFS of 2.8 months, and an mOS of 21.6 months. No effects were observed for LPS or the other subtypes.

3.4.3 Others

In addition to ICIs, immunotherapy involving immune cell therapy is also utilized in patients with STSs. CAR-T-cell therapy and TCR-T-cell therapy are still in their early stages and face various challenges. In the SPEARHEAD-1 study, afami-cel was used to treat patients with MRLPS or SS, resulting in 2 cases of CR, 8 cases of PR, and 11 cases of SD out of 25 patients. NY-ESO-1 is one of the most immunogenic TAAs, with a positivity rate of 89%–100% in MRLPS. Phase I/Ib studies of NY-ESO-1 TCR/IL-15 NK cells are currently underway. In recent years, the CMB305 vaccine has also been utilized in STS research, enhancing immune responses to the NY-ESO-1 antigen. A phase II trial (Chawla et al., 2022) evaluating CMB305 in combination with atezolizumab for MLS/SS patients revealed no significant extension of PFS or OS, but some patients exhibited anti-NY-ESO-1 immune responses, with seemingly favorable radiographic responses. IFN- γ alters the TME, increases antigen presentation, reduces T-cell exhaustion, and can convert tumors into “hot” tumors, potentially synergizing with PD-1 antibodies (Zhang et al., 2019). In an IB/II trial conducted by the University of Iowa (Monga et al., 2021), TVEC combined with neoadjuvant radiotherapy for STSs resulted in SD in 66.7% of patients, PR in 1 MLS patient, death due to PD in 2 patients, and pCR in 7 patients (24%), with 2-year PFS and OS rates reaching 57% and 88%, respectively, without postoperative local recurrence.

Emerging therapeutic modalities such as antibody-drug conjugates (ADCs) and oncolytic viruses demonstrate promising therapeutic potential. Initially deployed in hematologic malignancies, ADC-based therapies achieved their first breakthrough in solid tumors with HER2-positive breast cancer. As of 2024, no ADC clinical trials targeting liposarcoma have received regulatory approval (Xi et al., 2024). The leucine-rich repeat-containing protein 15 (LRRC15), overexpressed in sarcoma-associated cancer-associated fibroblasts, has emerged as a compelling anticancer target. LRRC15-directed ADCs may substantially improve clinical outcomes for sarcoma patients (Ray

et al., 2022). Preclinical evidence indicates that BB-1701—a novel eribulin-based ADC engineered for HER2 targeting—represents a potential therapeutic advancement for liposarcoma management (Wang et al., 2024). Talimogene laherparepvec (T-VEC), an oncolytic herpes simplex virus type 1, holds the distinction of being the first oncolytic virus approved by the US FDA and European Medicines Agency (Greig, 2016). In a phase IB/II trial involving 30 patients with locally advanced soft tissue sarcoma (STS), preoperative intratumoral T-VEC combined with concurrent external beam radiotherapy (EBRT) demonstrated no treatment-related herpes infections. The 2-year progression-free survival (PFS) and overall survival (OS) rates were 57% and 88%, respectively (Monga et al., 2021). A phase II trial enrolling 20 patients with locally advanced or metastatic sarcoma evaluated T-VEC plus pembrolizumab, yielding an overall objective response rate (ORR) of 35%, with 20% grade 3 treatment-related adverse events (TRAEs) and no grade 4 TRAEs (Kelly et al., 2020). Another phase II study of 39 pretreated advanced sarcoma patients investigated the TNT regimen (T-VEC + trabectedin + nivolumab), reporting an ORR of 7.7%, disease control rate (DCR) of 84.6%, median PFS of 7.8 months, and median OS of 19.3 months (Chawla et al., 2023). Novel combinatorial therapeutic strategies incorporating oncolytic viruses remain under active investigation.

3.5 Radiotherapy

Postoperative LPS is prone to recurrence, and radiotherapy can improve local control rates. Therefore, radiotherapy is strongly recommended for patients with high-risk localized recurrence profiles, while therapeutic de-escalation through radiation omission represents a viable strategy for those with low recurrence probability (Salerno, 2022). For most patients, preoperative delivery of radiation therapy is preferred. In patients initially thought to be at low risk for local recurrence and found to have unexpected adverse pathologic features at resection, postoperative radiation therapy is indicated. In select patients who received preoperative radiation and have close or positive margins, postoperative boost may be considered (Salerno, 2022).

MLS is sensitive to radiotherapy and is an important target for radiotherapy. Multiple studies have shown that neoadjuvant radiotherapy combined with surgical resection can achieve a 5-year local control rate of 96%–98% (Guadagnolo et al., 2008; Chung et al., 2009; Moreau et al., 2012). Research (Chen et al., 2021) has shown that the interaction between FUS-CHOP and chromatin remodeling complexes regulates sarcoma cell proliferation, explaining the sensitivity of MLS to radiotherapy. Phase II/III trials (Bonvalot et al., 2019) evaluating neoadjuvant radiotherapy combined with NBTXR3 versus radiotherapy alone in advanced STSs have shown a significant increase in the R0 resection rate (81% vs. 66%; $P = 0.042$). The standard neoadjuvant radiation therapy dose for MLS is 50 Gy/25 fractions. Low-dose radiation therapy may reduce the complications associated with preoperative radiation therapy while maintaining disease control. A phase II trial (Lansu et al., 2021a) revealed that low-dose preoperative radiation therapy (36 Gy) had comparable efficacy in nonmetastatic MLS and could reduce complications. Another phase II trial (Lansu et al., 2021b)

showed that moderate-dose preoperative radiation therapy (36 Gy) could improve the resectability of MLS while preserving clear margins and function.

Radiation therapy is also under investigation for RLPS. The STRASS phase III study (Lam et al., 2021) demonstrated that neoadjuvant radiation therapy reduced the risk of local recurrence in patients with resectable RLPS, with a 3-year ARFS rate of 71.6%. The TARPSWG study (Haas et al., 2019) enrolled 607 RLPS patients, and univariate analysis revealed that perioperative radiation therapy had local control advantages in all three cohorts, but no survival benefit was confirmed after adjustment. An analysis of 2082 RLPS patients from the American Cancer Database revealed that neoadjuvant radiation therapy conferred survival benefits, with a mOS of 129.2 months vs. 84.3 months, with more pronounced effects in those with involvement of adjacent organs. Multidisciplinary discussions are recommended to formulate initial treatment plans, and the selective use of RT may be considered for those at high risk of local recurrence (Istl and Gronchi, 2022; Callegaro et al., 2023).

In the radiotherapeutic management of liposarcoma, emerging modalities continue to undergo rigorous investigation. A clinical study validated the safety profile of proton and carbon ion particle therapy for dedifferentiated liposarcoma (DDLPS), demonstrating favorable overall survival (OS) and local control (LC) outcomes (Kubota et al., 2024). A retrospective analysis of stereotactic body radiotherapy (SBRT) in sarcoma pulmonary metastases revealed prolonged disease-free intervals among oligometastatic patients, with a median survival duration of 40.7 months (Lee et al., 2023). Evidence indicates stereotactic ablative radiotherapy (SABR) serves as a viable local control strategy for limited pulmonary oligometastatic disease, exhibiting minimal toxicity (Baumann et al., 2020; Baumann et al., 2016). A multicenter trial evaluating SABR in oligometastatic soft tissue sarcoma (STS) established its therapeutic efficacy and safety profile, with 20% of patients maintaining progression-free status at 2-year follow-up (Franceschini et al., 2024).

3.6 Alternative local therapeutic approaches

Emerging locoregional therapeutic modalities including percutaneous radiofrequency ablation (RFA), cryoablation, and high-intensity focused ultrasound (HIFU) are being increasingly utilized in liposarcoma management. A clinical case demonstrated sustained tumor-free survival exceeding 24 months following RFA treatment in a patient with third recurrence of retroperitoneal liposarcoma involving the left psoas muscle (Keil et al., 2008). Koichiro et al. conducted a retrospective multicenter analysis of percutaneous RFA in 52 recurrent bone and soft tissue sarcoma patients, reporting a 1-year overall survival (OS) rate of 73.4% with minimal major complication rate (0.9%), confirming RFA as a safe and effective option for advanced sarcomas (Yamakado et al., 2014).

A retrospective study of percutaneous cryoablation in 141 adults with recurrent/metastatic soft tissue sarcomas documented 217 ablation procedures achieving adequate ice-ball coverage in 82% (204/250) of lesions. The cohort exhibited a 2% complication rate (4/217) with favorable survival outcomes: 89% 1-year OS and 80% 2-year OS (Pal et al., 2024). Another real-world analysis of

67 recurrent/metastatic STS patients undergoing 189 cryoablation procedures for 104 lesions demonstrated an objective response rate (ORR) of 65.38% and disease control rate (DCR) of 86.54%, with survival analysis indicating prognostic improvement (Wu et al., 2021).

In HIFU applications, a study treating 29 lesions in 22 solid tumor patients achieved near-complete MRI-confirmed ablation in liposarcoma cases with symptomatic relief (Orgera et al., 2011). Yu et al. reported 51.8% ORR and 85.2% local control rate in 27 patients with locally unresectable sarcomas undergoing HIFU, with no severe treatment-related complications observed (Yu et al., 2015).

3.7 Multidisciplinary discussions

STSs are diverse and rare, and nonspecialist doctors should refrain from diagnosing and treating them. The MDT diagnostic and treatment model is essential in the management of STSs (Kawai et al., 2022).

4 Prognosis

4.1 Prognostic factor analysis of primary nonmetastatic extremity or trunk liposarcoma

For patients with nonmetastatic extremity or trunk liposarcoma, studies have shown that tumor size and subtype are independently associated with distant metastasis-free survival (DSD) and disease-specific survival (DR), whereas size, subtype, and R1 resection are independently associated with local recurrence (LR) (Bartlett et al., 2021). These findings suggest that the patterns, risks, and timing of postoperative recurrence vary by subtype, which can guide the development of targeted treatment measures for patients.

4.2 Prognostic factor analysis of patients with retroperitoneal liposarcoma

Large-scale population-based international cohort studies have consistently identified advanced age as an independent prognostic factor for overall survival (OS) and cancer-specific survival (CSS) in patients with retroperitoneal liposarcoma (Li et al., 2022; Singer et al., 2003). Gender-specific analysis reveals males exhibit inferior survival outcomes compared to females following primary resection of RLPS, particularly in subsets with low-grade histology or undergoing non-radical resection (R1/R2 resections) (Ren et al., 2024). An Asian multicenter cohort study of 211 patients demonstrated independent associations between American Society of Anesthesiologists (ASA) physical status classification, Clavien-Dindo complication grading system, and long-term OS (Zhuang et al., 2021).

Tumor-related characteristics significantly impact prognostic outcomes in retroperitoneal liposarcoma (RLS) patients. Retrospective cohort analysis demonstrates inferior disease-free survival (DFS) in dedifferentiated histology compared to well-differentiated subtypes (Osuna-soto et al., 2021). Histologic

subtype emerged as an independent predictor of progression-free survival (PFS) (Singer et al., 2003; Zhuang et al., 2021). The Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grading system and myogenic differentiation status constitute critical prognostic determinants (Gronchi et al., 2015). Additionally, tumor anatomical location and presence of necrosis may serve as independent pathologic prognostic indicators (Sun et al., 2021). Tumor rupture and major postoperative complications (Dindo-Clavien grade $\geq$ III) adversely affect overall survival (OS) (Brehat et al., 2023). Patients developing multifocal recurrence exhibit particularly dismal clinical outcomes (Deng et al., 2023a).

Surgical margin characteristics significantly influence oncologic outcomes in retroperitoneal liposarcoma (RLS). The presence of dedifferentiated (DD) components at resection margins correlates with diminished local recurrence-free survival (LRFS) (Dehner et al., 2021). A comparative effectiveness study demonstrated that total (ipsilateral) retroperitoneal lipectomy (TRL), when contrasted with conventional complete resection (CR), confers significant improvements in both recurrence-free survival (RFS) and overall survival (OS) for primary RPLS patients (Gao et al., 2024).

Recurrence patterns critically determine clinical prognosis in RLS management. Patients exhibiting DR patterns demonstrate more favorable survival trajectories compared to those with early multifocal recurrence (Deng et al., 2023b). Homsy. P et al. analyzed 107 RPL patients and reported that 72% experienced LR, whereas 15% experienced DR, indicating more local recurrence and fewer metastases (Homsy et al., 2020). After R0/R1 resection, histological type and grade were important predictors of DSS, with multifocal LR having a poorer prognosis and a higher DR rate with high-grade histology. Improt. L et al. studied 109 RPL cases, with a 5-year OS rate of 67%, a DFS rate of 53.2%, an LR rate of 25.7%, and a DM rate of 12.1%, with lung metastasis being the most common. Patients with complications had better DFS and OS, and HOI-3 was an independent risk factor for DM, OS, and DFS (Improt. et al., 2023). One study reported a 6-year DFS rate of 19.2% and an OS rate of 54.1% for RPS-LR1 patients, with recurrence patterns associated with histological subtypes, and the CCI for the second LR of LPS was the highest (60.2%–70.9%). Column charts predicting DFS and OS were established, incorporating multiple factors (Raut et al., 2019). The TARPSWG study analyzed RPS-R2 patients and reported a 70.5% incidence of second recurrence, with an LR accounting for 80.75%, predominantly LPS (Van houdt et al., 2020). Singaporean scholars proposed a five-gene prognostic model for retroperitoneal DDLPS, which better predicted overall survival than did clinical factors (Shannon et al., 2021).

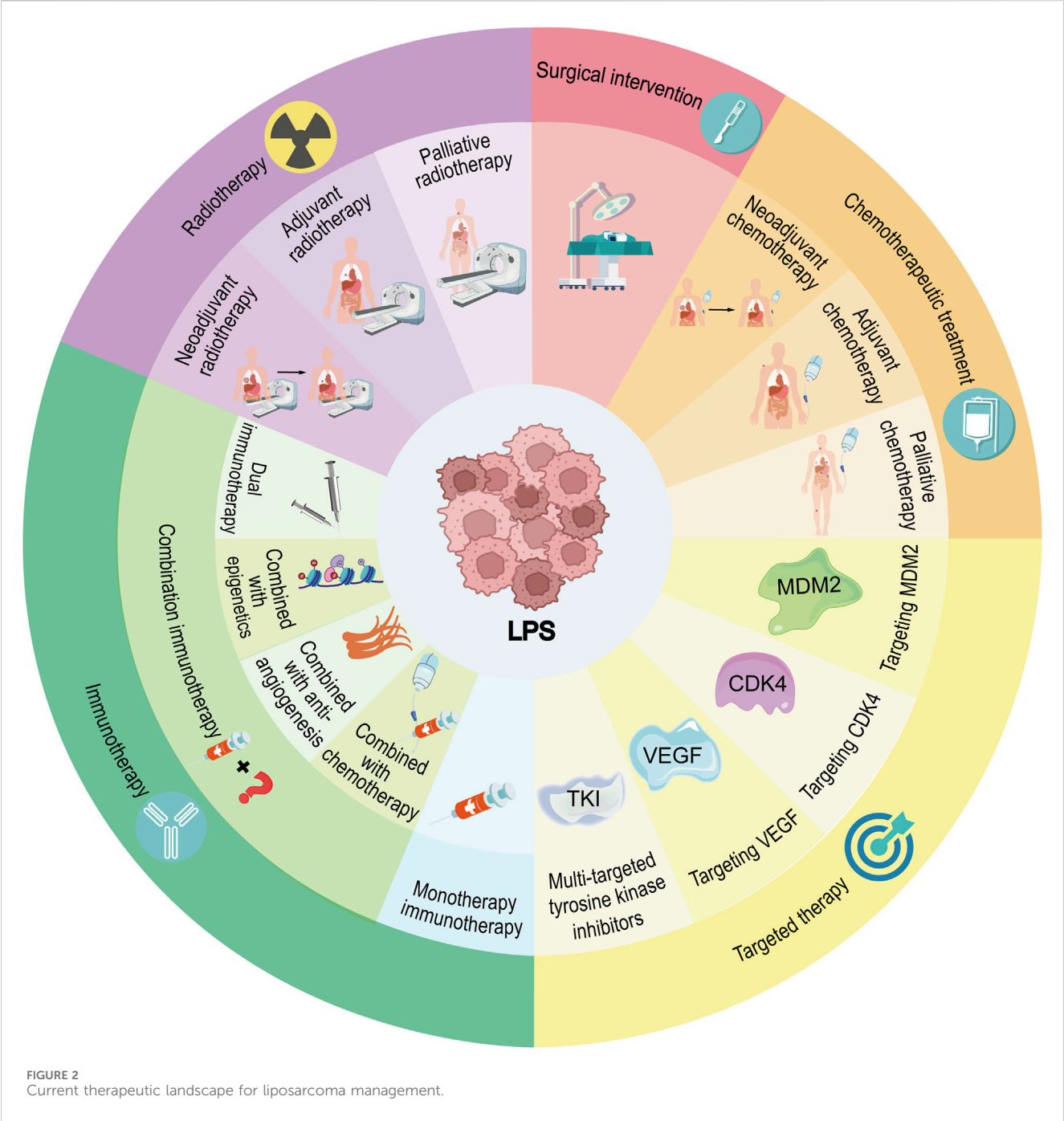
4.3 Potential prognostic significance of immune-related molecular markers and tumor-infiltrating immune cells in LPS

Miyake M et al. reported that PD-L1 expression was higher in retroperitoneal DDLPS and retroperitoneal LMS than in other sarcomas (Miyake et al., 2020). Serum LDH levels were moderately positively correlated with PD-L1 and PD-L2 expression. Higher PD-1 expression was associated with an increased risk of recurrence; High expression of Ki-67 and stage IIIB disease were independent predictors of RFS and DSS. The

Ki67 proliferation index has been established as an independent prognostic factor for recurrence, metastasis, and overall survival (OS) in retroperitoneal liposarcoma (RLS) patients undergoing complete resection (Gao et al., 2023). Schroeder BA et al. reported that high TCR clonality combined with a low T-cell fraction predicted a lower 3-year OS rate, that CD4⁺ T cells were associated with better outcomes, and that CD14⁺ monocytes were associated with poorer prognosis (Schroeder et al., 2021). In recent years, research on tertiary lymphoid structures (TLSs) in patients with STSs has increased, with more TLS patients showing longer OS and PFS, associated with increased expression of the TNFRSF14 and DUSP9 genes, and better immunotherapy outcomes (Xiang-Xu et al., 2023). Inflammatory biomarkers such as the NLR or PLR fail to accurately predict survival (Schwartz et al., 2020), with tumor-related factors remaining the best predictors. Kim KM et al. reported that baseline inflammatory markers such as IL-6 were associated with early recurrence of STSs (Van der laan et al., 2023). The nuclear expression of 4Ra and IL13Ra1 is associated with shortened OS and RFS (Kim et al., 2021). Next-generation sequencing (NGS)-based detection of circulating cell-free DNA (cfDNA) in sarcoma patients facilitates diagnostic refinement and longitudinal disease monitoring (Mc connell et al., 2020). Circulating tumor DNA (ctDNA) demonstrates clinical utility in tracking minimal residual disease (MRD) and early recurrence patterns (Braig et al., 2019). Thrombospondin-2 (Tsp2) encoded by THBS2 serves as an independent predictor of disease-free survival (DFS) and recurrence-free survival (RFS) in RLS cohorts (Xu et al., 2022). Fibroblast growth factor receptor substrate 2 (FRS2) exhibits high positivity rates in primary RPLS tumor specimens, demonstrating significant correlation with recurrence dynamics and survival outcomes (Chen and Miao, 2023).

5 Summary and prospects

Recent research has revealed significant findings and trends in the treatment and prognosis of liposarcoma. Various treatment modalities can be seen in Figure 2. Overall, liposarcoma treatment and prognosis are influenced by various factors, including the tumor type, grade, histological characteristics, and the immune environment. Surgical resection remains the primary treatment modality for tumors such as retroperitoneal liposarcoma (RPL) and retroperitoneal sarcoma (RPS), although the risk of local recurrence after surgery is high. Advances in medical technology are expected to enhance minimally invasive surgery and precision radiotherapy, reducing treatment-related complications and side effects and thus improving patient quality of life. Adjuvant radiotherapy before and after surgery, along with novel immunotherapy, may become integral parts of treatment strategies. Preoperative radiotherapy has shown efficacy in lowering the risk of local recurrence, but the effectiveness of perioperative radiotherapy remains uncertain. Immunotherapy has exhibited potential efficacy in some studies, particularly for patients with high PD-L1 and PD-L2 expression. Future research may delve deeper into the mechanisms and efficacy of immunotherapy and identify more precise prognostic markers to personalize treatment regimens, ultimately increasing patient survival rates and quality of life.



In addition to advancements in treatment, prognosis evaluation has become more precise. By integrating various factors, such as tumor characteristics, patient factors, and treatment response, we can establish more reliable prognostic models to assist physicians and patients in making informed treatment decisions. Further research in patients with retroperitoneal liposarcoma (RPL) and retroperitoneal sarcoma (RPS) suggests that peripheral blood inflammatory markers and specific biomarkers in tumor tissue may aid in predicting early recurrence and survival rates for postoperative patients. Additionally, with a deeper understanding of tumor immunology and genomics, coupled with the ongoing development of immunotherapy and targeted therapy, personalized

treatment, including targeted therapy and immunotherapy tailored to specific tumor subtypes, is poised to become a future trend. Our approach to treating liposarcoma will also become more personalized and precise. Genomic and biomarker studies will further our understanding of tumor development mechanisms and prognostic factors, providing a stronger scientific basis for personalized treatment. Furthermore, ongoing clinical trials will present opportunities for the development of novel treatment modalities and drugs, offering patients more options and improving treatment success and survival rates.

In the future, comprehensive assessment and personalized treatment plans based on multidisciplinary teams will be key to

improving the prognosis of patients with liposarcoma. Additionally, conducting more large-scale clinical trials and molecular biology research is expected to provide deeper insights and breakthroughs in the treatment and prognosis of this field. Strengthening multicenter clinical research and data sharing is also a future direction to promote a comprehensive understanding of the treatment and prognostic factors of liposarcoma, accelerate the clinical translation of new treatment methods, and continuously improve treatment outcomes and survival rates for patients. With advances in science and technology and further research, we hope to find more effective treatment methods and improve the quality of life and survival of patients with liposarcoma. We anticipate a brighter future for the treatment and prognosis of liposarcoma.

Overall, significant progress has been made in the treatment and prognosis research of liposarcoma, including the exploration and application of various treatment methods, such as surgery, radiotherapy, and immunotherapy, as well as the discovery and validation of new prognostic factors. However, many challenges and unknown factors remain. The application of novel treatment methods such as personalized treatment, immunotherapy, and targeted therapy has brought new hope for patients, but further research and clinical validation are needed to determine how to select and combine these treatment options better.

In the future, we expect further in-depth research into the pathogenesis, biological characteristics, and potential of targeted therapies for liposarcoma. With the continuous development of technology, the application of high-throughput technologies such as genomics, transcriptomics, and proteomics will provide us with more comprehensive and precise tumor classification and personalized treatment strategies. Moreover, the accumulation of clinical trials and practical experience will lead to more information on the effectiveness and safety of various treatment options, helping to guide clinical practice and improve patient prognosis. In the future, with the continuous advancement of technology and research methods, we can expect more accurate diagnostic methods, more effective treatment strategies, and more accurate prognosis evaluation models to emerge. Ultimately, we hope to provide liposarcoma patients with more effective and safer treatment methods, improving their quality of life and survival rate through various efforts.

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Supplementary material

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