European Journal of Cancer 125 (2020) 58–68

Available online at www.sciencedirect.com

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journal homepage: www.ejcancer.com

Original Research

A multicentric randomized phase II clinical trial evaluating high-dose thiotepa as adjuvant treatment to standard chemotherapy in patients with resectable relapsed osteosarcoma

Perrine Marec-Berard [a,*], Cécile Dalban [b], Nathalie Gaspar [c], Laurence Brugieres [c], Jean-Claude Gentet [d], Cyril Lervat [e], Nadège Corradini [f], Marie-Pierre Castex [g], Claudine Schmitt [h], Hélène Pacquement [i], Marie-Dominique Tabone [j], Mehdi Brahmi [k], Séverine Metzger [b], Jean-Yves Blay [l], David Pérol [b] on behalf of the OSII Thiotepa study group of the French Society of Pediatric Cancer - La Société Française des Cancers de l'Enfant (SFCE) and the French Sarcoma Group–Groupe d'Etude des Tumeurs Osseuses (GSF-GETO) Networks

[a] *Paediatric Department, Hematology and Oncology Pediatric Institute, Centre Léon Bérard, Lyon, France*
[b] *Department of Clinical Research and Innovation, Centre Léon Bérard, Lyon, France*
[c] *Department of Pediatrics and Adolescents Oncology, Gustave Roussy, Villejuif, France*
[d] *Department of Pediatric Hematology and Oncology, La Timone Hospital, Marseille, France*
[e] *Department of Pediatric Oncology, Centre Oscar Lambret, Lille, France*
[f] *Department of Pediatric Hematology and Oncology, CHU Nantes, Nantes, France*
[g] *Paediatric Department, Hospital Centre, Toulouse, France*
[h] *Pediatric Hospital, CHU Nancy, Vandoeuvre-les-Nancy, France*
[i] *Pediatric Oncology Department, Institut Curie, Paris, France*
[j] *Department of Pediatric Hematology and Oncology, A.Trousseau Hospital, APHP, Paris, France*
[k] *Department of Medical Oncology, Centre Léon Bérard, Lyon, France*
[l] *Department of Medical Oncology & Claude Bernard University, Centre Léon Bérard, Lyon, France*

Received 1 August 2019; received in revised form 1 November 2019; accepted 7 November 2019

Abbreviations: Overall survival, (OS); Progression-free survival, (PFS); Post-relapse survival, (PR-S); High-dose thiotepa, (HDTp); Standard chemotherapy, (SCT); Intent-to-treat analysis, (ITT); Hazard ratio, (HR); Eastern Cooperative Oncology Group Performance Status, (ECOG-PS); Upper limit of normal, (ULN).

* *Corresponding author*: Pediatric Department, Hematology and Oncology Pediatric Institute/Centre Léon Bérard, Lyon, France. 1, place Joseph Renaut, Lyon 69008, France.
E-mail address: perrine.marec-berard@ihope.fr (P. Marec-Berard).

https://doi.org/10.1016/j.ejca.2019.11.007

KEYWORDS
Osteosarcoma;
Osteogenic sarcoma;
Relapse;
High-dose chemotherapy;
Thiotepa

Abstract ***Background:*** The role of high-dose chemotherapy in relapsing osteosarcomas has not been established. We evaluated the efficacy and tolerance of high-dose thiotepa (HDTp) after standard chemotherapy (SCT) in patients with relapsed osteosarcoma.
Patients and methods: This randomised open-label phase II study enrolled patients 1–50 years, with local or metastatic relapse of a high-grade osteosarcoma, not progressive after two cycles of SCT, for whom a complete surgery can be achievable following treatment. The trial assigned enrolled patients in a 1:1 ratio to receive two additional courses of SCT + HDTp and autologous transplantation (Arm A), or SCT alone (Arm B). Surgery for complete resection was scheduled as soon as feasible. Primary endpoint was overall survival (OS). Secondary objectives included progression-free survival (PFS) and safety.
Results: From September 2009 to November 2016, 44 patients were randomised (A:22; B:22). In total, 54.5% were males, and the median age was 16 years (9-32years). The two-year OS rate was 66.7% (95% CI 42.5–82.5) (SCT + HDTp, Arm A) *versus* 50.0% (95% CI 28.2–68.4) for SCT alone (Arm B). Median OS was 27.4 and 24.8 months, respectively (hazard ratio [HR] 0.826, 95% CI 0.393–1.734; $p = 0.6123$). Median PFS was 15.6 (8.9–24.9) months in Arm A *versus* 7.2 (4.8–33.3) months in Arm B, $p = 0.3845$. Among the 22 patients treated with SCT + HDTp, 16 (72.7%) experienced at least one grade ≥3 adverse events *versus* 18/22 (81.8%) patients treated with SCT. No toxic death occurred.
Conclusion: Adjuvant HDTp failed to significantly improve OS and PFS in resectable relapsed osteosarcomas. Despite a trend of prolonged survival and an acceptable toxicity, thiotepa cannot be recommended.
Key message: HDTp and autologous transplantation added to SCT did not improve OS and PFS in patients with resectable relapsed osteosarcomas. Despite a trend of prolonged survival, thiotepa cannot be recommended.

1. Introduction

Osteosarcoma is an aggressive neoplasm that mostly affects adolescents and young adults [1]. These tumours have high propensity to metastasise, and relapse occurs in 40% [1,2].

With aggressive therapy, including poly-chemotherapy and surgery, about 60% of the patients with localised disease can become long-term survivors [3]. The presence of metastases at diagnosis and a poor histological response to neo-adjuvant chemotherapy are unfavourable prognosis factors. The prognosis of patients with relapsing high-grade osteosarcoma is poor [4–6]. Indeed, five-year survival of relapsing patients does not exceed 30% with a median post-relapse survival of 10–17 months. Median time to first relapse is of 19 months, time to second relapse of less than 12 months [4,5]. In relapsing patients, extended time to relapse (over 2 years) and complete response following salvage therapy are favourable prognostic factors [4,7–9].

The role of systemic treatment in recurrent osteosarcoma is still not demonstrated, especially in patients surgically free of disease; recent retrospective studies did not evidence clinical benefit from second-line chemotherapy [5–8,10] Moreover, the most appropriate design to evaluate therapies in osteosarcoma remains poorly defined [10]. Several drugs have been used in second-line protocols with limited therapeutic success, and the best second-line treatment in patients with refractory osteosarcoma has still to be determined [4]. High-dose Thiotepa (HDTp) 900 mg/m^2 with stem cell rescue had been used after standard chemotherapy (SCT) in several French centres in the last two decades, despite lack of robust prospective study results [11,12]. HDTp efficacy was first suggested in 1998 [11], and a retrospective series of 53 relapsing or refractory patients treated with HDTp from 1992 to 2004 reported interesting radiological response rate with 31% objective response and 46% stable disease, and 29% good histological responses [12]. The objective of the present trial is to evaluate the efficacy and tolerance of HDTp with stem cell rescue in patients with relapsing osteosarcoma in a randomised study.

2. Material and methods

2.1. Patients

The study enrolled patients aged 1–50 years, with high-grade osteosarcoma, with first relapse, or second relapse if the first relapse had been exclusively treated with surgery. A multidisciplinary committee identified patients eligible for SCT and stated that a complete tumour and metastases resection can be achieved prior or after SCT. Patients should not have received a prior high-dose chemotherapy (HDCT) with autologous stem

cell transplantation and should not be progressive after two SCT courses. Eligibility criteria included Lansky score ≥60% or Eastern Cooperative Oncology Group (ECOG) performance status ≤2, at least a 21-day period from any first-line chemotherapy administration when applicable, adequate haematologic function (absolute neutrophil count $\geq 1 \times 10^9$ cells/L, platelet count $>100 \times 10^9$/L), adequate hepatic function (total bilirubin ≤2 × upper limit of normal [ULN], aspartate aminotransferase and alanine aminotransferase ≤5 × ULN), adequate renal function (serum creatinine ≤1·5 × ULN) according to their age, considering that inclusion of patient with serum creatinine >1·5 × ULN with creatinine clearance >70 mL/min/1.73 m^2 was allowed, and adequate cardiac function (shortening fraction >28% and isotopic or echography assessment of left ventricular ejection fraction value > 50%) in the last seven days prior inclusion. Exclusion criteria were patients with multiple relapses for whom complete resection for any localisations cannot be performed immediately or after administration of chemotherapy, who had already received HDCT with autologous stem cell transplantation, for whom contraindications to the study treatment were detected, patients not eligible for leukapheresis, or for whom a two-year follow-up would be impossible for social, familial, geographical, or psychological reasons, or for whom poor patient compliance was expected.

2.2. Study design

This multicentre, open-label phase 2 study randomly assigned in a 1:1 ratio patients who had already received two courses of SCT to receive two additional courses of SCT and HDTp 900 mg/m^2 with stem cell rescue in Arm A *versus* two additional courses of SCT exclusively (Arm B). The first-line chemotherapy regimens were defined as per the investigator choice, according to the French sarcoma group recommendations in force at the time. Paediatric patients had been treated in clinical trials OS 94 [13] and OS 2006 [14]and adult patients in OSAD 93 [15] and OS 2006 clinical trials [14]. The maximum cumulative doses had been limited to 450 mg/m^2 adriamycin, 600 mg/m^2 cisplatin, and 120 g/m^2 ifosfamide, and second-line treatment regimens recommended for patients initially treated with low doses of adriamycin and cisplatin were regimens alternating adriamycin/cisplatin (AP) and etoposide/ifosfamide (EI) (regimen 1), or for patients with maximal doses of adriamycin and/or cisplatin reached during initial treatment four courses of EI exclusively (regimen 2), or from 2013 gemcitabine/docetaxel (GD) (regimen 3).

A complete resection of any tumour and metastatic site should be performed prior to SCT or as soon as feasible. The randomisation was stratified by number of lesions at relapse (1 lesion *versus* multiple lesions) using an interactive Web-based centralised registration platform. The randomisation list was generated by a statistician at the coordination centre using a permuted block design of block size four within each stratum. Clinicians and patients were not masked to treatment group allocation. The study was conducted according to the declaration of Helsinki and the International Conference on Harmonization on Good Medical Practices after local approval (Ethics Committee of Lyon Sud-Est IV) in 25 institutions. All patients provided written informed consent before enrolment. For minor patients, authorization was granted to the persons having the parental authority. The trial was registered with ClinicalTrials.gov, number NCT00978471.

2.3. Outcomes

The primary endpoint was overall survival (OS), defined as the time from randomisation until death from any cause. Secondary endpoints were progression-free survival (PFS), defined as the time from randomisation to the date of first documented event of tumour progression or death from any cause, whichever occurred first; post-relapse survival (PR-S), calculated as the time from relapse (before inclusion) to the date of death from any cause; and response rate (RR), defined as the proportion of patients who reached a complete or partial response during study treatment (after two cycles, four cycles, or at the end of thiotepa administration or end of treatment).

2.4. Procedures and response assessment

Patients were planned to receive four courses of SCT with (Arm A) or without (Arm B) HDTp 900 mg/m^2 with stem cell rescue. SCT used doxorubicin, ifosfamide, cisplatin, and etoposide, administrated in cycles separated by 21 days, based on a therapeutic scheme optimised according to the first-line treatment and histologic response achieved. This optimisation allows to maintain cumulative total doses of doxorubicin, ifosfamide, cisplatin, and etoposide to 450 mg, 120 mg, 600 mg, and 3000 mg, respectively.

Evaluations based on chest computerized tomography (CT) scan and magnetic resonance imaging (MRI), if clinically indicated, were performed at inclusion. Response assessments were performed from day 14 to day 21 of the second course of SCT before the randomisation, from day 14 to day 21 of the fourth course of SCT, following HDTp administration in the absence of prior resection, and eight weeks after thiotepa administration (Arm A) or after last SCT (Arm B) in the absence of prior resection (or 8 weeks after surgery when resection was performed after the last cycle of chemotherapy).

Histological response to treatment was assessed on resected sample. For bone samples, the classification used HUVOS-staging [16]. Tumour staging guides

further patient qualification as 'good' responders (<10% viable cells) and 'poor' responders (>10% viable cells). Non-bone tissue samples were classified into groups 1 to 3 according to the response observed (group 1 = adequate response to treatment with necrosis, fibrosis, and no or exceptional visible viable cells; group 2 = intermediary response impossible to classify, and group 3 = poor response to treatment with visible lesions and perennial cells still identified and no visible efficacy of the treatment). A centralised review could be required for classification. For multiple resection with divergent conclusions, patients were classified in group 2.

Toxicity was assessed and graded according to the Common Terminology Criteria Adverse Events (CTCAE version 4).

Patients were followed-up for clinical and radiological assessment at three and six months after treatment discontinuation, and then every six months during three years or up to two years after the randomisation date of the last patient. Survival data were updated for all patients prior to the database lock (4th June 2018). Data for the last three randomised patients were updated on 19th October 2018.

2.5. Statistical analysis

The study was designed with a 80% power to detect an improvement in two-year OS from 20% (SCT, Arm B) to 45% (SCT + HDTp, Arm A) with an one-sided α of 10% (HR 0.5). The sample size calculation imposed a recruitment of 33 evaluable patients per arm with a fixed follow-up duration of two years per patient, and the total number of required events was 37. The extended recruitment period led to re-estimating the sample size to 22 patients per arm to reach the 37 events required, taking into account a longer follow-up duration reached for the first patients (from 24- to 60-month follow-up), with 80% power and an one-sided α of 10% (HR 0.5). After the last patient randomised had reached a two-year follow-up duration, we performed the final analysis regardless the number of events (28/37 events were finally required). At this time point, the first patients randomised had more than seven-year follow-up.

Based on the intent-to-treat (ITT) principle, efficacy analyses were done including all randomised patients. All randomised patients who received at least one cycle of experimental treatment were assessed for safety.

OS, PFS, and PR-S were estimated with the Kaplan–Meier method and were described in terms of median and survival rates (at 1- and 2-year) in each arm, along with the associated two-sided 95% confidence intervals (CIs) for the estimates. Survival distributions were compared between the two study arms using a log-rank test, supported by a Cox regression to estimate the HR and its 95% CIs. Median follow-up (min–max) was calculated using the reverse Kaplan–Meier method. All analyses were performed using SAS software, version 9.4 (SAS Institute).

3. Results

From 9th July 2009 to 23rd November 2016, we enrolled 58 patients from 17 institutions (seven comprehensive cancer centres, 10 hospitals) (Fig. 1, Supplementary data S1). Following two initial courses of SCT, 44 (75.9%) patients were randomly assigned to receive additionally two courses of SCT + HDTp (22; Arm A), or two courses of SCT exclusively (22; Arm B), and analysed following the ITT principle (Fig. 1); 14 patients were not randomised for the following reasons: patient decision (N = 3), progression before randomisation (N = 8), impossibility to resect all metastases (N = 2), early death (N = 1). To note, one patient in the Arm A, who received HDTp, was subsequently confirmed as progressive at randomisation, and censored at the date of randomisation.

Table 1 describes the main demographic, baseline clinical, and tumour characteristics. The two treatment arms were well balanced, notwithstanding a greater number of patients with metastatic diseases at diagnosis in the experimental arm (A:9; B:5), and more patients with second relapse (A:7; B:1). The median number of SCT cycles received during the study was similar in both arms (A: 4.0 (2–6); B: 4.0 (3–6)).

In the experimental Arm A, 18 (82%) patients received four courses of SCT + HDTp. The median delay between the last SCT course and thiotepa administration was 47.5 (28–74) days. No dose modification was reported for HDTp. Four (18%) patients allocated to the Arm A received SCT exclusively and did not receive HDTp for the following reasons: consent withdrawal (N = 1), investigator decision (N = 1), progression (N = 2) both post-randomisation either immediately post-randomisation after two initial cycles of SCT (N = 1), or after four cycles of SCT (2 initial and 2 post-randomisation additional cycles) (N = 1)). The median dose of thiotepa administrated per patient was 921.3 (787–985) mg/m^2, and the median duration of hospitalisation was 15.5 (4–34) days. In Arm B, 19 (86%) out of 22 patients adequately received the required duration of study drug (four courses of SCT). The three remaining patients stopped the study treatment for progressions (N = 2), and one received six cycles of SCT (investigator decision N = 1).

3.1. Efficacy

With a median follow-up of 51.4 months, median OS was 27.4 (95% CI 21.5–NA) months in Arm A *versus* 24.8 (95% CI 12.1–NA) months in Arm B (HR for death 0.826, 95% CI 0.393–1.734; p = 0.6123). The estimated proportion of patients who were alive at 12

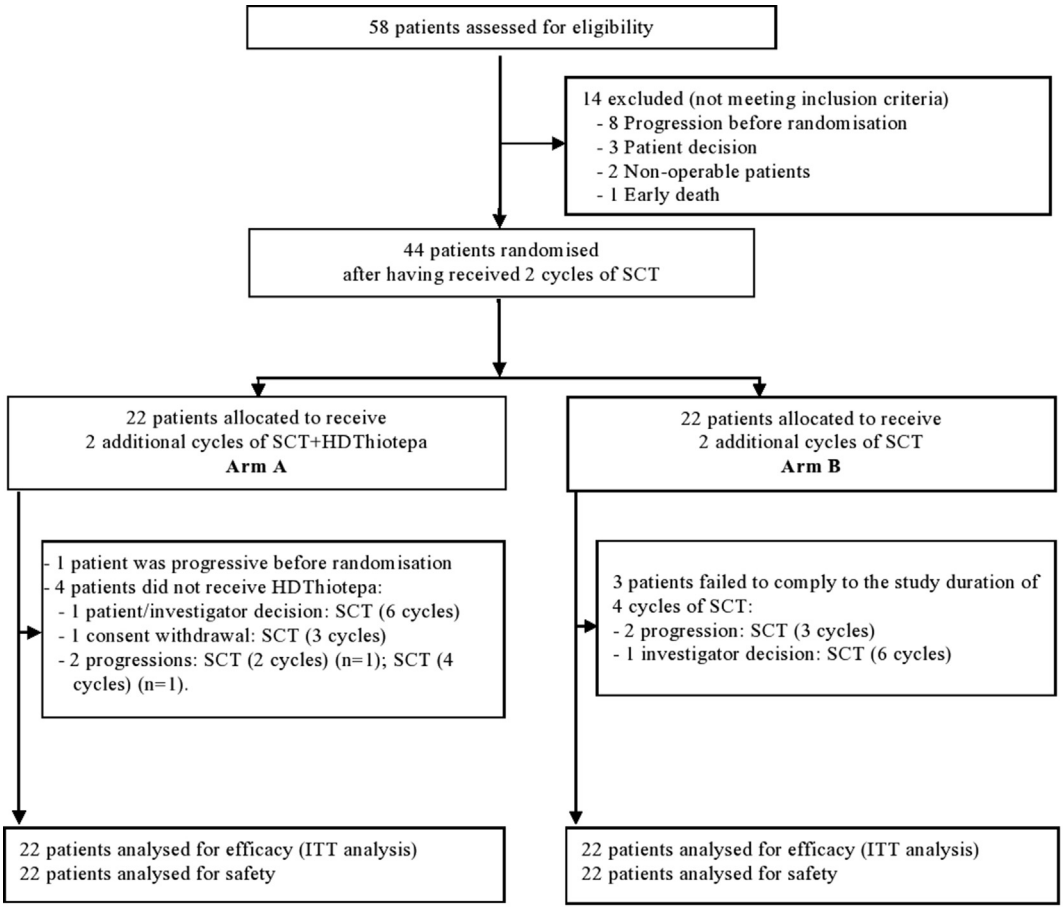

Fig. 1. Trial profile. Standard Chemotherapy (SCT). High dose (HD). Intent-to-treat (ITT) analysis ($N = 44$ randomised patients). Safety analysis included all patients who had received at least one cycle of chemotherapy and for whom at least one assessment was performed after randomisation ($N = 44$).

months was 95.2% (95% CI 70.7–99.3) in Arm A and 77.3% (95% CI 53.7–89.8) in Arm B. The two-year OS rate was 66.7% (95% CI 42.5–82.5) and was 50.0% (95% CI 28.2–68.4) in Arms A and B, respectively.

The median PFS was 15.6 (95% CI 8.9–24.9) months in Arm A and 7.2 (95% CI 4.8–33.3) months in Arm B (HR for progression or death 0.731, 95% CI 0.360–1.486, $p = 0.3845$). The estimated proportion of patients who were alive and progression-free at 12 months was 55.3% (95% CI 31.6–73.7) in Arm A and 31.8% (14.2–51.1) in Arm B. The two-year PFS rate was 35.2% (95% CI 15.8–55.4) and 31.8% (14.2–51.1) in Arms A and B, respectively.

The median PR-S was 29.9 (95% CI 24.2–NA) months in Arm A and 26.7 (95% CI 14.2–NA) months in Arm B (HR 0.850, 95% CI 0.405–1.784, $p = 0.6670$), with one-year PR-S rate of 100% and two-year PR-S rate of 76.2% (95% CI 51.9–89.3) in Arm A *versus* one-year PR-S rate of 86.4% (95% CI 63.4–95.4), and two-year PR-S rate of 54.5% (95% CI 32.1–72.4) in Arm B.

The overall response was 77.3% in each arm (A: 54.6–92.2%; B: 54.6–92.2%). The global strategy allowed to reach complete response (CR) at the end of study treatment in 16 patients (72%) in arm A and in 11 (50%) patients in Arm B. Seven patients (A:2; B:5) had progressive disease before the end of treatment.

3.2. Surgery

Out of the 44 patients, 42 (95.5%) patients had surgical resection of one ($N = 27$), two ($N = 13$), or three ($N = 2$) metastases (Fig. 2). Resection was performed before initiation of treatment in nine patients (A:6; B:3), after two cycles (A:5; B:9), three cycles (A:2; B:2), or four cycles (A:10; B:6) of SCT, and after HDTp administration in four out of the 18 patients receiving thiotepa. Among the nine patients having pulmonary surgery before SCT initiation, three had another surgery after treatment initiation (second pulmonary surgery [$N = 2$]; bone and second pulmonary site [$N = 1$]). Two patients were not operable because of progressive disease (see Fig. 3).

Five out of the 10 patients with bone resections were good responders according to the four grade HUVOS staging [16]. Pulmonary resections occurred in 33 of the 34 patients with pulmonary site, and one patient

Table 1
Main demographics and baseline characteristics (*non-exclusive reasons). Eastern Cooperative Oncology Group Performance Status (ECOG-PS). Data are median (range min–max) or n (%). † pelvis (A:0; B:1), intracerebral (A:1; B:0), sacrum (A:1; B:0); †† French protocol, including seven preoperative courses of high-dose methotrexate (HDMTX: 12 g/m^2) with leucovorin rescue and two courses of etoposide (300 mg/m^2) and ifosfamide (12 g/m^2). ††† Chemotherapy based on API AI + methotrexate (N = 1) (outside of clinical trial, according to EURAMOS study); Standard Chemotherapy (SCT); High Dose Thiotepa (HDTp); *non-exclusive reasons.

	Arm A (SCT + HDTp)	Arm B (SCT alone)	All randomised patients
	N = 22	N = 22	N = 44
Median age at inclusion (range)	16.5 (11–32)	16 (9–32)	16 (9–32)
Gender			
Male	12 (54·5%)	12 (54·5%)	24 (54.5%)
Female	10 (45.5%)	10 (45.5%)	20 (45.5%)
ECOG-PS			
0	16 (76.2%)	16 (72.7%)	32 (74.4%)
1	5 (23.8%)	3 (13.6%)	8 (18.6%)
2	0	3 (13.6%)	3 (7.0%)
Non-specified	1	0	1
Primary site at diagnosis			
Lower limb	20 (90·9%)	21 (95·5%)	41 (93·2%)
Other†	2 (9·1%)	1 (4·5%)	3 (6·8%)
Metastatic status at diagnosis			
Non-metastatic	13 (59·1%)	17 (77·3%)	30 (68.2%)
Metastatic	9 (40·9%)	5 (22·7%)	14 (31.8%)
Metastatic sites at inclusion*			
Lung	19 (86.4%)	16 (72.7%)	35 (79.5%)
Mediastinal	0	1 (4.5%)	1 (2.3%)
Lymph node	1 (4.5%)	0	1 (2.3%)
Bone	2 (9.1%)	3 (13.6%)	5 (11.4%)
Previous treatment			
OS 94 (NCT00180908)	0	1 (4.5%)	1 (2.3%)
OS 2005 ††	1 (4.5%)	2 (9.1%)	3 (6.8%)
OS 2006 (NCT00470223)	18 (81.8%)	18 (81.8%)	36 (81·8%)
Other †††	3 (13.6%)	1 (4.5%)	4 (9.1%)
Median delay from prior treatment to inclusion (months) (min–max)*	14.1 (4–211)	13.7 (2–81)	13.7 (2–211)
Histological response to the prior treatment			
Good responder	16 (72.7%)	12 (60.0%)	28 (66.7%)
Poor responder	6 (27.3%)	8 (40.0%)	14 (33.3%)
Non-specified	0	2	2
Number of relapse(s) at inclusion			
Fist relapse	15 (68.2%)	21 (95.5%)	36 (81.8%)
Second relapse	7 (31.8%)	1 (4.5%)	8 (18.2%)
Type of relapse at inclusion			
Local	2 (9.1%)	3 (13.6%)	5 (11.4%)
Metastatic	20 (90.9%)	17 (77.3%)	37 (84.1%)
Local and metastatic	0	2 (9.1%)	2 (4.5%)
Number of lesion(s) at randomisation			
One	8 (36.4%)	6 (27.3%)	14 (31.8%)
Multiple	14 (63.6%)	16 (72.7%)	30 (68.2%)

underwent interventional radiology. Histological response was specified in 29 patients, and nine of them achieved complete necrosis.

Three pulmonary surgeries were pneumectomies. Three complications occurred (A:3 [haemothorax (N = 1); pneumothorax (N = 2)]; B:1 [effusion with pneumothorax (N = 1)]).

3.3. Safety

The majority of patients in both treatment arms experienced AEs (Table 2) (17 [77.3%] patients in arm A; 19 [86.4%] patients in arm B), including at least one grade ≥3 AE (A: 16 [72.7%]; B: 18 [81.8%]). Nine patients experienced serious AEs (A:5; B:4). To note, several unexpected serious adverse events occurred in Arm A in one patient (pancytopenia grade 4, gastrointestinal disorders, including stomatitis grade 3, oesophagitis grade 3, anal inflammation grade 4); no toxic death was observed.

4. Discussion

This multicentric phase 2 study is the first and unique randomised trial designed to evaluate the efficacy of HDTp in patients with resectable relapsed osteosarcoma. Our results showed no statistically significant improvement in median OS (27.4 *versus* 24.8 months

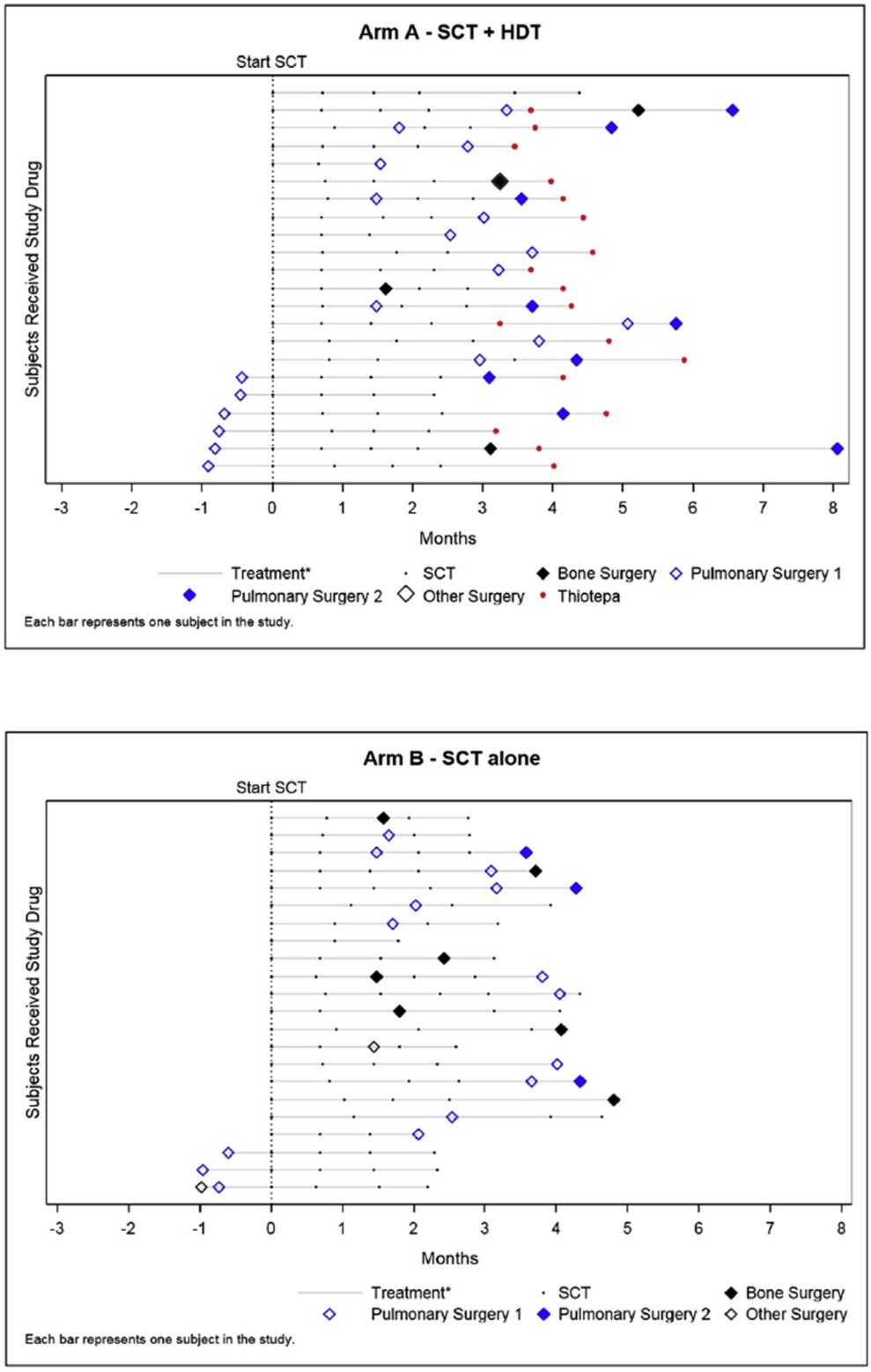

Fig. 2. Schematic representation of surgeries in patients receiving Standard Chemotherapy combined with High Dose Thiotepa (SCT + HDTp) and SCT alone.

[HR for death 0.826, 95% CI 0.393–1.734; $p = 0.6123$]) and PFS (15.6 *versus* 7.2 months [HR for progression or death 0.731, 95% CI 0.360–1.486, $p = 0.3845$]) in patients with SCT + HDTp with autologous stem cell rescue compared with patients receiving SCT exclusively. We showed that patient with relapsed osteosar-

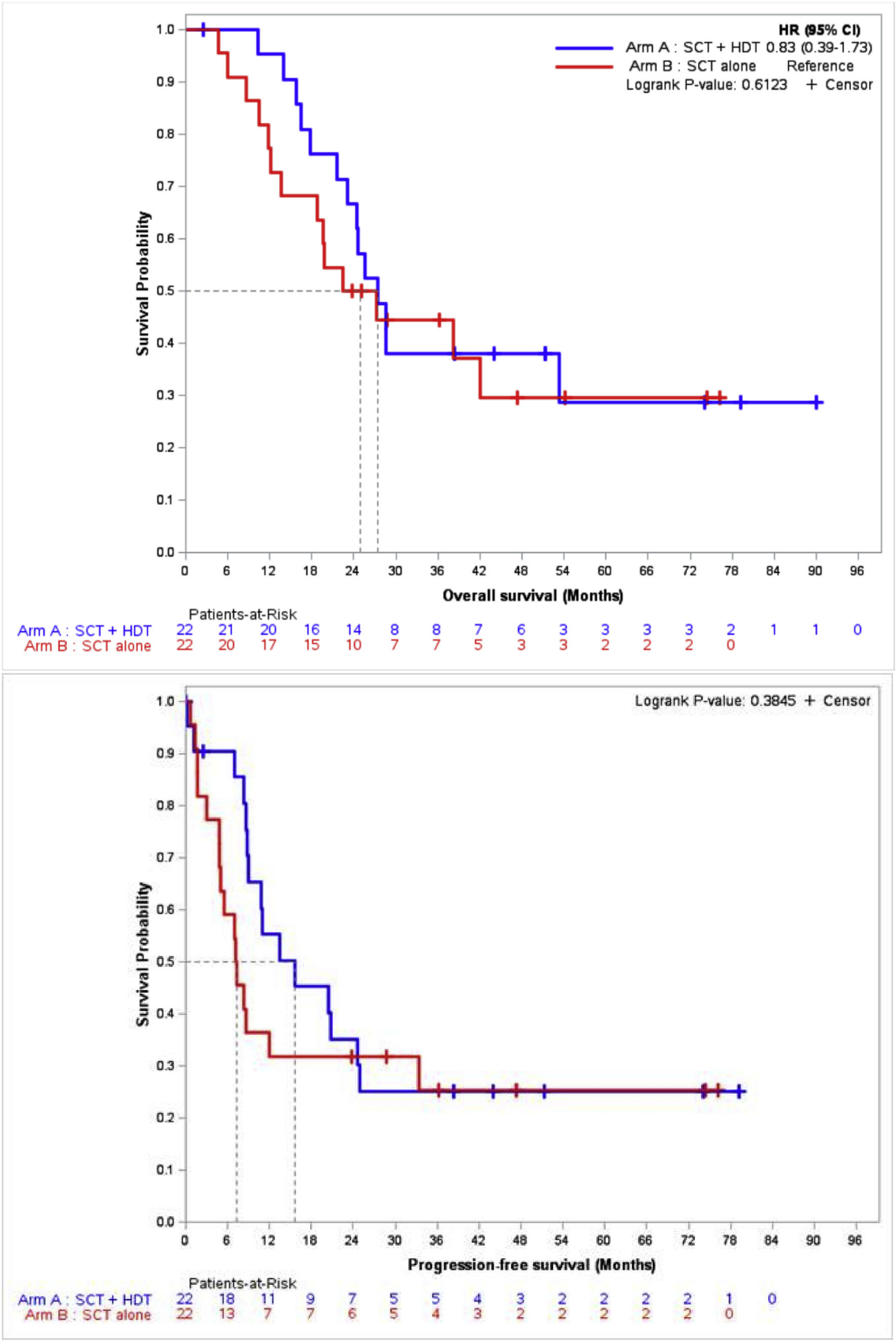

Fig. 3. Kaplan–Meier plots for Progression-Free Survival (PFS) and Overall Survival (OS) in all patients (intent to treat analysis). The data cut-off was 4th June 2018. Data for the last three randomised patients were updated on 19th October 2018.

Table 2
Tolerance. Grade ≥3 adverse events (≥10% in at least one arm).

Grade ≥3 events	Arm A (SCT + HDTp) $N = 22$	Arm B (SCT alone) $N = 22$
Mucositis/ Stomatitis	9 (40.9%)	0 (0%)
Anaemia	10 (45.5%)	10 (45.5%)
Febrile aplasia	6 (27.3%)	4 (18.2%)
Leukopenia	11 (50.0%)	14 (63.6%)
Neutropenia	8 (36.4%)	11 (50.0%)
Febrile neutropenia	3 (13.6%)	2 (9.1%)
Thrombopenia	12 (54.5%)	10 (45.5%)

coma reached a two-year OS rate of 67% in SCT + HDTp and 50% with SCT alone, much greater than our assumption at 20%.

The impact of a second-line chemotherapy in relapsed osteosarcoma remains matter of debate, and literature had never demonstrated a significant benefit so far [2,4]. At disease recurrence, complete surgical resection with clear margins is the first goal and options for second- and third-line therapy for refractory bone sarcoma previously treated with SCT are limited and much less defined [6,17,18]. Treatment choice considers prior disease-free interval and initial histological response and includes adriamycine, cisplatinum, ifosfamide, or cyclophosphamide, or other active drugs and combination. Clinical trials investigated the use of gemcitabine and docetaxel in recurrent osteosarcoma with contrasted results based on small number of patients [19–21]. No new drug had been FDA or EMA approved over the last decades.

The rational for the use of high-dose TTP was based on the experience of the *French Society of Children's Cancer* (SFCE) network who initiated a phase II study in 1999 and showed two partial responses and two long responders out of 21 children with osteosarcoma, acceptable toxicity, and favourable pharmacokinetics results (in press). A retrospective study conducted in 2009 reported 12 partial radiological responses (response rate 31%), and nine complete histological responses (31 surgery) in a series of 45 children with relapsing osteosarcoma treated with HDTp and autologous stem cell rescue after initial progression or in relapsed osteosarcoma. The OS at three years was 40% and the PFS was 24% [12]. We designed this study at the end of the 2000s, with the dose of 900 mg/m^2 based on the results of a phase II study reporting HDTp use for paediatric solid tumour treatments, with acceptable toxicities [11].

In the present study, we noted that the median duration of hospitalisation of 15 (4–42) days is not so long compared to duration observed with other HDCT with stem cell rescue regimens, and tolerance issues with mainly mucositis reported was considered as manageable [22]. No toxic deaths were reported. However, it remains questionable whether such treatments associated with long periods of hospitalisation and exposure to mucositis and fertility impairment are ethically justified in regards to the survival improvement.

The strength of this prospective investigation was to use a randomised design in a rare population of children and young adults with resectable relapsing osteosarcoma. Nevertheless, the study has been designed in 2000s, and at that time, defining best estimate assumptions was challenging. Indeed, our statistical hypothesis was premised on the results from phase 2 trials and retrospective series of relapsing osteosarcoma, namely, highly heterogeneous populations who had received either conventional chemotherapy [7–9,17,21] or HDCT [1,23,24]. We chose OS as the primary endpoint, but this endpoint was probably not appropriate and would have been reconsidered in favour to event-free survival or PFS for current trial design [10,25]. Primary objectives in more recently designed studies were six-month objective response (CR + PR) and six-month non-progression (CR + PR + SD), but these criteria were not usual in 2009.

Our assumptions planned more than 10 years ago an improvement in OS at two years (two-year OS) from 20% (in patients with SCT alone) to 45% (in patients with SCT + HDTp). These figures turn out to be totally inadequate several years later with more recent data. Indeed, the study showed that patients treated with SCT alone reached a two-year OS rate of 50%. Meanwhile, survival data have been refined, and our current findings are definitively in line with more recent results. It can be assumed that the absence of standard second-line chemotherapy may have contributed to increase difficulties in generating accurate assumptions. Our results highlighted an improved survival in patients with SCT, which may result from the enhanced homogenisation in treatment with four cycles of SCT compared to others series. It seems important to confirm that the median number of cycles of SCT was similar in both arms.

Overall, it has to be noted that patients' characteristics analysis showed less favourable prognostic factors in patients treated with the combination SCT + HDTp, despite the randomisation design. In particular, more patients were metastatic at inclusion than previously published series, more patients had already reached metastatic status at diagnosis, and more patients had second relapse of osteosarcoma. These criteria, which have been shown to correlate with poor prognosis, may have contributed to substantially reduce the difference in term of survival between arms, hence preventing to observe a significant improvement in efficacy of HDTp combined with SCT *versus* SCT alone. Nevertheless, no difference in HR was observed after adjustment on these criteria (adjusted HR on metastatic at diagnosis and on the first or second relapse was 0.888 [0.371–2.125]).

This study has some limitations. The recruitment process was slower than anticipated, and coupled with a

lack of power, the results did not allow to reach a significantly improved OS. Indeed, the study barely reached 57% statistical power with a calculation based on the true observed two-year OS rates. Even if the slow recruitment rate had been anticipated, it was even slower. We faced to an unforeseeable temporarily thiotepa supply suspension, which caused further delays. In addition, motivating and mobilising oncologists over the extended study period became challenging in the newly emerging competitive context of clinical trials providing access to innovative targeted therapies, especially in adults [26,27]. Indeed, encouraging results have been reported in locally advanced or metastatic relapsing osteosarcoma treated with different multi-tyrosine kinase inhibitors [28–32]. Davis and colleagues recently reported significantly improved median PFS in relapsed metastatic osteosarcoma after at least one prior line of therapy (average 2.3 prior therapy regimen) with regorafenib versus placebo (3.6 *versus* 1.7 months), and one-year OS was 53% *versus* 33%, with manageable toxicity [30]. A randomised, placebo-controlled phase II study evaluating efficacy and safety of regorafenib in patients with metastatic osteosarcoma who had received one to two previous lines of chemotherapy for metastatic disease showed a positive effect of regorafenib with a non-progressive rate at eight weeks of 65% *versus* 0% and a median PFS of 16 weeks *versus* four weeks with placebo [29]. Cabozantinib has been evaluated in a phase II non-randomised trial in patients with relapsed osteosarcoma and allows a median PFS of 6.2 months and median OS of 10.6 months with an overall response rate of 11.9% [31]. Lenvatinib has also been tested in a phase Ib-II trial, in combination with ifosfamide and etoposide, with encouraging preliminary data [32]. Tyrosine kinase inhibitors demonstrated clinically meaningful anti-tumour activity in patients with recurrent, progressive, metastatic osteosarcoma after failure of conventional chemotherapy, with a positive effect on delaying disease progression and should be evaluated in the setting of advanced disease, as well as potentially earlier in the disease course for patients at high risk of relapse. Tyrosine kinase inhibitors might offer a complementary therapeutic role to standard cytotoxic chemotherapy in the therapeutic armamentarium against osteosarcoma. These innovative therapeutic opportunities combined with the results of our trial did not favour future use of HDTp in relapsed osteosarcoma.

In conclusion, this phase II randomised trial has failed to demonstrate a significant benefit in adding HDTp and autologous transplantation to SCT compared to SCT alone in patients with relapsed osteosarcoma assessable to surgery. Additional large multicentre, randomised controlled trials and phase 2 trials are required in the treatment of relapsed osteosarcoma to select the most appropriate chemotherapy regimens and doses, including gemcitabine, docetaxel, and combinations, and further exploration of the therapeutic role of targeted agent such as regorafenib, cabozantinib, or lenvatinib combined with standard cytotoxic therapy in individual subtypes is genuinely required.

Conflict of interest statement

The authors have declared no conflicts of interest.

Acknowledgements

Thanks to all participants, investigators, and clinical research staff from each participating centres. Trial registration: EudraCT Number: 2009-009899-12 and ClinicalTrial.gov Number: NCT00978471. Thanks to any investigators who actively participated in the study, Drs Claire Berger, Philippe Cassier, Olivier Collard, Ludovic Mansuy, Anne Pagnier, Emmanuel Plouvier, Isabelle Ray-Coquard, Yves Requerre, Angélique Rome, Hervé Rubie, Christine Soler, and Estelle Thébaud. The authors thank Sophie Darnis PhD (Centre Léon Bérard) for medical editorial assistance with this manuscript.

Funding

This work was supported by la Ligue Nationale contre le Cancer and La Société Française des Cancers de l'Enfant (SFCE) and Chugai Pharma.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2019.11.007.

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