RESEARCH ARTICLE

# Carboplatin and Doxorubicin in Treatment of Pediatric Osteosarcoma: A 9-year Single Institute Experience in the Northern Region of Thailand

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## Abstract

**Background: Osteosarcoma is the most common primary bone tumor in childhood and adolescence. Carboplatin, a platinum-derived agent, is used as neoadjuvant chemotherapy for pediatric osteosarcoma because of its anti-tumor activity and had low toxicity as compared to cisplatin. Objective: To determine demographic data, prognostic factors and outcome of childhood osteosarcoma treated with a carboplatin-based chemotherapeutic protocol at Chiang Mai University. Method: A retrospective analysis was conducted on 34 osteosarcoma patients aged less than 18 years and treated between 2003 and 2011. Results: Overall limb-salvage and amputation rates were 23.5% and 70.6%, respectively. With the mean follow-up time of 29.5 months (1.5-108.9), the Kaplan-Meier analysis for 3-year disease-free survival (DFS) and 3-year overall survival (OS) were 20.2±7.7% and 47.1±9.5% respectively. Patients who had initial pulmonary metastasis were at significantly greater risk for developing recurrence (p=0.02, OR=7; 1.2-40.1) and had a tendency to have lower 3-year OS compared to those without initial pulmonary metastasis (28.1±13%, 63.1±12.3%, respectively, p=0.202). On univariate analysis, age at diagnosis >14 years and patients who were declined surgery were significantly associated with lower 3-year OS (p=0.008 and <0.05, respectively). However, age at diagnosis, sex, tumor size and histological subtypes were not found to significantly affect recurrence or survival. Conclusions: In our study, the survival rate was far lower than those reported from developed countries. These might indicate the ineffectiveness of carboplatin in combination with doxorubicin as frontline treatment of pediatric osteosarcoma, especially in those with initial pulmonary metastasis. Refinement in risk and treatment stratification and dose intensification for pediatric osteosarcoma constitutes a future challenge to improve outcomes, especially in metastatic patients who may need a more intensive regimen.**

**Keywords:** Pediatric osteosarcoma - risk factors - carboplatin - outcome - survival - Thailand

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## Introduction

Osteosarcoma is the most common primary bone tumor in children, and especially in adolescents. Approximately 60% of osteosarcoma cases occur in patients aged less than 20 years (Stiller et al., 2001; Fletcher et al., 2006; Stiller et al., 2006; Mirabello et al., 2009). In Thailand, the Thai Pediatric Oncology Group (TPOG) reported the crude annual incidence of osteosarcoma of 1.9 cases per million children (Wiangnon et al., 2011). Prior to the introduction of chemotherapy, 80-90% of patients with non-metastatic osteosarcoma died despite early radical surgery (Coventry and Dahlin, 1957; Dahlin and Coventry, 1967; Gaffney et al., 2006). Disease-free survival (DFS) rates increased from below 20%, with surgery only, to more than 40% with adjuvant chemotherapy (Cortes et al., 1972; Jaffe et al., 1973; Dahlin, 1978). The concept of multi-agent chemotherapy as neoadjuvant therapy has improved cure rates dramatically for patients with osteosarcoma. The recent literature has shown that 60-76% of newly diagnosed non-metastatic osteosarcoma can be cured (Delepine et al., 1996; Ferrari et al., 2001; Lewis et al., 2007). In Thailand, there has been no data regarding patients' characteristics and outcome of treatment in pediatric osteosarcoma reported to date. Doxorubicin (ADM) has been reported as an active agent against osteosarcoma (Cortes et al., 1972; Cortes et al., 1974). Carboplatin (CBDCA), a platinum-derived agent, has an anti-tumor activity against osteosarcoma (Meyer et al., 2001; Van Winkle et al., 2005; Daw et al., 2011) and is

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better tolerated because of its lack of severe side effects - especially ototoxicity and nephrotoxicity – as compared to those of cisplatin. The role of CBDCA in combination of doxorubicin as upfront window therapy in pediatric osteosarcoma still remains unclear.

Our institution, Chiang Mai University Hospital, is a tertiary-care referral and research center for the Northern Region of Thailand. The purposes of this study were to explain demographic data, identify the prognostic significance indicating treatment outcome and assess the role and efficacy of CBDCA in combination with ADM in pediatric osteosarcoma in Thailand's Northern Region. The ultimate aim of this purpose is to optimize the management of this high-curable disease.

## Materials and Methods

*Patients*

As a single-institution retrospective study, we enrolled pediatric patients ≤15 years of age with a diagnosis of osteosarcoma between January 2003 and December 2011, and followed up with treatment until 31st January 2012 at Chiang Mai University Hospital, Chiang Mai, Thailand. Patients will be identified by reviewing their medical records at the Registry of Division of Pediatric Hematology-Oncology, Department of Pediatrics, Chiang Mai University Hospital. Clinical data collected for each patient included clinical characteristics (age at diagnosis, sex, medical background and genetic predisposition to cancer), tumor characteristics (location, size, metastatic status at diagnosis), treatment (preoperative chemotherapy, surgery, postoperative treatment, adverse effects),outcome (remission, relapse, survival), laboratory investigations (complete blood count; CBC, blood chemistries, lactate dehydrogenase; LDH level, alkaline phosphatase; ALP) and radiological investigations (chest roentgenogram, bone scintigraphy, computerized tomography (CT) of chest and magnetic resonance imaging (MRI) scan of involved anatomical sites).

*Treatment protocol*

The treatment protocol for pediatric osteosarcoma in our institute is described in Table 1. After histological diagnosis of osteosarcoma had been made by tumor biopsy, chemotherapy was started regardless of osteosarcoma subtypes, size of primary tumor, metastatic status at diagnosis and patients' characteristics. We used a cycle of CBDCA (400 mg/m$^2$/dose on day 1) and ADM (20 mg/m$^2$/day on day 1-3) as neoadjuvant chemotherapy at intervals of 3-4 weeks. Granulocyte colony stimulating factor (G-CSF) (5 mcg/kg/dose) was administered daily for 8-10 days as primary prophylaxis of febrile neutropenia. CBC, blood chemistries, liver function test (LFT) and kidney function were monitored regularly before every cycle of chemotherapy. Surgical procedure, such as wide resection with intraoperative radiotherapy, limb-salvage surgery or amputation, was usually performed after three to four cycles of neoadjuvant chemotherapy, but depended on the feasibility of implementing a surgical procedure and patients' consent.

The characteristics of a resected tumor, including surgical margin, histological subtypes and tumor response (percentage of tumor necrosis), were mostly provided by pathological reports. The therapeutic effect was evaluated by clinical (size of primary tumor), radiographic (bone scintigraphy, CT of chest and MRI of primary tumor) and pathological parameters (surgical margin and percentage of tumor necrosis; Huvos score). A progressive disease (PD) was defined by evidence of subsequently new or worsening metastatic lesions including pulmonary nodule(s) by CT scan, nuclear uptake lesion(s) by bone scintigraphy or size of primary tumor by physical examination and/or imaging. For patients without PD, three to four more cycles of CBDCA/ADM (total of 6-8 cycles) were administered as adjuvant chemotherapy. For patients with PD, chemotherapeutic regimens were adjusted individually by attending oncologists. The alternative adjuvant chemotherapy is also described in Table 1.

*Statistical analysis*

The DFS rate was determined as the time from the date of diagnosis to the first event (disease progression and/or death from any cause or the most recent follow-up examination). Overall survival (OS) rate was calculated from the date of diagnosis to death from any cause or the last follow-up examination. The probability of OS and DFS were analyzed using Kaplan-Meier analysis and the log-rank test for comparisons based on demographic data, pathological diagnosis and treatment protocol. The statistical significance was defined by a p-value <0.05. Descriptive statistics were used to define the population. Influence of discrete variables was analyzed by the $\chi^2$ test or Fisher's exact test, and comparison of continuous variables using the Mann-Whitney test. All data were analyzed using the Statistical Package for the Social

**Table 1. Treatment Protocol of Pediatric Osteosarcoma**

| | Neoadjuvant chemotherapy | | | | | | | | Evaluation & Operation | Adjuvant chemotherapy | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | | | | | | | | | | | | | |
| Cycle | I | | | II | | | III | | IV | V | VI | VII | VIII |
| Week | 0 | | | 3 | | | 6 | | 9 | 12 | 15 | 18 | 21 |
| Day | 1 2 3 | | | 1 2 3 | | | 1 2 3 | | 1 2 3 | 1 2 3 | 1 2 3 | 1 2 3 | 1 2 3 |
| Chemo | C | | | C | | | C | | C | C | C | C | C |
| | A A A | | | A A A | | | A A A | | A A A | A A A | A A A | A A A | A A A |

*4th cycle of CBDCA/ADM pre-surgically is optional when surgery can not be performed or patient declined surgical intervention; ** For patients without PD, three to four more cycles of CBDCA/ADM (total of 6-8 cycles of CBDCA/ADM) were administered as adjuvant chemotherapy; C: Carboplatin 400 mg/m$^2$/day IV over 1 hours x1 day; A: Doxorubicin 20 mg/m$^2$/day IV over 30 min x3 days; ¶For patients with PD, chemotherapeutic regimens were adjusted individually by attending oncologists. The alternative protocols: (1) IE protocol: I) Ifosfamide 1,800 mg/m$^2$/day IV over 1 hours x 3 days; E) Etoposide 100 mg/m$^2$/day IV over 1-2 hours x 3 days. (2) ICE protocol: I) Ifosfamide 1,800 mg/m$^2$/day IV over 1 hours x 5 days; C: Carboplatin 560 mg/m$^2$/day IV over 1 hours x 1 day; E) Etoposide 100 mg/m$^2$/day IV over 1-2 hours x 5 days

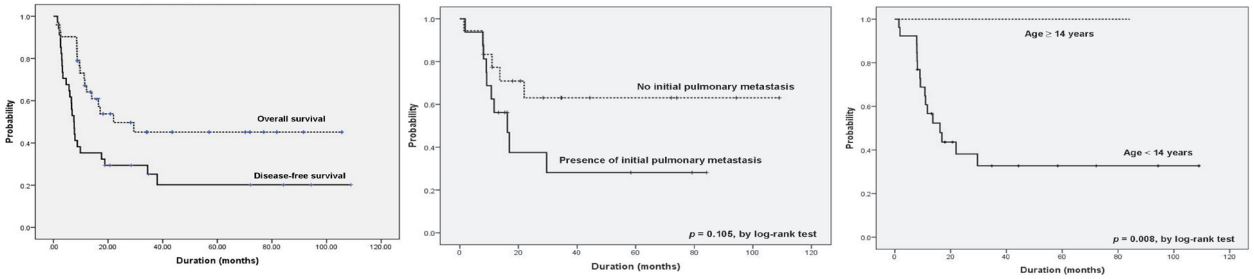


**Figure 1. Kaplan-Meier Survival Analysis for Pediatric Osteosarcoma Treated with the Combination of Carboplatin and Doxorubicin.** (A) Overall and disease-free survival of pediatric osteosarcoma regardless of metastatic status at diagnosis, (B) Kaplan-Meier estimated overall survival according to metastatic status at diagnosis and (C) Kaplan-Meier estimated overall survival according to age group at diagnosis

**Table 2. Patients' Characteristics According to Treatment Protocol**

| Patients' characteristics | All (N=34) |
|---|---|
| Gender, N (%) | |
| Male | 14 (41.20) |
| Female | 20 (58.80) |
| Age, mean (years), range | 11.77 (5.58-14.76) |
| Mean follow-up time (months), range | 29.4 (1.5-108.97) |
| Median time to treatment (months), range | 7.5 (3-22) |
| Primary sites, N (%) | |
| Knee | |
| Distal femur | 20 (58.80) |
| Proximal tibia | 8 (23.50) |
| Proximal fibula | 1 (2.90) |
| Ankle (Distal tibia) | 2 (5.90) |
| Hip | |
| Proximal femur | 0 |
| Iliac wing | 1 (2.90) |
| Shoulder (proximal humerus) | 1 (2.90) |
| Wrist (distal radius) | 1 (2.90) |
| Metastasis at diagnosis, N (%) | |
| Distant bony metastasis | 0 |
| Pulmonary metastasis | 16 (47.10) |
| Combined metastasis | 0 |
| Histology, N (%) | |
| Conventional type | 14 (41.10) |
| Telangiectatic type | 7 (20.60) |
| No data | 13 (38.20) |
| Chemotherapy, N (%) | |
| No chemotherapy | 2 (5.90) |
| Neoadjuvant chemotherapy | 31 (91.20) |
| Adjuvant chemotherapy | 1 (2.90) |
| Chemotherapy only | 2 (5.90) |
| Interval between chemotherapy cycle, mean (range), weeks | 4.94 (2.39-9.30) |
| Surgery, N (%) | |
| Amputation | 24 (70.60) |
| Rotatoplasty | 8 (23.50) |
| Declined surgery/Inoperable | 2 (5.90) |
| Histological response (Huvos grade), N (%) | |
| I / II / III / IV | 2 / 20 / 0 / 0 |
| No data | 10 |
| Local recurrence during chemotherapy | 13 (38.20) |
| Detection of lung metastases | |
| At initial presentation | 16 (47.10) |
| Unilateral lung nodules | |
| Bilateral lung nodules | |
| Newly detected during chemotherapy | 7 (20.60) |
| Newly detected after treatment | 0 |
| Progression during chemotherapy | 19 (55.90) |
| Stable disease during chemotherapy | 4 (11.76) |

Sciences (SPSS, Chicago, IL), version 17.0.

## Results

Over a 9-year period, a total of 36 patients with a diagnosis of pediatric osteosarcoma were enrolled. Two patients were excluded due to lost to follow up prior to starting any treatment. The patients' characteristics are shown in Table 2. The majority of patients were female (58.8%) with a median and mean age of 12.2 years (SD=2.44) and 11.77 years (5.58-14.76 years), respectively. The majority of patients were aged of between 9-14 years (64.7%). 16 patients (47.1%) had initial pulmonary metastasis at diagnosis. The most common primary site was at distal femur (58.8%) and overall likely to be affected around the knee joint (85.3%). Only one case had primary tumor at iliac wing. No primary tumor at skull, vertebrae or other axial site was presented in our series. The mean and median time from initial symptom to treatment were 2.2 months (0.75-5.50) and 1.87 months (SD=1.11), respectively. The most frequent presenting symptom was pain after minor trauma (64.7%). Overall limb-salvage and amputation rates were 23.5% and 70.6%, respectively. Two patients who were declined surgery subsequently died, despite chemotherapy. Among 31 patients who received neoadjuvant chemotherapy prior to surgery, tumor necrosis was defined in only 22 patients and all of them had tumor necrosis <90%. Only one patient underwent limb-salvage surgery before chemotherapy and died subsequently. Seven patients (20.6%) underwent surgical procedures after 1-2 neoadjuvant cycles according to the availability of the surgical schedule. Neither toxic death nor serious treatment-related toxicity was observed in our study, including renal, hepatic, neurological and gastrointestinal toxicity. None of the patients had an absolute granulocyte count less than 500/uL after administration of CBDCA and ADM.

With the mean and median follow-up time of 29.4 months (1.5-108.9) and 16.18 months (SD=29.3), the median survival time was 29.6 months. The Kaplan-Meier analysis for 1, 2, 3-year DFS and 1, 2, 3-year OS were 35.3±8.2%, 29.4±7.8%, 20.2±7.7% and 70.2±7.9%, 51.8±9.2%, 47.1±9.5%, respectively (as shown in Figure 1). Patients who had initial pulmonary metastasis were at significantly greater risk for developing recurrence (p=0.02, OR=7; 1.22-40.12) and had statistically significant lower 3-year DFS as compared to those without initial pulmonary metastasis (41.7±12.4 and

**Table 3. Patients' Characteristics and Survival Analysis**

| Characteristics | | No. of patients (%) | 3-year OS (%) | p-value# | 3-year DFS (%) | p-value# |
|---|---|---|---|---|---|---|
| Sex | Male | 14 (41.2) | 45.7±14.7 | | 10.7±9.4 | |
| | Female | 20 (58.8) | 48.4±12.6 | 0.788 | 26.3±11 | 0.32 |
| Age at diagnosis | ≤11.77 years | 14 (41.2) | 36.7±14 | | 28.6±12.1 | |
| (by mean age) | >11.77 years | 20 (58.8) | 54.6±12.4 | 0.205 | 18±9 | 0.59 |
| Age at diagnosis | ≤14 years | 26 (76.5) | 32.7±10.1 | | 26.9±8.7 | |
| | >14 years | 8 (23.5) | 100 | 0.008** | 18.8±15.8 | 0.64 |
| Primary tumor | Axial | 1 (2.9) | 0 | | 0 | |
| | Peripheral | 33 (97.1) | 49.7±9.6 | - | 26±7.9 | - |
| Histology | Variants | 7 (20.6) | 85.7±13.2 | | 42.9±18.7 | |
| | Conventional | 14 (41.2) | 50±13.4 | 0.173 | 28.6±12.1 | 0.933 |
| | No data | 13 (38.2) | 33.8±13.7 | 0.097 | 23.1±11.7 | 0.618 |
| Presence of metastases at diagnosis | Absence | 18 (52.9) | 63.1±12.3 | | 41.7±12.4 | |
| | Presence | 16 (47.1) | 28.1±13 | 0.105 | 6.3±6.1 | 0.015** |
| Maximal tumor length* | >10 cm | 16 (48.5) | 37.5±13.4 | | 18.8±9.8 | |
| | ≤10 cm | 17 (51.5) | 56.1±12.7 | 0.202 | 26.5±11.6 | 0.125 |
| Extent of surgery | Amputation/Rotatoplasty | 24 (70.6) | 55±10.8 | | 26.7±9.7 | |
| | Limb-sparing resection | 8 (23.5) | 43.8±18.8 | 0.822 | 15±15.3 | 0.677 |
| | Declined surgery/inoperable | 2 (5.9) | 0 | < 0.05** | 0 | < 0.05** |
| Surgical margins* | Adequate | 31 (96.9) | 48.5±10 | | 24.9±8 | |
| | Inadequate | 1 (3.1) | 100 | 0.434 | 100 | 0.274 |
| Histological response* | Good responders*** | 0 | - | | - | |
| | Poor responders | 22 | | | 11.4±9.2 | |
| Recurrence | Local recurrence | 13 (41.1) | 35.2±15.5 | 0.688 | | |
| | Pulmonary progression/recurrence | 19 (55.9) | 30.5±13 | 0.162 | | |
| Treatment-related toxicity | | 0 | | | | |

*Not all data available. **Statistically significance; p=0.05. ***Good responder is defined by tumor necrosis >90%.#Log-rank test

6.3±6.1, respectively, p=0.015), but had not reached statistical power for 3-year OS (28.1±13%, 63.1±12.3%, respectively, p=0.105). The median survival time of patients with initial pulmonary metastasis was 5.70 months (95%CI: 0.74-10.6). In univariate analysis, age at diagnosis <14 years and patients who were declined surgery were significantly associated with lower 3-year OS (p=0.008 and <0.05, respectively). The survival analysis according to surgical modality found no difference between limb-salvage operation and amputation, but patients who underwent amputation tended to have higher 3-year OS and DFS. However, age at diagnosis, sex, tumor size, histological subtypes and location of primary tumor were not found to be statistically significant, affecting recurrence or survival, as shown in Table 3.

## Discussion

Osteosarcoma is the most frequent primary bone tumor in children, especially during adolescence. In the modern era of intensive chemotherapy, 60-76% of newly diagnosed non-metastatic osteosarcoma can be cured. However, there is still no consensus on a standard chemotherapy approach. In Thailand, there was no data regarding a treatment regimen and outcome of treatment in pediatric osteosarcoma reported to date. The study conducted in the Northeastern Region of Thailand was the only such study to have been reported as a population-based survival study, but no treatment regimen was described (Wiromrat et al., 2012). This is the first study regarding the outcome of pediatric osteosarcoma in Thailand that was conducted according to treatment regimen.

Unlike the previous literatures, females were affected approximately 1.4 times more often than males, since this study was conducted in a tertiary-care center and, for that reason, might not be equivalent to a population-based study. More than 80% of cases had primary tumors around the knee joint, comparable to previous reports. At diagnosis, approximately half of the patients had pulmonary metastasis which was higher than those of Western countries (Link et al., 1986; Eilber et al., 1987). The age distribution had a correlation with previous studies worldwide with a peak age incidence in adolescents (Mirabello et al., 2009; Smith et al., 2010; Wiromrat et al., 2012).

Since there was no consensus in standard treatment of pediatric osteosarcoma, a combination of several chemotherapies was used in order to achieve certain therapeutic effects. CBDCA is a synthesized platinum compound with fewer side effects as compared to those of cisplatin. Preclinical data of CBDCA against osteosarcoma was rather indicative (Bergman et al., 1996; Crnalic et al., 1996; Robson et al., 2002) of its ability to induce regression of osteosarcoma. The efficacy of CBDCA was comparable to cisplatin in treatment of several solid tumors, especially in adults (Lokich and Anderson, 1998). We applied the combination of CBDCA and ADM as frontline treatment in all pediatric osteosarcoma cases, regardless of metastatic status at diagnosis. We found the presence of pulmonary metastasis and an age of less than 14 years at diagnosis to be predictors of unfavorable outcome. Our results were consistent with Bacci et al. (2005), whose studies showed the worse outcome in the age group of less than 14 years, while certain others reported a favorable outcome in those of age less than 14 years (Mankin et al., 2004; Lee et al., 2009; Hagleitner et al., 2011). However, the influence of age on outcome in pediatric osteosarcoma is still contradictive and might

result from the variability of treatment regimens and metastatic status in each study. Age-related tumor biology and pharmacogenomics of pediatric osteosarcoma need to be explored in order to explain these findings. The percentage of tumor necrosis induced by preoperative chemotherapy had a significant correlation with outcome of pediatric osteosarcoma in previous studies (Hudson et al., 1990). We received an unsatisfactory response since no good responder (tumor necrosis >90%) was observed in our study. Thus, this finding might indicate the inferiority of our chemotherapeutic protocol. We found no significant correlation between other reported prognostic factors such as site of metastasis, surgical modality, percentage of tumor necrosis, histopathological type and outcome. But these might be the result of the lack of complete histopathological data in some patients, including histopathological subtypes and percentage of tumor necrosis after preoperative chemotherapy in our retrospective study.

Our treatment regimen was to treat patients with osteosarcoma without cisplatin and, of significance, well tolerable without any severe myelosuppression and nephrotoxicity. The European Osteosarcoma Intergroup (Lewis et al., 2000) reported the 3-year DFS of 46% in pediatric osteosarcoma treated with cisplatin and ADM as compared to 20.2% in our study. Petrilli et al. (Petrilli et al., 1999) demonstrated the 3-year DFS of 65% in response to intraarterial CBDCA (600 mg/m$^2$/dose) as a preoperative chemotherapy for metastatic osteosarcoma, followed by the combination of ifosfamide, CBDCA and HDMTX as postoperative chemotherapy. Ferguson et al (Ferguson et al., 2001) described responses in at least one tumor site in 23% of patients with metastatic and/or unresectable osteosarcoma by intravenous CBDCA (1,000 mg/m$^2$/cycle). Meyer et al. (Meyer et al., 2001) used the combination of CBDCA (560 mg/m$^2$/dose for 1 day) and ifosfamide (2.65 g/m$^2$/dose for 3 days) as frontline treatment and observed a 3-year DFS of 60% for unresectable non-metastatic osteosarcoma and 5.9% for metastatic osteosarcoma. Our inferior outcome might have resulted from the inadequacy of CBDCA intensity in our protocol, no stratification of treatment for metastatic patients and early surgical procedure prior to receiving the benefit of preoperative chemotherapy. In order to optimize and stratify treatment for pediatric osteosarcoma, a more intensified combination of chemotherapy with other active agents against osteosarcoma, such as high-dose methotrexate (HDMTX), ifosfamide, cisplatin, should be included in the treatment protocol, especially for patients younger than 14 years who had pulmonary metastasis at diagnosis. However, some studies reported that a combination of ADM and cisplatin may be as efficacious as more complex regimens (Bramwell et al., 1992; Souhami et al., 1997). The role of HDMTX is still controversial, but some studies have shown the same promising results (Jaffe et al., 1973; Pratt et al., 1980; Saeter et al., 1991) as those of ifosfamide (Bacci et al., 2001).

This study showed that the combination of CBDCA and ADM has inferior antitumor activity in osteosarcoma in the context of high incidence of pulmonary metastasis at diagnosis in our study as compared to those of western countries. The refinement in risk, treatment stratification and dose intensification for pediatric osteosarcoma is a future challenge to improve outcomes, especially in metastatic patients who may need a more intensive regimen.

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