



A phase II trial of second-line pemetrexed in adults with advanced/metastatic osteosarcoma [☆]

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Abstract *Background:* Osteosarcoma is the most common primary malignant tumour in young adults. An effective treatment strategy for relapsed patients is still not defined. Pemetrexed is a multitargeted antifolate with a mode of action similar to, and a range of action broader than that of methotrexate. The primary objective of this phase II study was to determine tumour response rate in patients with high-grade, advanced/metastatic osteosarcoma. Secondary endpoints included progression-free survival (PFS), overall survival (OS) and safety.

Methods: Pemetrexed 500 mg/m² was administered on day 1 of 21-day cycles with folic acid and vitamin B₁₂ supplementation. At least 5 tumour responses in a targeted population of 32 were required to consider further investigation.

Results: Thirty-two patients (median age, 43.3 years; range, 18.6–76.0) with 1 prior chemotherapy regimen for high-grade advanced/metastatic osteosarcoma were enrolled. Thirty (93.8%) patients had an ECOG performance status ≤1 and 29 (90.6%) had metastases in the lung. One patient had partial response (3.1%) and 5 (15.6%) had stable disease. Median PFS and OS were 1.4 months (95% CI: 1.4–1.7) and 5.5 months (95% CI: 2.3–10.5), respectively. The most common drug-related grade 3/4 toxicities were leukopaenia, asthaenia and elevated alanine aminotransferase in 3 (9.4%) patients each. One patient died due to multi-organ failure considered possibly related to the study drug.

Conclusions: Pemetrexed 500 mg/m² administered on day 1 of 21-day cycles as second-line treatment to patients with advanced/metastatic high-grade osteosarcoma was generally well tolerated but did not meet minimal response expectations for further investigation in this patient population.

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1. Introduction

Osteosarcoma is the most frequent primary cancer of bone (incidence: 0.03–0.2 per 100,000 per year).¹ At

presentation, only 20–25% of patients have radiologically detectable metastases; however, almost all patients with high-grade osteosarcoma have micrometastases.² Treatment of osteosarcoma requires complex multimodality therapy (surgery with pre- and post-chemotherapy). The most active chemotherapeutic agents in the treatment of osteosarcoma are high-dose methotrexate, doxorubicin, cisplatin and ifosfamide.^{2,3} The long-term survival rate of newly diagnosed patients with high-grade osteosarcoma without detectable metastasis has improved considerably compared with that of surgery alone (>60% versus 10–20%).¹

The value of second-line chemotherapy and the best treatment strategy for relapsed patients with high-grade, advanced or metastatic disease are not well defined.¹ This is further confounded by the fact that few studies exist about the management of patients with relapsed osteosarcoma due to the relative rarity of the disease.¹ Consequently, outcomes to second-line treatment, such as tumour response rate, are not well established. Thus, metastatic osteosarcoma remains difficult to treat, with a lack of consensus regarding the choice of second-line therapy, and as such, new agents are continuously being evaluated for efficacy.

Pemetrexed, a multitargeted antifolate, is approved for use in combination with cisplatin for unresectable mesothelioma,⁴ as a single agent for second-line as well as maintenance treatment of non-squamous advanced/metastatic non-small cell lung cancer (NSCLC),^{5,6} and in combination with cisplatin for first-line treatment of advanced/metastatic non-squamous NSCLC.⁷ Its primary mechanism of action is the potent inhibition of the folate-dependent enzyme thymidylate synthase^{8,9}; dihydrofolate reductase and glycinamide ribonucleotide formyl transferase are secondary targets.⁹ Pemetrexed has demonstrated broad-spectrum activity in various solid tumours and has a broader spectrum of action than its anti-folate predecessor, methotrexate, by virtue of its multitargeted capability and its ability to interfere with pyrimidine and purine synthesis. Given the wider range of action of pemetrexed compared with methotrexate, we decided to conduct this phase II trial evaluating pemetrexed treatment in patients with relapsed osteosarcoma to address this unmet medical need.

The primary objective of our phase II study of second-line pemetrexed therapy in patients with high-grade, advanced or metastatic osteosarcoma was to assess tumour response rate. Secondary objectives included measurements of progression-free survival (PFS), overall survival (OS) and safety.

2. Materials and methods

2.1. Eligibility criteria

Patients were eligible to participate in the study if they were ≥ 18 years of age, with an estimated life

expectancy of at least 12 weeks and a histologically documented diagnosis of high-grade locally advanced or metastatic osteosarcoma that was not amenable to surgery, radiation or combined modality therapy with curative intent. Patients must have received one prior chemotherapy regimen for advanced disease; neoadjuvant and adjuvant chemotherapies were not counted towards this requirement. Pemetrexed was considered as second-line chemotherapy for advanced/metastatic disease. Prior radiation therapy was allowed to $< 25\%$ of the bone marrow and must have been completed at least 4 weeks before study enrolment with complete recovery from acute toxic effects. Patients were required to have ≥ 1 unidimensionally measurable lesion (at least one measurable lesion outside the field of any prior radiation therapy) that met the Response Evaluation Criteria in Solid Tumours (RECIST)¹⁰; a performance status (PS) of 0–2 on the Eastern Cooperative Oncology Group (ECOG) scale and adequate haematologic (absolute neutrophil [segmented and bands] count [ANC] $\geq 1.0 \times 10^9/L$, platelets $\geq 100 \times 10^9/L$ [in case of bone marrow disease: $\geq 75 \times 10^9/L$] and haemoglobin ≥ 9.0 g/dL), renal (calculated creatinine clearance [CrCl] ≥ 45 mL/min based on the standard Cockcroft and Gault formula), cardiac and hepatic functions.

Patients were excluded from the study if they had a serious concomitant systemic disorder (eg, active infection); a serious cardiac condition, such as myocardial infarction within 6 months of enrolment, angina or heart disease (New York Heart Association Class III or IV); a prior malignancy other than osteosarcoma, carcinoma in situ of the cervix or non-melanoma skin cancer unless treated ≥ 5 years before enrolment with no evidence of recurrence; central nervous system metastases unless the patient completed successful local therapy for this disease and had not been receiving corticosteroids for ≤ 4 weeks before enrolment; the presence of clinically detectable (by physical exam) third-space fluid retention; and the inability to discontinue aspirin, other non-steroidal anti-inflammatory agents or corticosteroids.

All patients provided written informed consent before study participation. The study was approved by the appropriate ethical review boards and conducted according to all applicable laws and regulations, good clinical practices and the ethical principles of the Declaration of Helsinki.

2.2. Study design and treatment plan

In this phase II, open-label, multicentre, single-arm study, pemetrexed was supplied by Eli Lilly and Company (Indianapolis, IN) at a dose of 500 mg/m^2 by a 10-minute intravenous infusion on day 1 of 21-day cycles. All patients received oral folic acid and vitamin B₁₂ supplementation per the pemetrexed label.¹¹ In addi-

tion, patients received 4 mg (or equivalent) of prophylactic dexamethasone orally twice daily on the day before, the day of and the day after each dose of pemetrexed. Patients were to continue treatment until disease progression or unacceptable toxicity.

Pemetrexed dose adjustments were based on toxicity. All toxicities were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 3.0. Doses were reduced by 25% for a platelet count of $\geq 50 \times 10^9/L$ and an ANC $< 0.5 \times 10^9/L$ or $< 1.0 \times 10^9/L$ plus febrile neutropenia (fever $\geq 38.5^\circ C$), or for grade 4 transaminase elevation, grade 3 or 4 diarrhoea or any grade diarrhoea requiring hospitalisation, or any other grade 3 or 4 non-haematologic toxicity (except nausea and/or vomiting). Doses were reduced by 50% for a platelet count $< 50 \times 10^9/L$ with or without bleeding and any ANC or for grade 3 or 4 mucositis.

A patient who required a dose reduction continued to receive the reduced dose for the remainder of the study. For a recurrence of any grade 3 or 4 toxicity after two dose reductions, the patient was discontinued from treatment. Treatment may have been delayed up to 42 days from day 1 of the treatment cycle to allow a patient sufficient time to recover from study drug-related toxicity.

2.3. Baseline and treatment assessments

Baseline patient evaluations included a medical history, physical examination and radiologic (computed tomography scan [CT], bone scan or magnetic resonance imaging [when indicated]) assessment of lesions, blood chemistry (bilirubin, alkaline phosphatase, transaminases and creatinine) and haematology panels (haemoglobin, leucocytes [WBC], platelets, neutrophils and lymphocytes) and recording of PS.

Tumour response was assessed at baseline with a CT scan ≤ 4 weeks before the first dose of study treatment, according to RECIST criteria and after every two cycles (every 6 weeks) using the same method as that used at baseline. Best response (complete response [CR] or partial response [PR]) must have been confirmed with a second assessment performed ≥ 28 days and ≤ 42 days after the first evidence of response. OS was defined as the time from the date of study enrolment to the date of death from any cause. PFS was defined as the time from the date of study enrolment to the first date of progressive disease (PD) or death from any cause.

Safety was evaluated before each cycle via assessment of adverse events and physical examinations. Chemistry and haematology panels were performed on days 8 and 15 (± 3 days) as indicated, within 4 days prior to each cycle, and if possible at the first post-study visit. All patients who were treated with at least one dose of the study regimen were evaluated for efficacy and safety.

2.4. Statistical considerations

The target sample size was based on tumour response rate, the primary outcome measure. A probability of less than 0.05 for achieving response in the targeted population was to be considered unacceptable for warranting further investigation (null hypothesis), whereas a probability of at least 0.2 was to be considered acceptable (research hypothesis). Based on this assumption, a sample size of 32 eligible patients was needed to reject the null hypothesis with a power of approximately 80% and a significance level of 5% (two-sided) using an exact binomial test.¹² Therefore, if ≥ 5 patients had tumour responses in the 32 eligible patients, it could be concluded that the results of the trial warranted further investigation of pemetrexed in patients with advanced/metastatic osteosarcoma.

Response rate and two-sided 95% confidence intervals (CIs) were provided. For time-to-event variables, Kaplan–Meier curves were generated, and quartiles and point probabilities were calculated. Interval estimates were calculated using 95% CIs.

3. Results

The study was conducted from 11th September 2007, to 30th March 2009, in 12 centres involving 5 countries (France, Germany, Italy, Spain and the United Kingdom). A total of 32 patients were enrolled in the study, all of whom received at least 1 dose of pemetrexed.

3.1. Patient characteristics

The vast majority of patients had an ECOG PS of 0 or 1 and prior surgery (93.8% for each) (Table 1). The predominant metastatic site was the lung (90.6% of patients). A total of 12 (37.5%) patients received prior methotrexate chemotherapy (two methotrexate monotherapy and 10 methotrexate in combination with other agents). Eleven of the 12 patients were discontinued from the study due to disease progression and 1 patient died. The best response to prior systemic therapy was CR or PR for 4 (12.5%) patients and stable disease (SD) for 10 (31.3%) patients. Nine patients (28.1%) received prior radiotherapy (with adjuvant/curative intent for 5 patients).

3.2. Efficacy

Efficacy results are summarised in Table 2. Of the 32 evaluable patients, 1 (3.1%) patient had a PR and an additional 5 (15.6%) patients had SD; one of these patients (SD) received prior systemic treatment with methotrexate (adjuvant/curative intent). The single responding patient presented with right lung metastasis and an ECOG PS of 1, and received prior chemotherapy

Table 1
Baseline patient characteristics (*N* = 32).

| Variable | <i>n</i> (%) ^a |
|---|---------------------------|
| Gender | |
| Male | 20 (62.5) |
| Female | 12 (37.5) |
| Age, median (min, max), years | 43.3 (18.6, 76.0) |
| ECOG performance status | |
| 0 | 16 (50.0) |
| 1 | 14 (43.8) |
| 2 | 1 (3.1) |
| Unknown | 1 (3.1) |
| Prior surgery | 30 |
| Resection, complete | 8 (26.7) |
| Resection, partial | 10 (33.3) |
| Ablative | 15 (50.0) |
| Conservative | 15 (50.0) |
| Other | 13 (43.3) |
| Target lesions | |
| 0 | 1 (3.1) |
| 1 | 9 (28.1) |
| 2 | 8 (25.0) |
| 3 | 4 (12.5) |
| 4 | 3 (9.4) |
| 5 | 7 (21.9) |
| Metastatic sites | |
| Lung | 29 (90.6) |
| Bone | 8 (25.0) |
| Soft Tissue | 6 (18.8) |
| Lymph Node | 3 (9.4) |
| Oesophagus | 1 (3.1) |
| Liver | 1 (3.1) |
| Thymus | 1 (3.1) |
| Reasons for prior systemic regimen ^b | |
| First-line metastatic | 25 (78.1) |
| Locally advanced | 19 (59.4) |
| Neoadjuvant | 16 (50.0) |
| Missing | 1 (3.1) |
| Best response to last prior systemic regimen ^b | |
| Complete response | 1 (3.1) |
| Partial response | 3 (9.4) |
| Stable disease | 10 (31.3) |
| Progressive disease | 15 (46.9) |
| Unknown | 3 (9.4) |

ECOG indicates Eastern Cooperative Oncology Group.

^a Unless otherwise specified.

^b Systemic therapy included chemotherapy, biological therapy and hormonal therapy.

for metastatic disease. The duration of the PR was about 9.5 months. The patient was discontinued from the study (physician decision) before documenting PD. Median PFS was 1.4 months (95% CI: 1.4–1.7). Median OS was 5.5 months (95% CI: 2.3–10.5), with 31.3% of the patients alive at 1 year. Kaplan–Meier curves for PFS and OS are in Figs. 1 and 2, respectively.

3.3. Drug administration

The 32 patients in the study received a median of 2 cycles (range, 1–14) of pemetrexed. A total of 20

Table 2
Efficacy (*N* = 32).

| | |
|-------------------------------------|----------------------------|
| Overall response rate, <i>n</i> (%) | 1 (3.1%) |
| Complete response (CR) | 0 |
| Partial response (PR) | 1 (3.1%) |
| Stable disease (SD), <i>n</i> (%) | 5 (15.6%) |
| Progressive disease | 22 (68.6%) |
| Unknown ^a <i>n</i> (%) | 4 (12.5%) |
| Progression-free survival | |
| No. patients (No. censored) | 28 (4) |
| Median, months | 1.4 (95% CI: 1.4, 1.7) |
| 3-Month rate | 17.2% (95% CI: 3.5, 31.0) |
| 6-Month rate | 6.9% (95% CI: 0.0, 16.1) |
| Overall survival | |
| No. patients (No. censored) | 26 (6) |
| Median, months | 5.5 (95% CI: 2.3, 10.5) |
| 6-Month rate | 46.9% (95% CI: 29.6, 64.2) |
| 1-Year rate | 31.3% (95% CI: 15.2, 47.3) |
| 2-Year rate | 21.9% (95% CI: 7.6, 36.2) |

CI indicates confidence interval

^a Three patients died before the first tumour assessment (after cycle 1), and 1 patient discontinued the study without any tumour assessments.

(62.5%) patients received ≤ 2 cycles of pemetrexed; most of these patients (15, 75%) were discontinued from treatment due to PD, 4 due to death and 1 due to subject decision. A total of 7 (21.9%) patients required at least 1 dose reduction or cycle delay because of adverse events: alanine aminotransferase (ALT) increased (dose reduction, 1 patient; cycle delay, 3 patients), obesity (dose reduction, 1 patient), asthaenia (cycle delay, 1 patient) and erythema (cycle delay, 1 patient). The median relative dose intensity for pemetrexed was 96.9% (range, 59.5–105.2%).

3.4. Safety

The most commonly reported drug-related grade 3/4 toxicity was leukopaenia in 3 patients (9.4%, grade 3 only); grade 4 haematologic toxicities, neutropenia and thrombocytopenia, were reported in 1 (3.1%) patient each (Table 3). Asthaenia and ALT increases were the most common non-haematologic toxicities reported in 3 (9.4%) patients each. A total of 5 (15.6%) patients received at least 1 transfusion: packed red blood cells (4 patients) and platelets (1 patient).

Six patients died during the study (four due to disease progression and two due to adverse events). The adverse events that resulted in death for two patients were sudden death and multi-organ failure, respectively. Pulmonary embolism was suspected as the cause of the sudden death. The multi-organ failure was considered possibly related to pemetrexed treatment. Five days after the first dose of pemetrexed, this patient developed skin toxanemia with erythema on the face and upper back that quickly extended to soles of the feet and right index finger (grade 3). The patient quickly experienced

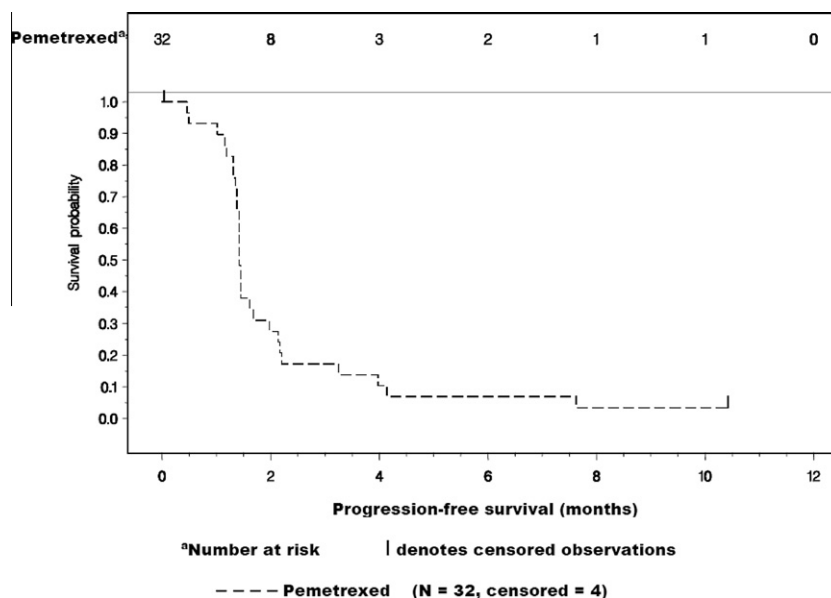


Fig. 1. Kaplan–Meier curve of progression-free survival.

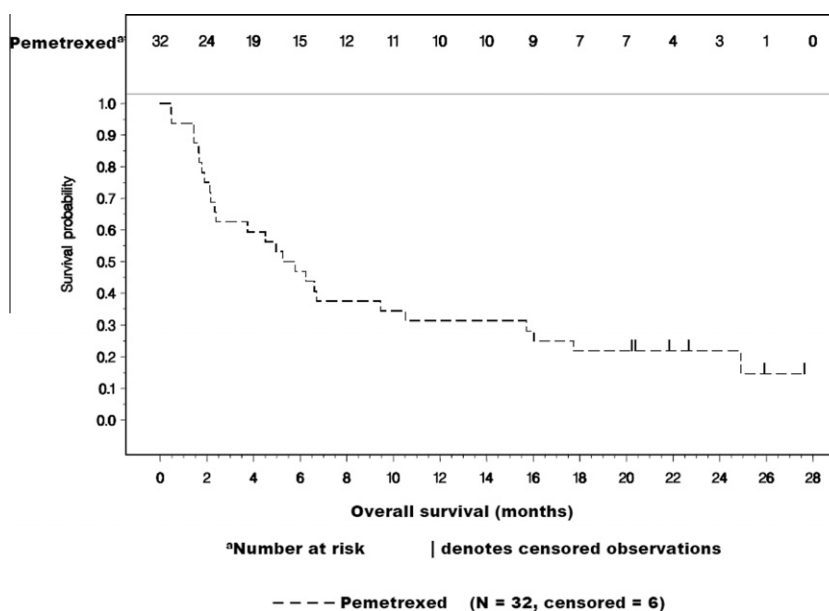


Fig. 2. Kaplan–Meier curve of overall survival.

general physical health deterioration with significant asthenia and anorexia, and increased pain but no fever. The patient continued in the study and died 9 days later due to multi-organ failure with renal and hepatic failure within the context of febrile neutropenia. The skin toxicity persisted until the patient's death.

Twenty-six additional patients were discontinued from the study: 22 due to PD, 2 due to physician decision, 1 due to subject decision and 1 due to an adverse event (asthenia, not drug related). A total of 17 (53.1%) patients received post-study therapy. Of the remaining 15 patients, who did not receive post-study

therapy, 6 died and 9 were discontinued from the study due to PD.

4. Discussion

Although the survival of patients with high-grade osteosarcoma has improved considerably since the introduction of chemotherapy, about 20–40% of patients with localised disease have local or distant recurrence.¹³ In patients with detectable metastatic osteosarcoma, the prognosis has only improved modestly, with only about 30% of these patients surviving long-

Table 3
CTCAE maximum grade 3/4 toxicities (*N* = 32).^a

| | Regardless of causality ^a | | Drug-related (possibly) | |
|------------------------------------|--------------------------------------|---------|-------------------------|---------|
| | Grade 3 | Grade 4 | Grade 3 | Grade 4 |
| Haematologic, <i>n</i> (%) | | | | |
| Leukopaenia | 3 (9.4) | 0 | 3 (9.4) | 0 |
| Anaemia | 3 (9.4) | 0 | 2 (6.3) | 0 |
| Neutropenia | 1 (3.1) | 1 (3.1) | 1 (3.1) | 1 (3.1) |
| Febrile neutropenia | 2 (6.3) | 0 | 2 (6.3) | 0 |
| Thrombocytopenia | 0 | 1 (3.1) | 0 | 1 (3.1) |
| Febrile bone marrow aplasia | 1 (3.1) | 0 | 1 (3.1) | 0 |
| Non-haematologic, <i>n</i> (%) | | | | |
| Asthaenia | 4 (12.5) | 0 | 3 (9.4) | 0 |
| Alanine aminotransferase increased | 3 (9.4) | 0 | 3 (9.4) | 0 |
| Toxic skin eruption | 0 | 1 (3.1) | 0 | 1 (3.1) |

CTCAE indicates Common Terminology Criteria for Adverse Events, version 3.0.

^a Additional events (grade 3 unless otherwise specified) reported in 1 patient were pericardial effusion (grades 3 and 4), dyspepsia, catheter-related infection, respiratory tract infection, sepsis, staphylococcal infection, urinary tract infection, lumbar vertebral fracture, blood alkaline phosphatase increased, transaminases increased, dizziness, headache, nerve compression, anxiety, haematuria, renal failure acute, ureteric obstruction, urinary retention and erectile dysfunction (grade 4 only).

term.¹ In the event of recurrence, long-term survival is poor (<20%).¹ Clearly, there is a need for new chemotherapeutic agents, especially in relapsed patients. Pemetrexed is a member of a new generation of multitargeted antifolates with a wider range of action compared with that of methotrexate.

Methotrexate, an analogue of folic acid, has been incorporated into treatment modalities for osteosarcoma since the introduction of chemotherapy treatment for this tumour, and has shown clinically relevant efficacy in osteosarcoma, especially when administered at high doses.³ Moreover, Wagener et al reported that low-dose methotrexate administration to 25 evaluable patients with metastatic osteosarcoma (of whom only 4 received prior chemotherapy) achieved CR in 3 (12%), SD in 12 (48%) and PD in 10 (40%) patients.¹⁴ In a more recent phase II study, a new antifolate, trimetrexate, was combined with high-dose methotrexate in an attempt to circumvent the mechanisms implicated in methotrexate resistance and improve outcomes to chemotherapy in patients with recurrent, high-grade osteosarcoma.¹⁵ The combination yielded a response rate of 13% in 38 evaluable patients. Of the 32 patients treated in our study, 12 (38%) received prior methotrexate, of whom one had SD and 11 progressed or died from the study disease. Although we could not determine if the patients in our study received low- or high-dose methotrexate, it is possible that prior methotrexate treatment may have played a role in the apparent resistance to pemetrexed treatment in these patients; however, as the overall response rate was low (3.1%), irrespective of the type of prior chemotherapy, it is difficult to draw any definitive conclusions in this regard.

In the current study, all patients were previously treated with systemic chemotherapy for advanced or metastatic osteosarcoma, and 90.6% presented with lung

metastasis. The primary efficacy outcome of best overall response (1 PR) was less than the expected 5 responders required by the study design for a successful outcome. Median PFS and OS times were low (1.4 and 5.5 months, respectively); however, 31.3% of these poor prognosis patients remained alive at 1 year. In addition, the patients fared well in terms of toxicity to pemetrexed treatment, with low rates of grade 3/4 toxicity (both haematologic and non-haematologic) and a median relative dose intensity of 96.9%.

The most active and widely used chemotherapies for the treatment of high-grade osteosarcoma are methotrexate, doxorubicin, cisplatin and ifosfamide. These 'conventional' agents are generally used in combination at various stages of the disease, although there is no clear evidence as to the best combination and timing of regimens in recurrent osteosarcoma.¹ In a study that evaluated ifosfamide in patients (<30 years old) with recurrent, metastatic osteosarcoma, the overall response rate was 10% (1 CR and 2 PR) in 30 pretreated patients compared with an overall response rate of 27% (1 CR and 8 PR) in 33 chemo-naïve counterparts.¹⁶ These results, in addition to our study findings, underscore the difficulty of obtaining robust outcomes in patients with recurrent osteosarcoma, particularly in pretreated patients.

Attempts to improve treatment outcomes in patients with high-grade osteosarcoma have involved high-dose intensification of conventional chemotherapy and/or the addition of carboplatin, etoposide and epirubicin to conventional chemotherapies, although patients with recurrent disease eventually relapse,^{17–19} with short remissions reported.¹⁷ These approaches have also been shown to increase the risk of toxicity.² Because osteosarcoma is a complex tumour, it is unlikely that one chemotherapy or treatment modality will be successful for all

patients, especially those with a poor prognosis and recurrent disease.

Pemetrexed administered at 500 mg/m² on day 1 of 21-day cycles as second-line treatment to patients with advanced or metastatic high-grade osteosarcoma was generally well tolerated. Although minimal response expectations were not met, about a third of the patients with relapsed osteosarcoma survived their disease at 1 year. Further exploration of novel, promising agents should continue and new strategies should be designed to increase the therapeutic efficacy of conventional therapies without increasing toxicity.

Conflict of interest statement

Hisham Rassam and Ulrike Boecker are employees of Eli Lilly and Company, a pharmaceutical company, and as employees, own stock in the company. Drs. Duffaud, Egerer, Ferrari and Bui-Nguyen have no financial disclosure to declare.

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