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European Journal of Cancer 38 (2002) 2243–2251

European Journal of Cancer

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Neoadjuvant chemotherapy for Ewing's tumour of bone: recent experience at the Rizzoli Orthopaedic Institute

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Received 3 December 2001; received in revised form 29 January 2002; accepted 9 May 2002

Abstract

The results achieved in 157 patients with non-metastatic Ewing's sarcoma of the bone treated at a single institution between 1991 and 1997 according to a new protocol (REN-3) are reported. Induction chemotherapy consisted of two cycles of 'VAC': vincristine (V), doxorubicin (A), cyclophosphamide (C) alternated with one cycle of 'VIAc': V, ifosfamide (I), actinomycin (Ac). After local treatment, patients received three more cycles of VAC, two of VIAc, three cycles of I plus etoposide (E) and two cycles with V, C and Ac. Local treatment was surgery in 53% of patients, surgery + radiotherapy in 25% and radiotherapy only in 22%. With a follow-up ranging between 4 and 10 years (mean: 7 years), 110 patients (70%) remained continuously event-free, 2 patients died of toxicity and 45 patients relapsed: 33 due to metastases and 12 due to local recurrence always associated with metastases. The 5-year event-free survival (EFS) and overall survival (OS) were 71.0 and 76.5% respectively. These results are significantly better that the ones achieved in our previous three studies in which a three-drug VAC regimen (REA-1), and 4-drug VACAc regimen (REA-2 and REN-1) was used, and in our most recent study (REN-2) which was based on a six-drug regimen as in the present study, but where I and Ac were used only after the local treatment. However, since REN-3 surgery was used in a significantly larger number of patients, we cannot say whether the better outcome of this study was due to the use of a six-drug regimen with an early delivery of ifosfamide and actinomycin, or to the wider use of surgery as local treatment or both.

Keywords: Neoadjuvant chemotherapy; Ewing's sarcoma; Surgery; Radiotherapy

1. Introduction

Today, the standard treatment for Ewing's sarcoma of bone (ESB) is neoadjuvant chemotherapy, i.e. chemotherapy is employed before and after the local treatment of the primary tumour. This multimodal approach combining surgery and/or radiotherapy with systemic chemotherapy dramatically improved the prognosis of localised ESB, with increased long-term survival rates from less than 10% [1,2] to 50% or more [3–12]. Despite these advances, several international centres are striving to identify better protocols of chemotherapy and better techniques of local treatment.

At our institution, combined treatment of ESB was started in 1972 and used until October 1991. Four studies were performed. In the first two studies, REA-1 and REA-2, chemotherapy was used as an adjuvant treatment [3] while in the others, REN-1 and REN-2, the treatment was neoadjuvant [4]. In REA-1 a three-drug regimen 'VAC' (vincristine (V), doxorubicin (A), cyclophosfamide (C)) was used, while in REA-2 a four-drug regimen 'VACAc' (VAC plus actinomycin (Ac)) was applied. The 5-year event-free survival rates (EFS) were 34 and 59%, respectively ($P < 0.009$). In the

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PII: S0959-8049(02)00148-X

following two studies, a four-drug regimen (VACAc) was applied in the REN-1 protocol, while in the second neoadjuvant study REN-2, we used a six-drug regimen (VACAc + ifosfamide (I) and etoposide (E)). However, these two new drugs were delivered only after the local therapy. The 5-year EFS rates were 49 and 51%, respectively, so we concluded that the addition of ifosfamide and etoposide to the VACAc regimen did not improve the cure rate in patients with ESB treated with neoadjuvant chemotherapy.

In these four studies, the rate of patients who received surgery as local treatment grew from 33% in the first study to 57% in the last one, resulting in a better prognosis for patients locally treated with surgery. Moreover, the outcome was significantly related to the grade of histological response to preoperative chemotherapy in patients treated with neoadjuvant chemotherapy [13,14].

With the purpose of increasing the rate of good response to primary chemotherapy, in November 1991 we started a new neoadjuvant, single-arm, non-randomised study (REN-3) with ifosfamide and actinomycin from the induction treatment. In addition to this, surgery was more widely used for local treatment. The chemotherapy of the REN-3 protocol was the same as that used by the Cooperative Study of Italian Pediatric Oncologists for the National Council of Research (SE 91-CNR protocol) whose preliminary results were published 3 years ago [15]. That protocol was performed between November 1991 and June 1997, and it included half the patients of this study, all younger than 30 years.

The aim of this paper was to report the results achieved with the protocol REN-3 in 157 patients treated at our institution up to September 1997, comparing these results with those of our previous adjuvant and neoadjuvant studies.

2. Patients and methods

2.1. Patient selection

Eligibility criteria to enter the study were the same as in previous adjuvant and neoadjuvant studies: (a) histological diagnosis of Ewing's family tumour, (b) primary lesion located in the bone, (c) age < 50 years, (d) no distant metastases at diagnosis, (e) no previous therapies, (f) interval no longer than 4 weeks between the biopsy and the beginning of treatment. All the patients included in this review were treated at the Rizzoli Orthopaedic Institute. There were 243 newly diagnosed cases of Ewing's tumours observed at the Institute over the study period. 38 patients fulfilling eligibility criteria, diagnosed and staged at the Insitute, received chemotherapy and/or surgery and /or radiotherapy elsewhere so they were excluded from the study. 48 patients were excluded for the following reasons: metastatic disease at presentation [25], primary tumour located in soft tissues [15], age > 50 years [5], previous treatment elsewhere [6], interval longer than 4 weeks between biopsy and start of treatment [2]. 5 patients had more than one cause of exclusion. 157 patients were therefore entered into this study.

2.2. Diagnosis

Diagnostic materials included stained slides, unstained slides and paraffin-embedded tissue blocks obtained from the original tumour by surgical biopsy. Haematoxylin and eosin, period acid-Schiff (PAS), and PAS-diastase. Gomori's silver impregnation technique for reticular fibres were done in all cases. The following antibodies were tested: vimentin, keratins 8 and 18, epithelial membrane antigen, S-100 protein, neuron-specific enolase desmin, actin, glial fibrillary acid protein, myelin associated glycoprotein (Leu-7), lymphocyte common antigen.

All specimens, as in the previous study, REN-2, were reviewed by two pathologists, who were blinded to the information regarding clinical outcome. Tumours were classified as typical Ewing's sarcoma (TES) and primitive neuroectodermal tumour (PNET). Acceptable histological features of TES included: round cell tumour with variable portions of large, clear cells and smaller, hyperchromatic cells lacking tumour stroma or spindle cells, tumour with a filigree pattern, larger tumour cells ('large cell variant'), haemorrhage with formation of vascular lakes or sinuses, geographical necrosis with perivascular sparing, metaplastic bone or cartilage formation. Tumours with malignant osteoid or cartilage were specifically excluded, as well as tumour with any of the following features: PAS negativity, intercellular stroma, spindle cell cytology, single cell differentiation (myoblasts and ganglion cells), a lobular architecture with cohesive cells.

The diagnosis of PNET was based on the positivity of a minimum of two neural differentiation antigens. Cytogenetic and ultrastructural studies were not considered, even if performed in most cases.

The initial evaluation of all patients included the recording of the medical history, physical examination, haematological studies and several chemical laboratory tests including serum lactate dehydrogenase (LDH). Radiographs of the chest and of the site of primary tumour were routinely made. Local imaging included computed tomography (CT) scan and magnetic resonance (MR). The presence of metastases was investigated using bone scintigraphy and CT scan of the chest. Tumour size was estimated by CT scan measures of the three diameters of the lesion and calculated according to the method reported by Gobel and colleagues in Ref. [16].

2.3. Induction chemotherapy and local treatment

Chemotherapy was performed according to the third neoadjuvant protocol for ESB (REN-3). Before local treatment, two cycles with V, A, C were given alternated with one cycle of V, Ac and I according to the doses and the schedule reported in Fig. 1.

Local treatment, scheduled 3 weeks after the beginning of the last cycle of induction chemotherapy, was chosen individually and consisted of surgery, surgery followed by radiotherapy or radiotherapy alone. The decision for local treatment was made case by case in order to achieve complete local control and attempt to retain the greatest level of function of the tumour-affected site. Retrospectively, it is impossible to know exactly the reasons for each decision. However, tumour site, size and resectability, presence of pathological fracture and patient's age were generally considered. Amputation was recommended in cases of non-curable pathological fractures, and lesions of the distal femur, leg or foot in young growing children. In these patients, radiotherapy would have caused limb-length discrepancy and worse functional impairment than amputation.

For local control, radiotherapy alone was given at a dose of 60 Gy. As reported in detail in other papers [3,4], before 1991 patients received conventional fractionated irradiation, whereas hyperfractionated irradiation was used after 1991 [17]. In patients surgically treated, those with inadequate surgical margins always received postoperative full-dose radiotherapy at (55–60 Gy). In patients with adequate margins, radiotherapy was given at a reduced dose (40–45 Gy), and only if allowed by the type of surgical reconstruction.

2.4. Pathological evaluation of surgically treated patients

After surgery, all gross specimens were carefully observed and surface-labelled histological sections taken. Surgical margins were evaluated according to Enneking and colleagues [18]. Radical and wide margins were considered adequate, while marginal and intralesional margins were considered inadequate. The response to chemotherapy was determined by a thorough histological examination of an entire coronal section of the tumour according to a method previously reported in Ref. [13] and classified as grade I (evidence of macroscopic foci of viable tumour cells), grade II (only isolated microscopic nodules of viable tumour cells), and grade III (no nodules of viable cells).

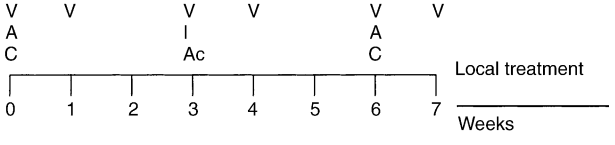

Fig. 1. Third neoadjuvant protocol for Ewing's sarcoma of bone (REN-3): induction chemotherapy.

2.5. Maintenance chemotherapy and follow-up

After local treatment, maintenance chemotherapy was delivered by alternating cycles of V/A/C, V/I/Ac, E/I and V/C/Ac, according to the schedule and the doses reported in Fig. 2.

Chemotherapy was resumed approximately one week after the operation in patients locally treated by surgery, and 3–4 weeks when postoperative radiotherapy was as well.

During and after chemotherapy administration, patients were followed with a physical check-up and standard radiographs of the chest and involved bones, performed every 3 months for 4 years, then twice a year. Additional studies, including biopsies, were done if indicated by the clinical and radiological examinations.

2.6. Statistical analyses

The primary end point of this study was event-free survival (EFS), defined as the period from the start of chemotherapy to the most recent follow-up evaluation, local or systemic recurrence or death unrelated to tumour and due to treatment complications or second malignancy. Relapses were classified as local (local recurrence), systemic (metastases with or without local recurrence). The following pretreatment variables were considered for potential prognostic value: patient's sex and age, tumour size and site, presence of anaemia and fever, serum levels of LDH. As regards treatment-associated factors, the EFS was analysed according to the interval between the onset of symptoms and start of treatment, type of local treatment and, in patients treated by surgery, also according to the surgical margins and histological response to induction chemotherapy. The cut-off values for age (±15 years), tumour volume (±100 ml) and interval between the onset of symptoms

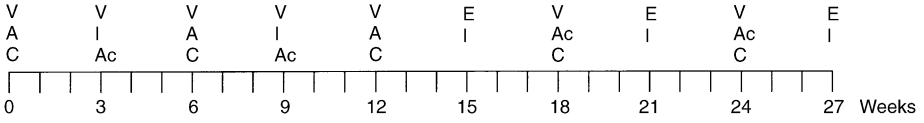

Fig. 2. Third neoadjuvant protocol for Ewing's sarcoma of bone (REN-3): maintenance chemotherapy. V: vincristine 1.4 mg/m^2. Top dose 2 mg; A: doxorubicin 40 mg/m^2/day in a 4-h infusion for 2 days; C: cyclophosphamide 1200 mg/m^2 in 30 min; I: ifosfamide 1800 mg/m^2/day in 1-h infusion for 5 days. Ac: actinomycin: 1.25 mg/m^2. Top dose 2 mg. E: etoposide 100 mg/m^2 in 1-h infusion for 5 days.

and start of treatment (±2 months) were chosen to allow a comparison of our data with those reported by others [6, 8,9, 19–21]. Overall survival (OS) was also evaluated, but these data must be considered with some caution. In fact, after relapse no homogeneous treatments were given, as several patients moved to other institutions and were treated by different therapies.

The Kaplan–Meier product limit estimate, was used to calculate EFS and OS. The log-rank test was used to calculate differences between groups. The distribution frequency of different parameters was compared among groups of patients by means of the Chi-square test. Significance was set at $P < 0.05$.

No patients were lost to follow-up, and there were no events other than relapse, second malignancy or deaths of chemotherapy toxicity and radiotherapy complications.

3. Results

The characteristics of the 157 patients in the study are reported in Table 1. There were 102 males (65%) and 55 females (35%), the median age was 17 years and the tumour was located in the femur (35 cases), tibia (28), pelvis (22), fibula (18), humerus (15), scapula (12), ribs (8), foot (5), spine (5) radius (3), sacrum (2), clavicula (2), ulna (1) and hand (1). Fever and anaemia at presentation were present in 11 (7%) and 17 (11%) of the patients, respectively, and the serum LDH was high in 42 cases (27%). 59 patients had a tumour volume < 100 ml and the mean interval between the onset of symptoms and diagnosis was 4.7 months (0.5–24 months). 33 patients, were classified as PNET, while the remaining 124 were classified as TES.

3.1. Local treatment and histological responses

Local treatment (Table 2) was surgery in 84 patients (53%), radiotherapy in 34 (22%) and surgery + radiotherapy in 39 (25%). 34 patients underwent surgery before radiotherapy, while 5 patients had radiotherapy at the end of preoperative chemotherapy and they were surgically treated only at the end of the maintenance treatment.

The rate of patients treated with radiotherapy was significantly higher for tumours located in the central axis in comparison with tumours in the extremities (20/51, 39% versus 14/106, 13%; $P < 0.0001$). For tumour of the central axis, the use of radiotherapy for local control was significantly higher in the pelvis and sacrum in comparison with other sites (16/24, 67% versus 4/27, 15%; $P < 0.0002$).

As regards surgery (123 patients), there were amputations in 5 patients (4%), resections in 117 (95%) and rotation plasty in 1 (Table 3). 86 patients with a tumour of the extremities were treated by limb salvage: patients with a tumour located in the fibula (16), in the proximal radius (1) and in the distal ulna (1) did not need reconstruction. In the other 68 resected patients, the affected skeletal segment was reconstructed after the resection. The reconstruction of a major joint was necessary in 52 cases and it was achieved by means of megaprosthesis (30 patients) or allograft–prosthetic composite (22

Table 1
Patients' characteristics and rate of 5-year event free survival (EFS) and local recurrence (LR)

Variable		No. of cases All = 157	% EFS 72	*P* value	% LR	*P* value
Gender	Male	102 (65%)	70	NS	7.8	NS
	Female	55 (35%)	76		7.2	
Age (years)	⩽15	64 (41%)	81	<0.045	3.1	NS
	>15	93 (59%)	66		10.7	
Tumor site	Extremity	106 (68%)	72	NS	6.8	NS
	Central axis	51 (32%)	71		9.3	
Tumor size	<100 ml	59 (38%)	84	<0.001	1.7	0.062
	⩾100 ml	98 (62%)	62		11.2	
Fever	Yes	11 (7%)	20	<0.001	9	NS
	No	146 (93%)	79		7.5	
Anaemia	Yes	17 (11%)	70	NS	11.8	NS
	No	140 (89%)	72		6.5	
Serum LDH[a]	Normal	112 (73%)	80	⩽0.0001	3.6	<0.004
	Elevated	42 (27%)	50		19	
Histology	Typical ES	124 (79%)	76	0.034	5.6	NS
	PNET tumour	33 (21%)	57		15.5	
Time to diagnosis	<2 months	51 (32%)	70	NS	8.5	NS
	>2 months	106 (68%)	72		6.5	

NS, non significant; LDH, lactate dehydrogenase; ES, Ewings' sarcoma; PNET, primitive neuroectodermal tumour; EFS, event-free survival; LR, local recurrence.

[a] Data missing for 3 patients.

patients). A joint arthrodesis was performed in 3 cases (knee: 2, hip: 1). 13 patients had various intercalary segments of the lower limb long bones reconstructed. In these cases, biological reconstructions were used more than massive bone allografts and vascularised autotransplants.

The surgical margins were radical in 2 patients, wide in 104, marginal in 16 and intralesional in 1.

The histological response to chemotherapy was grade I in 60 patients (49%), grade II in 29 (24%) and grade III in 34 (28%). The grade of histological response did not correlate with tumour size or the site.

3.2. Event-free survival

In September 2001, approximately 4 years after the end of the study, the median follow-up was 7 years (range 4–10 years). The median time to last follow-up was 6.5 years (4–9 years).

110 patients (70%) were continuously event-free, 2 patients (1%) died of treatment-related causes and 45 patients (29%) relapsed, 4 during maintenance chemotherapy and 41, 3 to 84 months (median 19 months) after the end of treatment. The actuarial 5 year EFS and OS rates were 71%, CI: 71.6%–84.8% (Fig. 3) and 76.5%, CI: 72.2%–85.8% respectively (Fig. 4).

As reported in Table 1, no relationships were found between EFS and patients' gender, site of tumour or presence of anaemia at the time of diagnosis. The univariate analyses showed the 5-year EFS was related to the serum level of LDH (80% for normal value versus 50% for patients with a high level; $P<0.0001$), tumour size (84% for tumours less than 100 ml versus 62% for tumours equal to or more than 100 ml; $P<0.001$), fever at diagnosis (20% versus 79%; $P<0.001$), histology (76% for TES versus 57% for PNET tumours; $P=0.034$) and patients' age (81% for patients 15 years old or younger and 66% for older patients; $P<0.045$). As illustrated in Tables 2 and 3, EFS did not correlate with the interval between the onset of the symptoms and the start of treatment, type of local treatment, type of surgery and surgical margins. EFS was significantly related to the type of histological response to induction chemotherapy (88% for grade I response, 76% for grade II and only 53% for grade 3; $P<0.001$).

The prognostic factors significant in the univariate analyses were then included in multivariate analyses and only tumour volume, fever at presentation, serum level of LDH and histological response to chemotherapy were found to be independent predictive factors of EFS (Table 4).

3.3. Relapse pattern

There were 33 systemic relapses and 12 combined relapses. No isolated local recurrences were seen. The first site of metastases was the lung in 18 patients, other bones in 15, both in other bone and lung in 8, the nervous central system in 3 and the soft palate in 1.

Table 2
Rate of 5-year event-free survival (EFS) and local recurrence (LR) according to treatment-associated variables

Variable		No. of cases All = 157	% EFS 72	*P* value	% LR	*P* value
Time to diagnosis	<2 months	51 (32%)	70	NS	8.5	NS
	>2 months	106 (68%)	72		6.5%	
Local treatment	R×T	34 (22%)	59		11.7%	
	Surgery	84 (53%)	78	NS	5.9%	NS
	Surgery + R×T	39 (25%)	72		7.7%	

R×T, radiotherapy.

Table 3
Rate of 5-year event-free survival (EFS) and local recurrence (LR) according to treatment-associated variables in patients locally treated by surgery

Variable		No. of cases (%) All = 123	% EFS 76.4	*P* value	% LR 6.5	*P* value
Type of surgery	Amputation[a]	6 (5)	50	NS	17	
	Resection	117 (95)	76		6.5	
Surgical margins[b]	Adequate	106 (86)	77	NS	5.6	NS
	Inadequate	17 (14)	76		11.7	
Histological response to chemotherapy	Grade I	34 (28)	88		5	
	Grade II	29 (23)	76	<0.001	6.9	NS
	Grade III	60 (49)	53		8.2	

[a] Including a rotation plasty.
[b] Adequate = radical (2 cases) and wide (104 cases); inadequate = marginal (16 cases) and intralesional (1 case).

The mean time to recurrence was 21.7 months (range 5–90 months), and it was shorter in local relapse (13.9 months, range 13–24 months) if compared with systemic relapse (24.6 months, range 5–90 months). For patients who developed metastases, the time to relapse was shorter in the 8 patients with contemporary bone and lung metastases (11.8 months), than in patients with metastases located only in the lung (28.1 months), bone (17.3 months), or other sites (26 months).

Sites of local relapse were: femur (4), pelvis (2) tibia, fibula, humerus, rib, spine, and scapula. According to pretherapeutic factors (Table 1), the rate of LR was related only to serum values of LDH at presentation (19% for high values versus 3.6% for normal values; $P<0.004$). According to local treatment, the rate of local recurrence was 5.9% for patients treated only by surgery, 7.7% for patients treated by surgery followed by radiotherapy and 11.7% for patients treated by radiotherapy only (Table 2). These differences were not statistically significant. In patients treated with surgery, the rate of local recurrence according to surgical margins was 12.5% (2/16) for marginal operations and 5.8% (6/104) for patients with wide surgical margins. This difference was not statistically significant. According to the histological response to chemotherapy, the rate of local recurrence was almost the same for the different grades of response (8.2%, 6.9%, 5%).

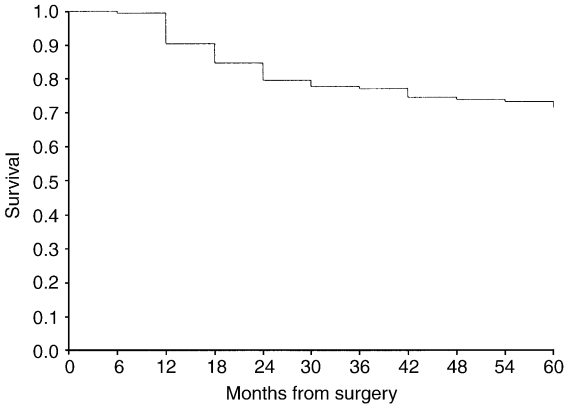

Fig. 3. Five-year event-free survival (71.0%).

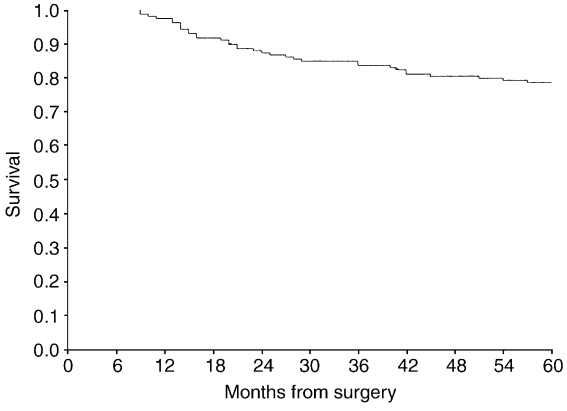

Fig. 4. Five-year overall survival (76.5%).

3.4. Postrelapse outcome

The 45 patients who relapsed were treated in different ways: surgical resection of metastases or local recurrence, radiotherapy, further chemotherapy coupled with surgery and/or radiotherapy, further chemotherapy, and palliative treatments. At the time of this analysis, 7 patients were alive and free of disease 9–66 months from the time of relapse, 1 was alive with uncontrolled disease 12 months after relapse, and 37 died. The mean time to death for these patients was 21.7 months (6–90 months). The overall 5-year survival was 76.5% (CI: 72.2%–85.8%).

Patients who experienced early relapse (less than 3 years after the initial diagnosis) had a lower percentage of EFS after relapse in comparison with patients who relapsed later than 3 years after initial diagnosis (3/37, 8% versus 5/8, 64%; $P<0.001$).

3.5. Compliance with the protocol and systemic toxicity

Of the 2036 cycles of chemotherapy performed, only 142 (7%) had to be delayed for more than 1 week (mean 1.4, range 1.2–2.8) due to persistent myelosuppression (136) or to complications related to the surgical treatment of the primary lesion (6).

The mean dose intensity was 92.8% of the projected dose intensity, and 85 patients (54%) had 90% or more of the projected dose intensity.

According to age, the mean dose intensity was: 95.8% for the 63 patients aged 15 years or younger; 90.1% for patients aged between 16 and 30 years, and 96.2% for the 11 patients older than 30 years. The 2 who died of toxicity were not considered.

A patient died of sepsis during myelosuppression that followed the first post-treatment cycle with VAC. Another patient, locally treated by surgery and radiotherapy, developed a broncoalveolar carcinoma 8 years after the beginning of the treatment.

Table 4
Probability of relapse according to several variables: multivariate analyses

Variable		Odds ratio (95% CI)	*P* value
Serum LDH	Elevated	2.484 (1.498–4.119)	$P=0.0004$
	Normal	1	
Necrosis	Grade I and II	2.083 (1.208–3.594)	$P=0.008$
	Grade IIII	1	
Fever	Present	3.460 (1.352–8.858)	$P=0.009$
	Absent	1	
Volume	>100 ml	2.484 (1.329–3.910)	$P=0.002$
	>100 ml	1	

95% CI, 95% Confidence Interval.

Table 5
Non-metastatic Ewing's sarcoma of bone: summary of the results achieved at Rizzoli Institute in the different adjuvant and neoadjuvant studies

Protocol	Patients (*n*)	Type of chemotherapy	Drugs used	% of surgical local treatment	5-year EFS rate (%)	5-year OS rate (%)	Rate of local relapse (%)	Rate of histological response grade III	
REA 1 (1972–1978)	85	Adjuvant	VAC	33	34	41	33		–
REA-2 (1979–1982)	59	Adjuvant	VACAc	49	59	71	19		–
REN-1 (1983–1988)	108	Neoadjuvant	Induction: VAC Maintenance: VACAc	63	49	56	21	23/67	34%
REN-2 (1988–1991)	82	Neoadjuvant	Induction: VAC Maintenance: VACAc, I + E	57	51	55	7	13/48	27
REN-3 (1991–1997)	157	Neoadjuvant	Induction: VAC, Ac, I Maintenance: VACAc, I + E	78	71	76	8	60/123	49%

V, vincristine; A, doxorubicin; C, ciclophosphamide; I, ifosfamide; Ac, actinomycin; E, etoposide.

Grade 4 haematological toxicity was observed after 270 cycles (13%), and hospitalisation was necessary in 40 cases (2%) to treat neutropenic febrile episodes or bleeding. According to age, the rate of grade-4 haematological toxicity was 10% for younger patients ($\leqslant$15 years), 14% for patients aged between 16 and 30 years, and 4% for patients older than 30 years. Although the cumulative dose of doxorubicin was 400 mg/m^2, there were no clinical cases of cardiotoxicity.

3.6. Local complications and sequelae

There were two major surgical complications related to the treatment of the primary tumour. Both these complications were observed in patients with tumour located in the femur and locally treated by conservative surgery only. These major complications consisted of an infection and a postoperative detachment of the extensor apparatus after prosthetic reconstructions. Both events required further surgery, leading to amputation in the first patient. Minor surgical complications requiring further surgery were seen in many other cases.

The major local complication after radiotherapy was the death of 1 patient due to typhlitis during pelvic irradiation. Other complications were transient oesophageal ulcers after radiation therapy of the eight dorsal vertebral in 1 patient, and radiation dermatitis in 7 patients. At present, long-term sequelae after irradiation of humerus and pelvis resulted in ankylosis (elbow and hip) in 2 patients, and amenorrhea persisting after 4 and 6 years in 2 patients. Radioinduced sarcoma has not yet been observed, but the follow-up is still too short.

3.7. Comparison with patients of the previous protocol

As shown in Table 5, the 5-year EFS and OS of patients of the present study was significantly higher than the four previous studies. It must be stressed that in this study there was also a higher rate of patients locally treated by surgery or surgery + radiotherapy (78% versus 33, 49, 63 and 57%; $P < 0.0001$). Moreover, in patients locally treated by surgery, the rate of grade III histologic response after neoadjuvant chemotherapy with VACAc, was significantly higher than in the previous neoadjuvant studies in which patients preoperatively received only VCA (50% versus 27%; $P < 0.04$) and versus 27% ($P = 0.01$).

Considering all the 49 evaluable patients with ESB treated at our Institute with adjuvant and neoadjuvant chemotherapy to evaluate the 5-year EFS according to local treatment, regardless of the protocol of chemotherapy, the 293 patients locally treated by surgery or surgery followed by radiotherapy had a much better EFS than the 195 patients treated by radiotherapy only (5-year EFS = 39% versus 65%; $P < 0.0001$). This result could have been biased by the fact that patients treated with radiotherapy often have lesions located in central sites that have a worse prognosis. However, if we analyse a more homogeneous group of patients, for example, only those with the tumour located in the extremities, the difference in EFS between patients surgically treated and patients treated by radiotherapy only is still highly significant (40% versus 67%; $P < 0.0001$).

4. Discussion

Adjuvant and neoadjuvant chemotherapy have dramatically improved the outcome for patients with ES family tumours of bone [3–12]. The multimodal treatment of these tumours still raises two questions: does the substitution of cyclophosphamide with ifosfamide in the classic VACAc regimen, or the addition of ifosfamide to the four drugs of the VACAc treatment, improve the efficacy of chemotherapy? And what is the best local treatment?

The substitution of cyclophosphamide with ifosfamide improved the cure rate in the multicentric English ET-1 study concerning 142 patients treated in several institutions [6,7], while it did not give any benefits according to the multicentric study of the French Society of Pediatric Oncology, concerning 65 patients

treated in 18 different centres [20]. The German Cooperative Ewing Sarcoma Study group [21], in the CESS 86 multicentric study used ifosfamide instead of cyclophosphamide in the VACAc regimen to treat a group of 'high risk' patients with large and/or central-axis tumours. The results of this study, including 52 'standard risk' patients, and 241 'high risk' patients from 92 different centres, indicated a benefit for the 'high risk' group treated with intensive ifosfamide instead of cyclophosphamide. It must be stressed that none of studies reported above were randomised, and the efficacy of ifosfamide was calculated by comparison with previous studies performed by the same groups.

As said before, in our previous, single-arm, non-randomised study, REN-2, we added ifosfamide and etoposide to the classic VACAc regimen, but we could not demonstrate any advantage in comparison with the previous REN-1 study in which the standard VACAc was used. In the REN-2 study, however, ifosfamide and etoposide were both used after local therapy.

As regards the best local treatment for ESB, data from the literature are contrasting. Some series showed surgery improved the rate of long-term survival more than radiotherapy [9,10,22,23], while other studies did not show any differences [5,19] or just an improvement for local control [24]. However, there are no randomised trials comparing radiotherapy with surgery. Moreover, patients treated with radiotherapy usually present with larger lesions in central sites, which have a worse prognosis. This fact could bias the outcome in favour of surgical resection.

In the REN-3 study, ifosfamide and etoposide were added to the classic VACAc regimen. In comparison with the REN-2 study, ifosfamide was administered in the induction treatment (combined with V and Ac), while etoposide was used only in the postoperative treatment. In addition to this, the six drugs used were delivered in four different combinations: V/A/C, V/I/Ac, E/I, V/Ac/C. As regards local treatment, in comparison with our previous studies, a significantly larger number of patients were locally treated by surgery.

The results achieved with REN-3 protocol are significantly better than those of our previous adjuvant studies [3] and neoadjuvant studies [4].

We used a four-drug VACAc regimen in the REN-1 study and a six-drug regimen (VACAc + postoperative I and E) in REN-2. In patients locally treated by surgery, the rate of a good histological response achieved in this study was significantly higher than in the two previous studies in which a VACAc regimen was used preoperatively: 49% versus 31%; $P < 0.004$. The rate of 5-year EFS was also significantly higher: 71% versus 49% of REN-1 protocol ($P < 0.0009$), and 51% of the REN-2 protocol ($P < 0.004$), as well as the 5-year OS rate (78% versus 56% ($P = 0.004$) and 55% ($P = 0.0009$)), respectively.

Multivariate analysis showed the EFS of this study to be significantly lower for larger tumours, and for patients with fever and high serum levels of LDH at the time of diagnosis. These data confirm previous results [25,26]. Moreover, for surgically treated patients there was a strict correlation between the grade of necrosis given by preoperative chemotherapy and prognosis. This has also been previously observed [9,12,14].

It must be stressed that local treatment in this study was quite different from the local treatment used in our two previous neoadjuvant studies. Many more patients were treated by surgery, and this may have contributed to the improved outcome of patients treated with the REN-3 protocol. In fact, if we evaluate all ESB-patients treated at our institution with adjuvant and neoadjuvant chemotherapy between 1972 and 1997, prognosis is significantly better for patients locally treated by surgery or surgery + radiotherapy, than for patients treated by radiotherapy only. This is likely due to the fact that the outcome of ESB probably differs according to the anatomical site [9,10,26], and patients suitable for radiotherapy are generally those who have lesions located in the central axis with a very poor prognosis. For these reasons, it is almost impossible to state whether the improvement of EFS and OS obtained with the REN-3 protocol was due to the early addition of I in the chemotherapy regimen or to the higher number of patients locally treated by surgery. Probably both factors contributed to the promising results.

Although our report referred to a 16-year period and non-randomised patients, it considered an extremely homogeneous group of patients, all treated at the same institution by the same team of surgeons, radiotherapists, and medical oncologists, and this gave reliability, especially in terms of local treatment and pathological evaluation, which cannot be reached in multicentric studies. Since ESB is a very rare tumour, we believe it will be almost impossible to perform a single-centre randomisation for both the chemotherapy protocol and type of local treatment. Such a study should be multicentric and this would introduce the variable of experience of the various centres in treating bone tumours, especially with regard to surgery. For this reason we think our results are of some value.

We found that the addition of ifosfamide and etoposide to the classic VACAc regimen improved the prognosis of patients with ESB. Moreover, it gave an indication for an early use of ifosfamide. These data seem to be confirmed by the preliminary results of the American Intergroup for Ewing's Sarcoma Study IESS-3 [27], comparing a four-drug regimen (VACAc) to a six-drug regimen (VACAc + I and E) including approximately 400 patients, from 1988 and 1992. The awaited definitive results of this study which was closed 10 years ago, may or may not confirm the importance of the addition of ifosfamide and etoposide to the VACAc

regimen in the treatment of ESB. Right now, we can state that the local treatment of primary ESB should be surgery wherever possible.

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