Clinical Study

Oncology

Oncology
DOI: 10.1159/000502548

Received: July 19, 2019
Accepted after revision: August 7, 2019
Published online: September 11, 2019

Histologic Response and Toxicity following Interval-Compressed Four-Drug Therapy Given Preoperatively in Children and Young Adults with Osteosarcoma: A Retrospective Study

Ji-Man Kang[a] Hee Young Ju[a] Jungnam Joo[b] Jee Young Sung[c] Seog Yun Park[d] June Hyuk Kim[e] Hyun Guy Kang[e] Mimi Kwon[a] Meerim Park[a] Hyeon Jin Park[a] Byung-Kiu Park[a, c]

[a]Center for Pediatric Oncology, National Cancer Center, Goyang, Republic of Korea; [b]Division of Cancer Epidemiology and Management, National Cancer Center, Goyang, Republic of Korea; [c]Division of Clinical Research, National Cancer Center, Goyang, Republic of Korea; [d]Department of Pathology, National Cancer Center, Goyang, Republic of Korea; [e]Orthopaedic Oncology Clinic, National Cancer Center, Goyang, Republic of Korea

Keywords
Osteosarcoma · Neoadjuvant chemotherapy · Interval compression · Histologic response · Toxicity

Abstract
Objectives: The histologic response to chemotherapy is an important prognostic factor in osteosarcoma. Thus, we attempted to develop an effective neoadjuvant regimen to achieve an improvement in histologic response. ***Methods:*** Twenty-nine patients with a high-grade osteosarcoma received 2 courses of neoadjuvant chemotherapy non-randomly with either the MAP regimen (methotrexate 12 g/m^2, cisplatin 120 mg/m^2, and doxorubicin 75 mg/m^2) or MAPI regimen (MAP plus ifosfamide 9 g/m^2). We applied interval compression to MAPI by shortening the preoperative period to be aligned with that of MAP. Adjuvant chemotherapy was tailored according to the necrosis rate of resected tumor specimens. Necrosis rate, toxicity, and survival outcome were compared retrospectively between the 2 groups. ***Results:*** The median interval between the beginning of neoadjuvant chemotherapy and surgery was 97.0 days in the MAPI group (17 patients) and 90.5 days in the MAP group (12 patients; $p = 0.19$). The good histologic response (>90% of necrosis) was observed in 71% of MAPI and in 42% of MAP ($p = 0.12$). Major toxicities of grade 3 or worse were not different between the 2 groups. The probability of 5-year progression-free survival and overall survival of the MAPI group were 74 and 83%, and those in the MAP group were 50 and 75%, showing no difference. ***Conclusions:*** Interval-compressed MAPI therapy given in a similar duration of the preoperative phase to that of conventional MAP therapy showed a marginal trend toward a better histologic response without a significant increase in major toxicities. Regarding the proportion of good histologic response, 71% is one of the highest values ever reported in the literature. The results warrant further testing in a prospective way in a larger cohort.

J.-M.K. and H.Y.J. contributed equally to this work.

Byung-Kiu Park, MD, PhD
Division of Clinical Research
National Cancer Center, 323 Ilsan-ro
Ilsandong-gu, Goyang 10408 (Republic of Korea)
E-Mail bkpark@ncc.re.kr

Introduction

Osteosarcoma is rare, but represents the most common primary bone cancer in children and young adults. The incidence rate is 2.4–4.0 per million in all ages and 4.4–5.3 per million in the age group 0–24 years, and men are affected 1.5 times as often as women [1, 2]. The long-term survival rate was <20% in patients with non-metastatic disease before the 1970s, when only a surgical treatment was performed, but the 5-year survival rate was greatly increased to >60% after the introduction of systemic chemotherapy [3–5]. However, there has been no further improvement in outcome over the past 3 decades since neoadjuvant chemotherapy, surgical resection, and adjuvant chemotherapy were established as a standard treatment [6]. In an effort to improve survival, various drugs have been tried at various doses in different combinations within study groups [6–8]. Currently, the most commonly used, active drugs for osteosarcoma are high-dose methotrexate, doxorubicin, cisplatin, and ifosfamide. Besides the combinatory regimen (MAP) of high-dose methotrexate (M), doxorubicin (Adriamycin; A), and cisplatin (P), one of the active neoadjuvant regimens, ifosfamide (I), has been added in an attempt to achieve a better outcome [7, 8]. However, these trials have not yielded a convincing survival benefit. A randomized study of interval-compressed chemotherapy for localized Ewing sarcoma, another chemo-sensitive pediatric bone cancer, demonstrated that chemotherapy administered at a shorter interval was more effective, with no increase in toxicity [9]. We explored whether a similar approach could be applicable to osteosarcoma.

Major prognostic factors of osteosarcoma include age and stage at initial diagnosis, location of the primary lesion, complete resection of all bulk disease, and histological response to neoadjuvant chemotherapy [6, 10, 11]. Most of these factors are predetermined at the time of initial diagnosis. However, the histological response to chemotherapy is presumably dependent on the number and combination of chemotherapeutic drugs administered preoperatively. Although contradictory results have been reported, the histologic response to neoadjuvant chemotherapy, as reflected in tumor necrosis, is still regarded as one of the important prognostic factors [6, 8, 10, 11]. Attempts to improve outcome with an intensified regimen postoperatively in patients with a poor histologic response have generally not succeeded [10, 12]. Therefore, we tried a novel approach to obtain a better histologic response by using an interval-compressed MAPI (MAP plus I) regimen. To this end, we compared the necrosis rate achieved by interval-compressed MAPI versus conventional MAP. We also investigated whether MAPI employed in the preoperative period was more toxic, but caused a survival gain compared to MAP.

Patients and Methods

Study Population

This is a retrospective observational study. From January 2002 to December 2015, patients treated for newly diagnosed osteosarcoma at the Center for Pediatric Oncology of the National Cancer Center (NCC), Korea, were included. All these patients were confirmed histologically to have a high-grade osteosarcoma with a diagnostic biopsy. However, patients who received neoadjuvant chemotherapy other than the study protocol or underwent surgery before chemotherapy were excluded. This study was approved by the Institutional Review Board (IRB) of the NCC (IRB No. NCC2017-0206), and the requirement for informed consent was waived because of the retrospective nature of the study.

Chemotherapy and Surgery

The patient population was divided into 2 groups: the MAP group and the MAPI group. MAP was used before November 2009, but the histologic response and oncologic outcome were not improved during the 8-year period of MAP therapy. From November 2009, we therefore decided to attempt a novel interval-compressed MAPI, the concept of which was supported by 2 studies [9, 13]. The MAP group received 2 courses of neoadjuvant chemotherapy, and each course consisted of intravenous administration of methotrexate 12 g/m^2 over 4 h (maximum dose 20 g), cisplatin 120 mg/m^2 (4-h infusion of 60 mg/m^2 per day on days 1 and 2) followed by doxorubicin 75 mg/m^2 (37.5 mg/m^2 per day on days 1 and 2) at the time point illustrated in Figure 1. Leucovorin (folic acid) rescue at a dose of 15 mg/m^2 was done starting 24 h from methotrexate infusion and continued until the methotrexate concentration was <0.1 μM. Leucovorin rescue was adjusted with the dosing nomogram according to the methotrexate concentration. The MAPI group also received 2 courses of chemotherapy where ifosfamide 9 g/m^2 (4-h infusion of 3 g/m^2 per day for 3 days) with mesna uroprotection was added to the 3 drugs used in MAP. Despite 4 drugs being used in MAPI, the treatment duration in the preoperative phase was designed to be the same (i.e., interval compression) as that in MAP (Fig. 1). To begin chemotherapy, patients needed an absolute neutrophil count ≥750/μL and a platelet count ≥75,000/μL. Patients were also required to have normal liver, renal, and cardiac function. After each course, the blood count was monitored every 2 days, starting on day 7 from the beginning of the chemotherapy infusion. Use of myeloid growth factor was begun when an absolute neutrophil count fell below 500/μL, and continued until it went up to 1,000/μL. Following 2 courses of chemotherapy, patients underwent resection of the primary tumor. Surgery was planned during weeks 11–12 after the start of neoadjuvant chemotherapy. Adjuvant chemotherapy was scheduled 2–3 weeks after surgery. Adjuvant chemotherapy was tailored according to the histologic

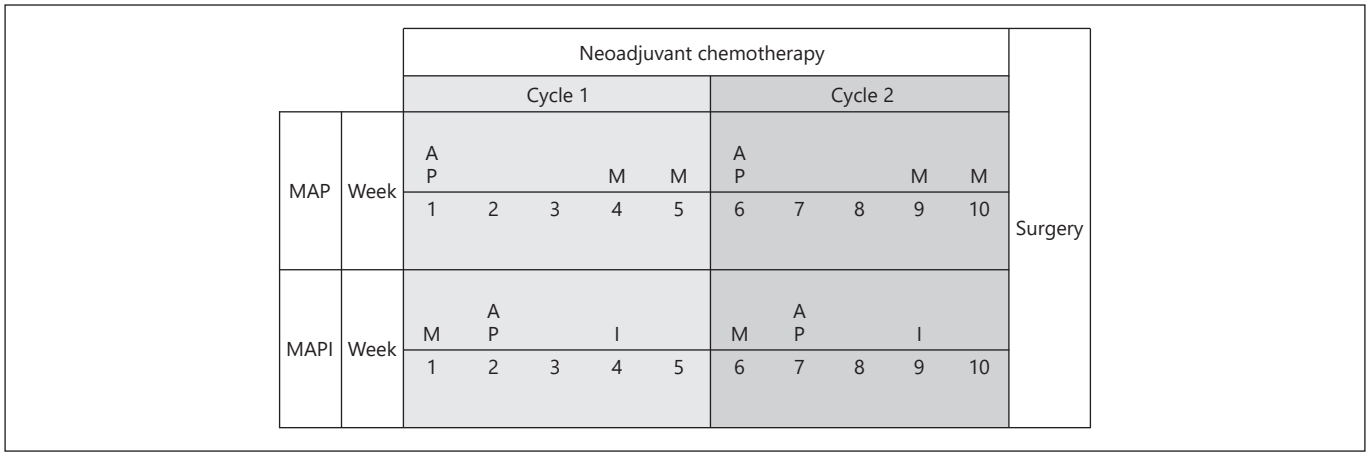

Fig. 1. Treatment scheme of the 2 neoadjuvant regimens. After a histologic diagnosis was made with the biopsy specimen, patients underwent neoadjuvant chemotherapy with a MAP or MAPI regimen. For MAP therapy, cisplatin (P) followed by doxorubicin (A) was given at weeks 1 and 6, and methotrexate (M) was given at weeks 4, 5, 9, and 10. For MAPI therapy, M was given at weeks 1 and 6, P followed by A was given at weeks 2 and 7, and ifosfamide (I) was given at weeks 4 and 9. After the completion of 2 courses of either MAP or MAPI chemotherapy, surgical resection of the tumor was performed. The dose for each agent was as follows: M, 12 g/m^2/day; A, 37.5 mg/m^2/day on days 1 and 2; P, 60 mg/m^2/day on days 1 and 2; I, 3 g/m^2/day for 3 days.

response determined at surgery: postoperative treatment for the MAP group followed the Children's Oncology Group (COG) AOST0331 regimen, that is MAP or MAPIE (MAP plus ifosfamide and etoposide), and that for the MAPI group is illustrated in online supplementary Figure S1 (for all online suppl. material, see www.karger.com/doi/10.1159/000502548). In the MAPI group, good responders received 2 courses of adjuvant chemotherapy, while poor responders completed 3 courses. For a metastatic disease, removal of metastases was performed, if resectable, following at least one course of adjuvant chemotherapy. If the disease progressed or relapsed while on adjuvant treatment or after treatment completion, various salvage regimens, including ifosfamide, carboplatin, and etoposide (ICE), gemcitabine and docetaxel (GD), topotecan and cyclophosphamide (TC), vincristine, irinotecan, and temozolomide (VIT), or sorafenib, were applied.

Collection of Clinical Information

We collected clinical data, including age at diagnosis, sex, site of primary tumor, stage, existence of pathologic facture at diagnosis, histology, period from the first day of neoadjuvant chemotherapy to the day of surgery, completeness of tumor resection, tumor size, necrosis rate of resected tumor, and survival outcome. Diagnosis, histologic subtype, and histologic response were reviewed by a pathologist who is an expert in sarcoma histology.

The histologic response to neoadjuvant chemotherapy was assessed by measuring the degree of necrosis of the surgical specimen as previously described [14]. The histologic response was categorized as good if there was >90% necrosis and poor for a lesser degree of necrosis in the surgical specimen. Progression-free survival (PFS) and overall survival (OS) were used as an outcome indicator. The length of PFS or OS was defined as the period from the time of diagnosis until first event (disease progression, relapse for PFS, death for OS) or last patient contact, whichever occurred first.

Toxicity Monitoring

All toxic events from the beginning of neoadjuvant chemotherapy until surgery were collected. Toxic events while on adjuvant chemotherapy were not evaluated because some of the MAP group received MAPIE after surgery (Fig. 2). Toxicity was examined by chart review. Hematologic toxicities included neutropenia, febrile neutropenia, thrombocytopenia, and anemia. Non-hematologic toxicities included electrolyte, liver enzyme, and creatinine abnormalities, mucositis, vomiting, and cardiac or neurologic dysfunction. All toxic events were classified according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Statistical Analysis

Demographic and clinical data were compared using Fisher's exact test or χ^2 test for categorical variables and Mann-Whitney test for continuous variables, as appropriate. Categorical variables are presented as frequencies (percentages) and continuous variables were expressed as medians (ranges). Logistic regression analysis was performed to evaluate the associated factors with histologic response. Relationships between potential risk factors for survival (PFS and OS) were tested by Cox proportional-hazards regression. Relationships between survival and the treatment regimen were assessed using the Kaplan-Meier method and compared using the log-rank test. $p < 0.05$ was considered to indicate statistical significance. Compliance with the protocol was evaluated for each patient and was expressed in terms of relative received dose (RRD; ratio of received cumulative dose by protocol-planned cumulative dose) of each drug. In addition, the received dose intensity (RRD/I) was calculated for each drug by dividing the RRD by the relative chemotherapy duration (ratio of actual duration of chemotherapy treatment by protocol-planned duration) [7]. The *t* test was used for comparing protocol compliance between the 2 regimens.

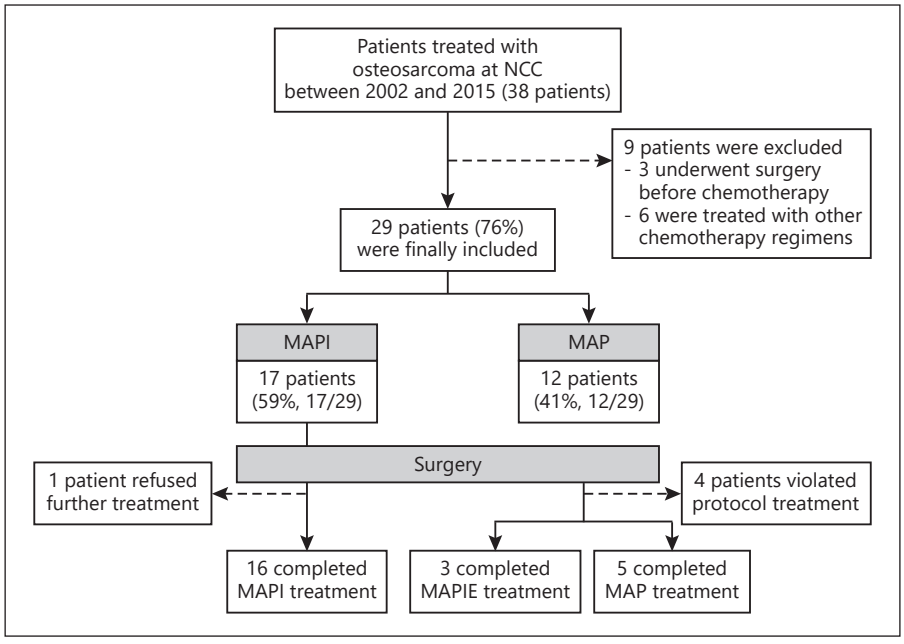

Fig. 2. Flow diagram of study populations.

All statistical analyses were performed using SPSS version 19 (SPSS, Chicago, IL, USA) and GraphPad Prism version 7.03 (GraphPad Software, La Jolla, CA, USA). The data were frozen on July 31, 2017.

Results

Patient Characteristics

Between January 2002 and December 2015, a total of 38 patients were treated for osteosarcoma at the NCC. Nine patients were excluded: 3 patients underwent surgery before chemotherapy and 6 did not receive the study regimen (Fig. 2). As a result, 29 patients were finally included, 17 of whom (58.6%, 17/29) received MAPI and 12 (41.4%, 12/29) undertook MAP. As was addressed in Patients and Methods, patients diagnosed from November 2009 to December 2015 were assigned into the MAPI group. Three of the 29 underwent an early part of neoadjuvant chemotherapy in other institutions and were subsequently referred to our institution. Sixteen completed adjuvant MAPI treatment, and 8 did either adjuvant MAPIE or MAP treatment. The remaining 5 of the 29 patients did not have protocol treatment.

Overall, the baseline clinical characteristics looked similar between the groups (Table 1), but small patient numbers might have mitigated statistical meaning. The median age at diagnosis was 16.1 years (range 9.9–36.7) and 55.2% ($n = 16$) were male. Limbs were predominantly involved as a primary site in both groups. Osteoblastic histology was a dominant type in both groups. The median period between the first day of neoadjuvant chemotherapy and the day of surgery was 97.0 days (range 77–126) and 90.5 days (range 76–133) in the MAPI and the MAP groups, respectively, with no difference between the groups ($p = 0.19$). Macroscopically, primary tumor was completely removed in 94.1 and 83.3% in the MAPI and the MAP group, respectively. The reason for the failure of complete resection in 3 patients was the involvement of an adjacent neurovascular bundle. Tumor volume ≥200 mL was observed in 17.6% of the MAPI group and 25.0% of the MAP group.

Treatment Compliance

All 29 patients completed preoperative treatment, and 26 patients (excluding 3 whose initial treatment was done at other institutions) were evaluated for treatment compliance. The RRD of each drug in the 2 groups did not differ ($p = 0.37$; Table 2). The median relative chemotherapy duration was 1.07 (range 0.90–1.44) for MAPI and 1.08 (range 0.94–1.31) for MAP ($p = 0.83$; data not shown). The average value of RRD/Is of each drug in MAPI was 0.89 ± 0.03 and that in MAP was 0.91 ± 0.13 ($p = 1.00$).

Histologic Response

The median necrosis rate of surgical specimens was 95.0% (range 40–100) in the MAPI group and 87.5% (range 3–100) in the MAP group ($p = 0.22$; Fig. 3a). When

Table 1. Patient characteristics (n = 29)

	MAPI (n = 17)	MAP (n = 12)	p value
Male	11 (64.7)	5 (41.7)	0.3
Age at diagnosis, years	16.1 (10.2–36.7)	15.8 (9.9–24.7)	0.6
10–17	12 (70.6)	9 (75.0)	1.00
≥18	5 (29.4)	3 (25.0)	
Primary site			0.4
Limb	13 (76.5)	12 (100.0)	
Proximal/diaphysis/distal	2/5/6	5/1/6	
Axial[a]	4 (23.5)	0 (0.0)	
AJCC stage			0.2
2	13 (76.5)	7 (58.3)	
3	1 (5.9)	0 (0.0)	
4	3 (17.6)	5 (41.7)	
Lung metastases	1 (5.9)	4 (33.3)	0.1
Extrapulmonary metastases[b]	2 (11.8)	1 (8.3)	1.0
Pathologic fracture at diagnosis	1 (5.9)	1 (8.3)	1.0
Histologic subtype			0.5
Osteoblastic	15 (88.2)	12 (100.0)	
Chondroblastic	2 (11.8)	0 (0.0)	
Days from the start of chemotherapy to operation	97 (77–126)	90.5 (76–133)	0.2
Surgical resection			0.6
Complete	16 (94.1)	10 (83.3)	
Incomplete	1 (5.9)	2 (16.7)	
Tumour volume ≥200 mL	3 (17.6)	3 (25.0)	0.7

Data are presented as n (%) or the median (range). MAPI, methotrexate, doxorubicin, cisplatin, and ifosfamide; MAP, methotrexate, doxorubicin, and cisplatin; AJCC, American Joint Committee on Cancer.

[a] One pelvic bone and 3 mandibles.

[b] Two bones and 1 lymph node.

we divided histologic response into a good versus poor response based on the necrosis rate of 90% as a cut-off, 12 (70.6%) of the MAPI group and 5 (41.6%) of the MAP group had a good response (Fig. 3b), which showed a marginal trend toward a difference but no significant difference between the groups in univariate analyses (p = 0.12; online suppl. Table S1). Protocol compliance had no impact on necrosis rate, given that the average RRD and RRD/I were similar between the 2 treatment groups (Table 2).

Survival Outcomes

With a median follow-up of 32.3 months, the 5-year probability of PFS was 74.0% (SE ±11.3) in the MAPI group and 50.0% (SE ±14.4) in the MAP group (online suppl. Fig. S2a). There was no difference in PFS between the 2 groups in the univariate analyses (hazard ratio, HR 0.75; 95% CI 0.22–2.50; p = 0.64; online suppl. Table S2). OS probability at 5 years was 82.9% in MAPI and 75.0% in MAP (median follow-up 32.3 months in the MAPI group and 89.0 months in the MAP group; online suppl. Fig. S2b). Separate analysis for non-metastatic osteosarcoma patients also yielded no difference in survival outcomes between the 2 groups in univariate analyses (p = 0.83 in PFS, p = 0.66 in OS; data not shown).

Toxicity Profile

Because 3 patients were initially treated at other institutions, toxicities were assessed in 16 of 17 patients in the MAPI group and 10 of 12 patients in the MAP group. There was no mortality or grade 5 toxicity during the preoperative period in both groups. Overall, toxicities of grade 3 or 4 were not different between the groups (p = 0.27; Table 3). Grade 3 or 4 neutropenia was the most commonly observed toxicity (23/26, 88.5%) which was observed in 16 (100%) patients in MAPI and 7 (70%) patients in MAP. Although grade 4 neutropenia (absolute neutrophil count $\leq 0.5 \times 10^3/\mu L$) was observed more frequently in MAPI than MAP (100 vs. 50%, p = 0.014), episodes of febrile neutropenia were not different between the 2 groups (75 vs. 60%, p = 0.44). No significant difference in ≥grade 3 anemia (81 vs. 50%) or thrombocytopenia (87.5 vs. 50%) was demonstrated between the 2 groups (p = 0.13 and 0.1, respectively). With regard to ≥grade 3 non-hematologic toxicities, no difference was observed between the groups (p = 0.67). Elevated alanine aminotransferase was most common (75.1% in MAPI vs. 60.0% in MAP) among ≥grade 3 non-hematologic toxicities, followed by mucositis or vomiting. The occurrence of grade 3 electrolyte disturbance, such as hypophosphatemia, hyponatremia, or hypokalemia, did not show a difference between the 2 groups. Grade 3 or above hypercreatinemia was not demonstrated in either group. However, a lesser grade hypercreatinemia was frequently observed (56.3% in MAPI and 50.0% in MAP). Grade 3 or above neuropathy occurred in 1 patient in the MAP group, but none in the MAPI group. Grades 1–2 left ventricular dysfunction (low shortening fraction; SF) or hypertension was noted in each patient of the MAPI group. One patient in the MAP group developed grade 1–2 hypertension.

Discussion

In the current study, the preoperatively given MAPI regimen intensified by shortening the interval between courses yielded only a marginal trend (p = 0.12) toward a better histologic response but no significant difference compared with the conventional MAP regimen. Nonetheless, we do not exclude the possibility that the small cohorts hindered us from reaching a statistical difference

Table 2. Treatment compliance (n = 26)

Drugs	MAPI		MAP		p value (RRD)	p value (RRD/I)
	RRD[a]	RRD/I[b]	RRD	RRD/I		
MTX	1 (0.81–1)	0.89 (0.61–1.11)	1 (0.75–1)	0.93 (0.63–1.06)	0.71	0.91
ADM	1 (0.83–1.03)	0.92 (0.69–1.11)	1 (0.76–1)	0.91 (0.72–1.06)	0.49	0.77
CDDP	1 (1–1.02)	0.93 (0.69–1.11)	1 (0.97–1)	0.93 (0.74–1.06)	0.27	0.87
IFOS	1 (0.5[c]–1)	0.82 (0.55–1.11)				
Average	1	0.89	1	0.91	0.37	1.00

Data are presented as the median (range). MAPI, methotrexate (MTX), doxorubicin (ADM), cisplatin (CDDP), and ifosfamide (IFOS); MAP, methotrexate, doxorubicin, and cisplatin; RRD, relative received dose; RRD/I, received dose intensity.

[a] The ratio of received cumulative dose by protocol-planned cumulative dose of each drug.

[b] The ratio of RRD by the relative chemotherapy duration (ratio between actual duration of chemotherapy treatment and protocol-planned duration).

[c] The final ifosfamide dose was skipped due to earlier surgical intervention in 1 patient.

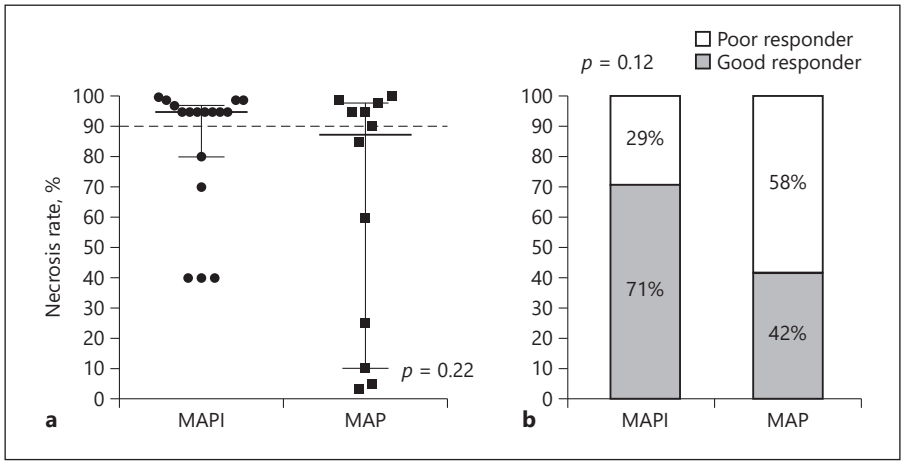

Fig. 3. Histologic response to neoadjuvant chemotherapy. **a** Necrosis rate of surgical specimens following MAPI or MAP chemotherapy. The dotted line represents 90% necrosis, and each dot plot indicates an individual specimen, with solid lines showing the median and 95% CIs. **b** Proportion of the MAPI group versus MAP group showing a good histologic response (>90% necrosis).

with regard to histologic response between the 2 groups. Indeed, the value of 71% for good response in the MAPI group is remarkable. For example, in the European and American Osteosarcoma Studies (EURAMOS)-1 trial, where 2,260 patients from 4 international study groups were registered, preoperative MAP yielded a good histologic response in 979 (50%) of the 1,975 patients [15]. In addition, the MAPI regimen was not accompanied by a significant increase in major toxicities (although retrospective collection of toxicities was made) with the exception of grade 4 neutropenia compared with the MAP regimen. Furthermore, protocol compliance was comparable between the 2 groups. However, PFS and OS were not different between the 2 groups.

Previous studies showed that final outcome was not improved even with intensified regimens postoperatively, or with use of non-cross-reacting adjuvant drugs to neoadjuvant ones for patients responding poorly to neoadjuvant chemotherapy [12, 16]. These reports led us to try preoperative MAPI in a compressed interval for a possible survival gain. We attempted a rather novel approach based on the following speculations. First, the success story of non-metastatic Ewing sarcoma by using an interval-compressed strategy could be extrapolated into osteosarcoma [9]. Both are chemosensitive bone tumors where an intensified chemotherapy is being tried. Hematopoietic stem cell transplantation has been investigated in both Ewing sarcoma and osteosarcoma, although a beneficial effect of transplantation has not been clearly documented yet [17]. Second, we chose the MAPI regimen as it produced at least a comparable outcome with other regimens, including MAP, and even a better one in a few studies [18, 19]. For example, MAPI was tested in a pilot study conducted by the Italian and the Scandinavian Sar-

Table 3. Chemotherapy-related adverse events (n = 26)

	MAPI			MAP			p value (≥grade 3)
	grades 1–2	grade 3	grade 4	grades 1–2	grade 3	grade 4	
Any toxicity	0/16 (0.0)	0/16 (0.0)	16/16 (100.0)	2/10 (20.0)	2/10 (20.0)	6/10 (60.0)	0.27
Non-hematologic toxicity	3/16 (18.8)	10/16 (62.5)	3/16 (18.8)	2/10 (20.0)	6/10 (60.0)	1/10 (10.0)	0.67
Neutropenia	NA	0/16 (0.0)	16/16 (100.0)	NA	2/10 (20.0)	5/10 (50.0)	0.06
Febrile neutropenia	NA	12/16 (75.0)	0/16 (0.0)	NA	6/10 (60.0)	0/10 (0.0)	0.44
Anemia	NA	13/16 (81.3)	0/16 (0.0)	NA	5/10 (50.0)	0/10 (0.0)	0.13
Thrombocytopenia	NA	2/16 (12.5)	12/16 (75.0)	NA	1/10 (10.0)	4/10 (40.0)	0.10
Hearing	5/16 (31.3)	0/16 (0.0)	0/16 (0.0)	0/4 (0.0)	0/4 (0.0)	0/4 (0.0)	
Left ventricular systolic dysfunction	1/16 (6.3)	0/16 (0.0)	0/16 (0.0)	0/10 (0.0)	0/10 (0.0)	0/10 (0.0)	
Hypertension	1/16 (6.3)	0/16 (0.0)	0/16 (0.0)	1/10 (10.0)	0/10 (0.0)	0/10 (0.0)	
Abnormal creatinine concentration	9/16 (56.3)	0/16 (0.0)	0/16 (0.0)	5/10 (50.0)	0/10 (0.0)	0/10 (0.0)	
Abnormal bilirubin concentration	12/16 (75.0)	0/16 (0.0)	0/16 (0.0)	5/10 (50.0)	0/10 (0.0)	0/10 (0.0)	
Abnormal ALT concentration	3/16 (18.8)	9/16 (56.3)	3/16 (18.8)	2/10 (20.0)	5/10 (50.0)	1/10 (10.0)	0.44
Hypophosphatemia	2/16 (12.5)	2/16 (12.5)	0/16 (0.0)	2/10 (20.0	0/10 (0.0)	0/10 (0.0)	0.51
Hyponatremia	NA	1/16 (6.3)	0/16 (0.0)	NA	1/10 (10.0)	0/10 (0.0)	1.00
Hypokalemia	NA	1/16 (6.3)	0/16 (0.0)	NA	2/10 (20.0)	0/10 (0.0)	0.54
Hypomagnesaemia	NA	0/13 (0.0)	0/13 (0.0)	NA	0/5 (0.0)	0/5 (0.0)	
Mucositis	NA	4/16 (25.0)	0/16 (0.0)	NA	2/10 (20.0)	0/10 (0.0)	1.00
Vomiting/anorexia	NA	4/16 (25.0)	0/16 (0.0)	NA	2/10 (20.0)	0/10 (0.0)	1.00
Neuropathy/ encephalopathy	1/16 (6.3)	0/16 (0.0)	0/16 (0.0)	0/10 (0.0)	1/10 (10.0)	0/10 (0.0)	0.39
Allergic reaction	0/16 (0.0)	0/16 (0.0)	0/16 (0.0)	2/10 (20.0)	0/10 (0.0)	0/10 (0.0)	

Data are presented as n/N (%). MAPI, methotrexate, doxorubicin, cisplatin, and ifosfamide; MAP, methotrexate, doxorubicin, and cisplatin; NA, non-applicable; ALT, alanine aminotransferase.

coma groups, and the regimen achieved a higher event-free survival (73%) and OS (87%) than other reported studies [18]. In that study, with the exception of ifosfamide (15 g/m^2), doses of MAPI in the preoperative setting were the same as ours. However, in a subsequent MAPI study performed by the same group [20], high-dose (15 g/m^2) ifosfamide caused major renal and hematologic toxicities with mortality. In our study, ifosfamide was used at a dose of 9 g/m^2 instead.

The most worrisome issue of this approach was the occurrence of far greater toxicities than those caused by a conventional regimen. Therefore, we examined whether the interval-compressed MAPI was tolerable. During the preoperative period, no significant difference in overall toxicities was noted between the MAPI and MAP therapy (Table 3). In addition, no difference in ≥grade 3 hematologic toxicities was observed with the exception of grade 4 neutropenia between the MAPI and the MAP group. Albeit with a higher incidence of grade 4 neutropenia in the MAPI group, occurrence of febrile neutropenia did not differ between the 2 groups. Furthermore, life-threatening complications of febrile neutropenia were not recorded in both groups. Similar results were recorded in a EURAMOS-1 trial where toxicities between MAP and MAPIE were compared [12]. The trial compared postoperatively given MAPIE (MAP plus ifosfamide and etoposide) and MAP in terms of event-free survival in osteosarcoma patients with a poor histologic response. The most common grade 3–4 toxicities were neutropenia, thrombocytopenia, and febrile neutropenia (90 vs. 89; 83 vs. 78; 73 vs. 50% in MAPIE and MAP, respectively). These figures appear to be similar to ours. With regard to ≥grade 3 non-hematologic toxicity, a similar incidence was recorded between our MAPI and MAP groups. The incidence of grade 4 non-hematologic toxicity was 18.8% in MAPI and 10.0% in MAP, which was close to EURA-

MOS-1 results (24% in MAPIE and 12% in MAP) [12]. Grade 1–2 left ventricular dysfunction (SF, 24%) occurred in a 13-year-old boy in the MAPI group. His baseline SF was 46% by echocardiogram, and it declined to 24% after he received a cumulative doxorubicin dose of only 150 mg/m^2 even with concomitant use of dexrazoxane, a doxorubicin cardioprotectant. Fortunately, his SF was recovered to 31% in 6 months. All the patients in both groups completed planned preoperative therapy.

Our data showed a seemingly higher rate of good histologic response in the MAPI group than that in the MAP group (70.6% in MAPI vs. 41.6% in MAP, $p = 0.12$), although the value failed to achieve statistical significance. Indeed, the rate of good histologic response obtained from our interval-compressed MAPI is one of the highest among the values ever reported, which are typically around 20–75% [21]. Regarding the presence of metastases and complete surgical resection, the 2 most important prognostic factors besides histologic response [12], our MAPI group had metastases in 17.5% and complete resection in 94.1% (Table 1). Those in the EURAMOS-1 were 23 and 98%, respectively [15], indicating that MAPI appears to be similar to EURAMOS-1 for these 2 factors. However, the proportion of good histologic response was 71 and 50% in our MAPI group and the EURAMOS-1, respectively.

A randomized trial (ISG/OS-1) for non-metastatic osteosarcoma patients, comparing histologic response and survival between MAPI and MAP therapy, conducted by an Italian group, demonstrated no difference in the necrosis rate ≥90% and event-free survival rate (42 vs. 48 and 55 vs. 64%, respectively) [7]. The drug dosages of MAPI used in the study were slightly lower than ours: the methotrexate and cisplatin doses were the same, but the doxorubicin and ifosfamide doses in their study compared to ours were 70 vs. 75 mg/m^2 and 6 vs. 9 g/m^2, respectively. Another difference is that they used the sequence of M-P/A-M-I/P-I/A unlike our M-PA-I/M-PA-I sequence as a preoperative chemotherapy. An even greater difference is that we applied interval compression (2-week interval between PA and I administration) and, thus, the duration of the preoperative phase between our MAPI and MAP group was similar (97 vs. 90.5 days, respectively; $p = 0.19$). We therefore speculate that greater intensity in a shorter preoperative phase resulted in a very high rate of good histologic response with our MAPI regimen. To confirm the relationship between a high necrosis rate and our MAPI regimen, we are commencing a prospective multicenter study (NCT 03390946).

A high necrosis rate with our MAPI therapy was not translated into higher PFS or OS. For this finding, we reasoned that small cohort numbers failed to generate a valid statistical meaning. Secondly, various salvage regimens with recurrence or progression were delivered into the 2 groups precluding informative OS analysis. Finally, we cannot exclude the possibility that a good histologic response does not always lead to an improved outcome as argued by several investigators [13, 22, 23]. In a randomized trial of 497 eligible patients with non-metastatic osteosarcoma, conducted by the European Osteosarcoma Intergroup, cisplatin and doxorubicin were delivered every 2 weeks for 3 courses or 3 weeks for 2 courses as a neoadjuvant therapy [13]. Although the value of >90% necrosis was higher in the former treatment group compared with the latter one (50 vs. 36%), clinical outcome was not different between the 2 groups: 5-year PFS and 5-year OS rates were 41 and 58% in the former versus 39 and 55% in the latter, respectively. This is the only study in which an intensified treatment using the interval compression strategy was applied. However, to our knowledge, no trials with an interval-compressed regimen with >2 drugs, containing methotrexate, have been conducted, which otherwise might have left a possibility for a better outcome. Furthermore, many studies still support the notion that histologic response is one of the important prognostic factors [6, 8, 10, 11, 19]. To draw a more solid conclusion on the prognostic implication of histologic response to the interval-compressed MAPI therapy, we are planning a multi-institutional prospective study using the same protocol.

This study has several limitations. First, this is a retrospective study with a small cohort number. Second, MAP was used in the earlier period of the study when less effective supportive care might have affected an outcome. Moreover, the follow-up period was shorter for the MAPI group. Finally, there may be missing data for toxicities considering the retrospective collection of data. However, it is most unlikely that at least data for ≥grade 3 toxicities were not collected.

With all these limitations, this study nonetheless suggests that the addition of ifosfamide to conventional MAP as a neoadjuvant regimen and its delivery in an interval-compressed manner allows the possibility of achieving a high percentage of good histologic response in children and young adults with osteosarcoma. To our knowledge, the value of 71% for good histologic response in our patient cohort is one of the highest reported in the literature from around the world. Moreover, this intensified regimen is not likely to be associated with a significant in-

crease in major toxicities as compared with conventional regimens. Finally, there is a little hope for improving the outcome for osteosarcoma with the current MAP therapy, at least in the near future. Therefore, a prospective study using the same design in larger subjects is quite warranted.

Conclusions

In the current situation in which outcomes for osteosarcoma have been stationary for the past 3 decades and are unlikely to be improved in the near future with current MAP therapy, the novel interval-compressed MAPI was tried in an attempt to improve the histologic response. Although only a marginal trend toward a better histologic response was observed with the novel regimen compared with MAP, the value of 71% with MAPI for good histologic response is one of the highest reported from worldwide collaborative groups over the past several decades. In addition, we do not exclude the possibility that small cohort numbers precluded reaching a statistical significance. Furthermore, MAPI was not accompanied by a significant increase in major toxicities as compared with MAP. Thus, the present data need to be confirmed in a multi-institutional prospective trial in a large cohort.

Acknowledgements

We thank the patients and the investigators who participated in the study.

Statement of Ethics

This study was approved by the Institutional Review Board (IRB) of the National Cancer Center, Korea (IRB No. NCC2017-0206) in September 2017, and the requirement for informed consent was waived because of the retrospective nature of the study.

Disclosure Statement

The authors have no conflicts of interest to declare.

Funding Sources

This work was supported by the Korean Association for Children with Leukemia and Cancer (KACLC; 1841930-1) and the Korean Foundation for Cancer Research (1732050).

Author Contributions

J.-M.K. and H.Y.J.: conception and design, collection and assembly of data, data analysis and interpretation, writing, and approval of the final report. J.J., M.P., and H.J.P.: conception and design, data analysis and interpretation, editing, and approval of the final report. J.Y.S.: data analysis and interpretation, editing, and approval of the final report. S.Y.P.: conception and design, collection and assembly of data, data analysis and interpretation, editing, and approval of the final report. J.H.K. and H.G.K.: conception and design, collection and assembly of data, editing, and approval of the final report. M.K.: collection and assembly of data, and approval of the final report. B.-K.P.: conception and design, collection and assembly of data, data analysis, and interpretation, writing and editing, approval of the final report, and responsibility for overall content.

References

1 Mirabello L, Troisi RJ, Savage SA. International osteosarcoma incidence patterns in children and adolescents, middle ages and elderly persons. Int J Cancer. 2009 Jul;125(1):229–34.
2 Mirabello L, Troisi RJ, Savage SA. Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. Cancer. 2009 Apr;115(7):1531–43.
3 Eilber F, Giuliano A, Eckardt J, Patterson K, Moseley S, Goodnight J. Adjuvant chemotherapy for osteosarcoma: a randomized prospective trial. J Clin Oncol. 1987 Jan;5(1):21–6.
4 Rosen G, Caparros B, Huvos AG, Kosloff C, Nirenberg A, Cacavio A, et al. Preoperative chemotherapy for osteogenic sarcoma: selection of postoperative adjuvant chemotherapy based on the response of the primary tumor to preoperative chemotherapy. Cancer. 1982 Mar;49(6):1221–30.
5 Link MP, Goorin AM, Miser AW, Green AA, Pratt CB, Belasco JB, et al. The effect of adjuvant chemotherapy on relapse-free survival in patients with osteosarcoma of the extremity. N Engl J Med. 1986 Jun;314(25):1600–6.
6 Janeway KA, Barkauskas DA, Krailo MD, Meyers PA, Schwartz CL, Ebb DH, et al. Outcome for adolescent and young adult patients with osteosarcoma: a report from the Children's Oncology Group. Cancer. 2012 Sep;118(18):4597–605.
7 Ferrari S, Ruggieri P, Cefalo G, Tamburini A, Capanna R, Fagioli F, et al. Neoadjuvant chemotherapy with methotrexate, cisplatin, and doxorubicin with or without ifosfamide in nonmetastatic osteosarcoma of the extremity: an Italian sarcoma group trial ISG/OS-1. J Clin Oncol. 2012 Jun;30(17):2112–8.
8 Bielack S, Kempf-Bielack B, Schwenzer D, Birkfellner T, Delling G, Ewerbeck V, et al. [Neoadjuvant therapy for localized osteosarcoma of extremities. Results from the Cooperative osteosarcoma study group COSS of 925 patients]. Klin Padiatr. 1999 Jul-Aug;211(4):260–70.
9 Womer RB, West DC, Krailo MD, Dickman PS, Pawel BR, Grier HE, et al. Randomized controlled trial of interval-compressed chemotherapy for the treatment of localized Ewing sarcoma: a report from the Children's Oncology Group. J Clin Oncol. 2012 Nov;30(33):4148–54.
10 Ferrari S, Serra M. An update on chemotherapy for osteosarcoma. Expert Opin Pharmacother. 2015;16(18):2727–36.

11 Gorlick R, Janeway K, Lessnick S, Randall RL, Marina N; COG Bone Tumor Committee. Children's Oncology Group's 2013 blueprint for research: bone tumors. Pediatr Blood Cancer. 2013 Jun;60(6):1009–15.
12 Marina NM, Smeland S, Bielack SS, Bernstein M, Jovic G, Krailo MD, et al. Comparison of MAPIE versus MAP in patients with a poor response to preoperative chemotherapy for newly diagnosed high-grade osteosarcoma (EURAMOS-1): an open-label, international, randomised controlled trial. Lancet Oncol. 2016 Oct;17(10):1396–408.
13 Lewis IJ, Nooij MA, Whelan J, Sydes MR, Grimer R, Hogendoorn PC, et al.; MRC BO06 and EORTC 80931 collaborators; European Osteosarcoma Intergroup. Improvement in histologic response but not survival in osteosarcoma patients treated with intensified chemotherapy: a randomized phase III trial of the European Osteosarcoma Intergroup. J Natl Cancer Inst. 2007 Jan;99(2):112–28.
14 Salzer-Kuntschik M, Brand G, Delling G. [Determination of the degree of morphological regression following chemotherapy in malignant bone tumors]. Pathologe. 1983 May; 4(3):135–41.
15 Whelan JS, Bielack SS, Marina N, Smeland S, Jovic G, Hook JM, et al.; EURAMOS collaborators. EURAMOS-1, an international randomised study for osteosarcoma: results from pre-randomisation treatment. Ann Oncol. 2015 Feb;26(2):407–14.
16 Smeland S, Müller C, Alvegard TA, Wiklund T, Wiebe T, Björk O, et al. Scandinavian Sarcoma Group Osteosarcoma Study SSG VIII: prognostic factors for outcome and the role of replacement salvage chemotherapy for poor histological responders. Eur J Cancer. 2003 Mar;39(4):488–94.
17 Ek ET, Choong PF. The role of high-dose therapy and autologous stem cell transplantation for pediatric bone and soft tissue sarcomas. Expert Rev Anticancer Ther. 2006 Feb; 6(2):225–37.
18 Bacci G, Ferrari S, Longhi A, Picci P, Mercuri M, Alvegard TA, et al.; Italian Sarcoma Group/Scandinavian Sarcoma Group. High dose ifosfamide in combination with high dose methotrexate, adriamycin and cisplatin in the neoadjuvant treatment of extremity osteosarcoma: preliminary results of an Italian Sarcoma Group/Scandinavian Sarcoma Group pilot study. J Chemother. 2002 Apr; 14(2):198–206.
19 Fuchs N, Bielack SS, Epler D, Bieling P, Delling G, Körholz D, et al. Long-term results of the co-operative German-Austrian-Swiss osteosarcoma study group's protocol COSS-86 of intensive multidrug chemotherapy and surgery for osteosarcoma of the limbs. Ann Oncol. 1998 Aug;9(8):893–9.
20 Ferrari S, Smeland S, Mercuri M, Bertoni F, Longhi A, Ruggieri P, et al. Neoadjuvant chemotherapy with high-dose ifosfamide, high-dose methotrexate, cisplatin, and doxorubicin for patients with localized osteosarcoma of the extremity: a joint study by the Italian and Scandinavian Sarcoma Groups. J Clin Oncol. 2005 Dec;23(34): 8845–52.
21 Anninga JK, Gelderblom H, Fiocco M, Kroep JR, Taminiau AH, Hogendoorn PC, et al. Chemotherapeutic adjuvant treatment for osteosarcoma: where do we stand? Eur J Cancer. 2011 Nov;47(16):2431–45.
22 Ferrari S, Mercuri M, Picci P, Bertoni F, Brach del Prever A, Tienghi A, et al. Nonmetastatic osteosarcoma of the extremity: results of a neoadjuvant chemotherapy protocol (IOR/OS-3) with high-dose methotrexate, intraarterial or intravenous cisplatin, doxorubicin, and salvage chemotherapy based on histologic tumor response. Tumori. 1999 Nov-Dec; 85(6):458–64.
23 Bacci G, Ferrari S, Bertoni F, Ruggieri P, Picci P, Longhi A, et al. Long-term outcome for patients with nonmetastatic osteosarcoma of the extremity treated at the istituto ortopedico rizzoli according to the istituto ortopedico rizzoli/osteosarcoma-2 protocol: an updated report. J Clin Oncol. 2000 Dec;18(24):4016–27.