Original Article

Results of a Randomized, Prospective Clinical Trial Evaluating Metronomic Chemotherapy in Nonmetastatic Patients With High-Grade, Operable Osteosarcomas of the Extremities: A Report From the Latin American Group of Osteosarcoma Treatment

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BACKGROUND: Metronomic chemotherapy (MC) consists of the administration of a low dose of chemotherapy on a daily or weekly basis without a long break to achieve an antitumoral effect through an antiangiogenic effect or stimulation of the immune system. The potential effect of MC with continuous oral cyclophosphamide and methotrexate in patients with high-grade operable osteosarcomas (OSTs) of the extremities was investigated. **METHODS:** Patients with high-grade OSTs who were 30 years old or younger were eligible for registration at diagnosis. Eligibility for randomization included 1) nonmetastatic disease and 2) complete resection of the primary tumor. The study design included a backbone of 10 weeks of preoperative therapy with methotrexate, adriamycin, and platinum (MAP). After surgery, patients were randomized between 2 arms to complete 31 weeks of MAP or receive 73 weeks of MC after MAP. The primary endpoint was event-free survival (EFS) from randomization. **RESULTS:** There were 422 nonmetastatic patients registered (May 2006 to July 2013) from 27 sites in 3 countries (Brazil, Argentina and Uruguay), and 296 were randomized to MAP plus MC (n = 139) or MAP alone (n = 157). At 5 years, the EFS cumulative proportions surviving in the MAP-MC group and the MAP-alone group were 61% (standard error [SE], 0.5%) and 64% (SE, 0.5%), respectively, and they were not statistically different (Wilcoxon [Gehan] statistic = 0.724; P =.395). The multivariate analysis showed that necrosis grades 1 and 2, tumor size, and amputation were associated with shorter EFS. **CONCLUSIONS:** According to the current follow-up, EFS with MAP plus MC is not statistically superior to EFS with MAP alone in patients with high-grade, resectable OSTs of the extremities. ***Cancer*** **2017;123:1003-10**. © *2016 American Cancer Society*.

KEYWORDS: chemotherapy, metronomic, nonmetastatic, osteosarcoma, survival.

INTRODUCTION

Long-term survival for patients with localized osteosarcomas (OSTs) of the extremities has improved to approximately 70% because of the introduction of multiagent neoadjuvant chemotherapy followed by surgery and adjuvant chemotherapy.[1] Large cooperative groups have successfully completed international clinical trials, and this has led to improved standardization for the treatment of OST.[2-5] High-dose methotrexate, adriamycin, and cisplatin (MAP) make up the standard backbone of chemotherapy in these trials. However, over the past 3 decades, efforts to move toward more effective chemotherapeutic regimens have failed to further improve patient outcomes.

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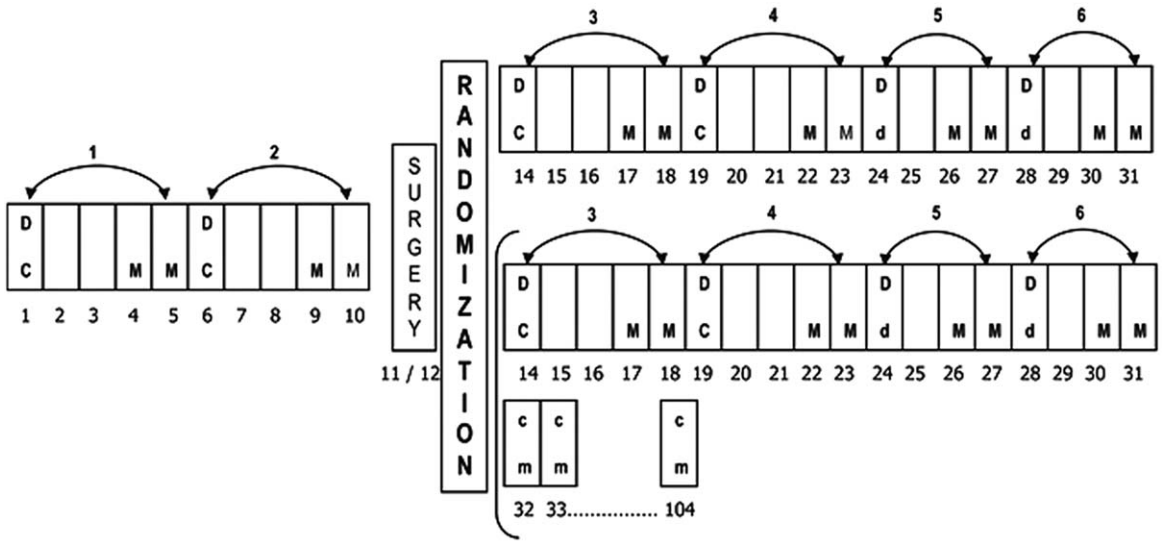

Figure 1. Treatment scheme. c indicates oral cyclophosphamide (25 mg/m^2 daily after chemotherapy until week 104); C, intravenous cisplatin (60 mg/m^2 on days 1 and 2); d, intravenous dexrazoxane (375 mg/m^2 on days 1 and 2); D, intravenous doxorubicin (37.5 mg/m^2 on days 1 and 2); m, oral methotrexate (1.5 mg/m^2 twice daily twice per week after chemotherapy until week 104); M, intravenous methotrexate (12 g/m^2 on day 1).

In pediatric tumors such as OSTs, in situ tumor angiogenesis and the levels of circulating angiogenic factors correlate with metastatic disease and a poor prognosis[6]; therefore, low-dose metronomic chemotherapy (MC), which can prevent tumor angiogenesis, represents an attractive, inexpensive, and low-toxicity antiangiogenic strategy.[7-9] The implementation of MC as a maintenance antiangiogenic therapy is very well established in acute lymphoblastic leukemia[10] and has been investigated in pediatric cancer patients.[11-17]

The addition of a metronomic combination of vinblastine, celecoxib, and cyclophosphamide to standard chemotherapy was recently reported in children with newly diagnosed metastatic Ewing sarcoma.[17] Although there was increased toxicity in irradiated sites, there was also increased event-free survival (EFS) for patients with isolated pulmonary metastases in comparison with historical controls. More recently, European and American Osteosarcoma Study 1 (EURAMOS-1) investigated whether the addition of a pegylated formulation of interferon-α-2b (IFN-α-2b) maintenance therapy after postoperative MAP could improve outcomes for patients with OSTs and a good histologic response to preoperative MAP. Results showed that MAP plus IFN-α-2b was not statistically different from MAP alone.[18]

New maintenance treatment regimens targeting residual disease represent a promising opportunity for improving the survival rate as recently demonstrated by the CAIRO3 trial in metastatic colorectal cancer.[19] The current trial was designed to examine whether the addition of a low-dose regimen of scheduled antiangiogenic chemotherapy to cyclophosphamide and methotrexate as maintenance therapy after postoperative MAP would improve EFS in patients with nonmetastatic OSTs of the extremities. Cyclophosphamide and methotrexate were selected as the metronomic agents because of their availability, presumably low toxicity, low cost, and preliminary data suggesting multitargeted therapeutic effects.[20]

MATERIALS AND METHODS

Inclusion/Exclusion Criteria

Patients 30 years old or younger who were newly diagnosed with histologically confirmed high-grade OSTs were eligible for registration. Patients could not have metastatic disease on a chest computed tomography scan or radioisotope bone scan. For randomization, we excluded patients with axial tumors; patients without a complete macroscopic resection of the primary tumor; pregnant or lactating patients; and patients with inadequate heart, renal, or liver function. Participants and/or their legal guardians, as appropriate, provided written informed consent for the registration and random assignment. Regulatory and ethics approvals were obtained according to national requirements.

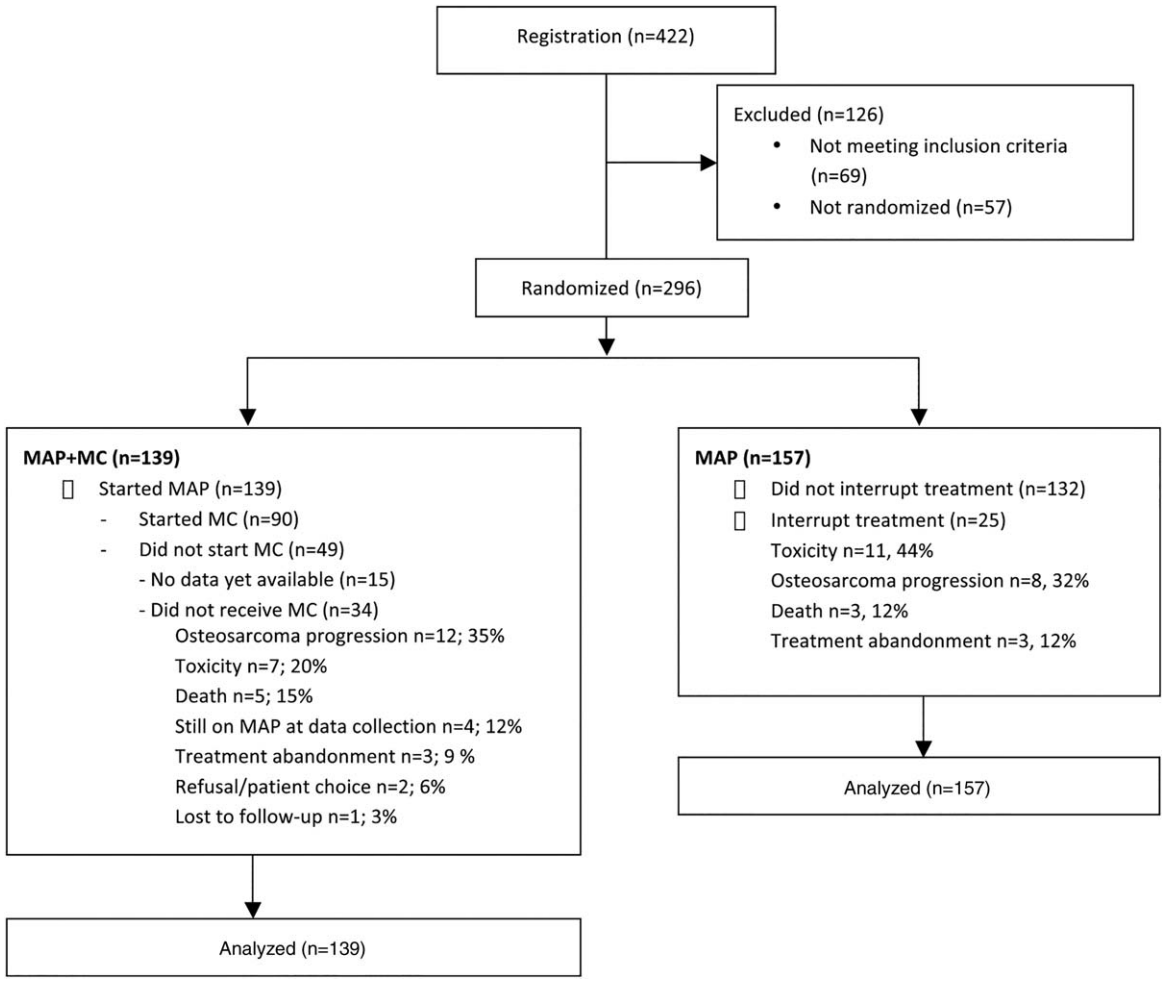

Figure 2. Consolidated Standards of Reporting Trials diagram. MAP indicates methotrexate, doxorubicin, and cisplatin; MC, metronomic therapy (oral cyclophosphamide and methotrexate).

Trial Treatments and Procedures

Details of the treatment are given in Figure 1. It consisted of induction with 2 MAP chemotherapy courses (5-week cycles with doxorubicin [75 mg/m^2], cisplatin [120 mg/m^2], and methotrexate [12 g/m^2]) during weeks 1-10) followed by surgery of the primary in week 11 or 12. The institutional orthopedic surgeon, in collaboration with the pediatric oncology team, chose the appropriate procedure for each case. Limb salvage surgery was encouraged whenever possible with a variety of techniques, such as nonconventional endoprosthesis, resection of expendable bones, and plates and bone graft fixation (autograft or allograft). After the primary tumor removal, a global assessment of necrosis was made by the institutional pathologist with the scoring system designed by Huvos et al.[21]

Adriamycin and cisplatin were administered in weeks 1 and 6, and methotrexate was administered in weeks 4, 5, 9, and 10. After surgery, consenting patients were randomly assigned to complete 31 weeks of MAP with cisplatin omitted in the last 2 cycles or to the same regimen followed by 73 weeks of MC (continuous oral low-dose chemotherapy with cyclophosphamide and methotrexate; Fig. 1). Treatment allocation was performed with permuted blocks of size 10.

Assessments

During the MAP treatment, clinical and toxicity assessments were performed according to the Common Terminology Criteria for Adverse Events (version 3.0). Patients were assessed for local and distant recurrence at predefined intervals by physical examination, computed tomography of the chest, and radiography of the primary site. Radiographically detected relapse was also imaged by computed tomography, magnetic resonance imaging, and/or bone scans and, if appropriate, was confirmed by histology.

Statistical Design

The primary outcome measure was EFS, which was defined as the time from random assignment until a first event (recurrence, disease progression, secondary malignancy, or death) or censoring at the last contact. The secondary outcome measures included overall survival (OS), which was defined as the time from randomization until death or last contact. To detect a 10% difference in 5-year EFS with a 2-sided 5% significant level and 80% power, a sample size of 156 patients was needed for each of the randomization branches.

Two different paradigms for the estimation of the MC impact were considered: 1) the traditional intention-to-treat approach (hence, missing values were imputed[22] via multiple imputation as recommended by Rubin et al[23]; 5 imputed data sets were created and pooled to produce the estimates) and 2) the complier average causal effect (CACE), which is defined as the treatment effect for subjects who would comply regardless of the assigned treatment. The main insight underlying CACE analysis is that we can reach an unbiased estimate of the difference in outcomes for compliers in the intervention group and those who would have engaged with treatment in the control group.[24] A detailed description of CACE and its assumptions can be found in the literatue.[25] All the analyses were performed in Mplus. The Kaplan-Meier method was used to estimate survival functions, log-rank tests were used for differences between survival curves, and Cox models were used to estimate treatment effects, with the suitability checked by tests for the proportionality of hazards. The consistency of the treatment effect was examined with interaction tests (χ^2) in subgroups defined post hoc: age, sex, tumor size, histological response to preoperative chemotherapy, and type of surgery. The median follow-up was calculated with reverse censoring on death.

RESULTS

Patients

Between May 2006 and July 2013, 422 patients diagnosed with nonmetastatic OST from 27 institutions in 3 countries (Brazil, Argentina, and Uruguay) were recruited for the study. The whole population of 422 patients was used for describing demographic data. The male-to-female ratio was 1.14, and the mean age at onset was 14 years with a range of 0 to 29 years. Conservative surgery or amputation was performed for 362 patients, and the histologic response was determined for 272 patients. The proportion of good responders (necrosis grades 3 and 4) was 39.7%, and the proportion of poor responders (necrosis grades 1 and 2) was 60.3%. One hundred twenty-six patients were deemed ineligible: 16 because of an axial location of the tumor, 53 because no local control was reported (23 died before surgery, 12 were on treatment, 11 discontinued the treatment, 5 refused, and 2 had a nonresectable tumor), and 57 because of no randomization. Thus, 296 patients were eligible, with 139 patients assigned to the MAP-MC group and 157 patients assigned to the MAP-alone group (Fig. 2); the mean age at enrollment was 14 years, and the mean time to diagnosis was 3.3 months. Table 1 shows the characteristics of the randomly assigned patients.

TABLE 1. Characteristics of the Randomly Assigned Patients

Variable	MAP + MC (n = 139)	MAP (n = 157)
Age (standard deviation), y	13.23 (4.61)	13.85 (4.10)
Tumor size (standard deviation), cm	10.76 (4.87)	11.06 (5.19)
Male sex, %	52.5	59.9
Surgery (amputation)	35.5	38.2
Primary tumor site, No.		
Femur	77	95
Tibia	36	35
Humerus	12	18
Other	14	9

Treatment

Surgery

Of the 296 patients, 36.8% (n = 109) had an amputation (13 before chemotherapy), 50% (n = 148) had a nonconventional endoprosthesis, 8.8% (n = 26) had plates plus bone graft fixation (autograft or allograft), and 4.4% (n = 13) underwent a resection of expendable bones.

Postoperative MAP

In both treatment arms, MAP was delivered accordingly to the schedule shown in Figure 1.

Maintenance therapy

Of the 139 patients randomly assigned to MAP plus MC, 35% (n = 49) did not start MC. The reported reasons were as follows: no data yet available (31%; n = 15), OST progression (24%; n = 12), toxicity (14%; n = 7), death (10%; n = 5), MAP still being received at data collection (8%; n = 4), treatment abandonment (6%; n = 3), refusal/patient choice (4%; n = 2), and loss to follow-up (2%; n = 1).

Efficacy

The mean EFS time was 51.58 months (standard error [SE], 2.7 months; 95% confidence interval [CI], 46.27-56.98 months) for the MAP-MC group and 58.81

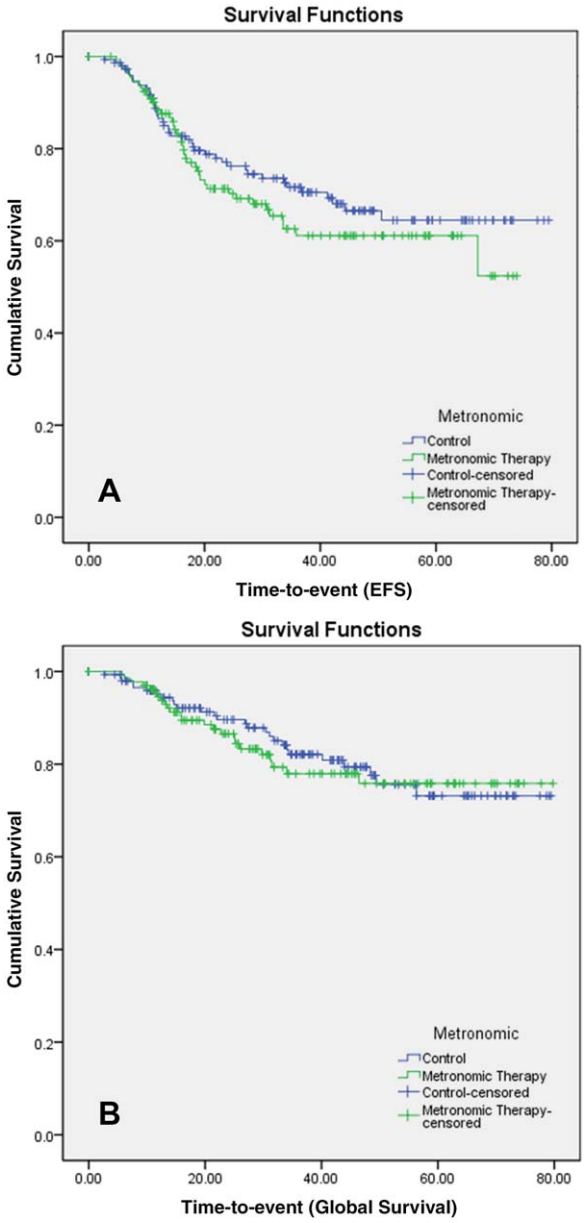

Figure 3. Kaplan-Meier estimates of (A) EFS and (B) OS in the intent-to-treat population.

TABLE 2. Summary of the Cox Regression Model for Overall Survival: Prognostic Factors

Covariate	Standard Error	*P*	Hazard Ratio
Metronomic therapy	0.283	.957	0.985
Surgery type (amputation)	0.303	.010	2.17
Necrosis grade (GI/II)	0.477	.003	4.132
Tumor size	0.027	.039	1.055

TABLE 3. Summary of the Cox Regression Model for Event-Free Survival: Prognostic Factors

Covariate	Standard Error	*P*	Hazard Ratio
Metronomic therapy	0.231	.406	1.211
Surgery type (amputation)	0.229	.001	2.148
Necrosis grade (GI/II)	0.303	<.001	3.22
Tumor size	0.021	.007	1.059

TABLE 4. Cox Regression: Complier Average Causal Effect Analysis

Covariate	Estimate (β)	Standard Error	*P*	Hazard Ratio
Metronomic therapy	−0.084	2.665	.975	0.9194
Surgery type (amputation)	0.816	1.294	.528	2.26
Necrosis class (GI/GII)	1.387	0.981	.157	4.00
Tumor size	0.054	0.054	.315	1.055

months (SE, 2.64 months; 95% CI, 53.63-63.99 months) for the MAP-alone group. The mean OS time was 65.82 months (SE, 2.53 months; 95% CI, 60.85-70.79 months) for the MAP-MC group and 66.16 months (SE, 2.268 months; 95% CI, 61.72-70.61 months) for the MAP-alone group. Log-rank tests were performed to determine whether the randomization allocation status influenced EFS and OS. At 5 years, the EFS cumulative proportions surviving in the MAP-MC group and the MAP-alone group were 61% (SE, 0.5%), and 64% (SE, 0.5%), respectively, and they were not statistically different (Wilcoxon [Gehan] statistic = 0.724; $P = .395$). The OS rate was 76% (SE, 0.04%) for the MAP-MC group and 73% (SE, 0.05%) for the MAP-alone group (Wilcoxon [Gehan] statistic = 0.377; $P = .539$; Fig. 3B). In a univariate analysis, the characteristics that were significantly associated with OS and EFS were the tumor size, type of surgery, and histological response. A multivariate analysis of the independent prognostic factors demonstrated that the grade of necrosis, the surgery type, and the tumor site were also independently associated with OS and EFS. The final model is presented in Tables 2 and 3.

An as-treated analysis was performed to take into account noncompliance (Table 4). Two Cox regression models were built: one for OS and another for EFS. For the former, the same covariate structure obtained in the final multivariate Cox regression was maintained; for the latter, SEs were not able to be estimated because the model estimation per se did not terminate normally on account of a nonzero derivative of the observed data log likelihood.

The CACE results for OS under covariate metronomic therapy showed a negative β value, which is

associated with an increase in survival; however, this estimate was statistically nonsignificant (HR, 0.919; P = .975), so statistically there was an absence of evidence showing that patients who were compliant with metronomic therapy had longer survival than control-condition individuals who could have complied if they had been assigned to the intervention condition.

Mortality

There were 27 deaths (19%) reported for the MAP-MC group. The causes of death were as follows: disease progression (81%; n = 22), regimen-related toxicity (11%; n = 3), second malignant neoplasm (4%; n = 1), and unspecified (4%; n = 1). For the MAP-alone group, 24 deaths (15%) were reported: 20 (83%) were due to disease progression, and 4 (17%) were due to regimen-related toxicity.

DISCUSSION

The low OS rate is a strong argument for international collaboration and has led to the establishment of several multi-institutional cooperative groups.[26] According to a search of the medical literature, this is the first randomized clinical trial performed for patients diagnosed with OST in Latin America.

Although OST survival rates were improved with the use of this regimen in comparison with results previously reported by the group,[4,27-29] according to the current follow-up data, EFS with MAP plus MC is not statistically superior to EFS with MAP alone in patients with high-grade, resectable OSTs of the extremities.

This result must be considered cautiously because 35% of the patients randomized to maintenance therapy did not start it, and another 10% stopped the treatment for reasons other than tumor progression, toxicity, or death. Anyway, the CACE approach was used to estimate the true effect in the presence of noncompliance. However, there was also an absence of evidence showing that patients who were compliant with MC had longer survival in comparison with potential compliers who could have complied if they had been assigned to the MAP-MC group. Compliance is a major concern when one considers cancer treatments and more specifically long-term treatments. EURAMOS-1 also investigated whether the addition of maintenance therapy with IFN-α-2b after postoperative MAP could improve outcomes for patients with OST. The results showed that MAP plus IFN-α-2b was not statistically different from MAP alone, but the interpretation is limited because one-quarter of the patients who were allocated to IFN-α-2b never started it.[18] More generally, compliance is a major issue with MC because patients have to take several pills a day. A recent survey has shown that compliance is similarly a challenge for recent targeted oral therapies. Strategies have to be developed to improve patient compliance.[30,31] In other tumors, recent reports have demonstrated its potential negative impact on survival for children with acute lymphoblastic leukemia.[32]

The current EFS results do not support the routine use of cyclophosphamide and methotrexate as metronomic agents after standard chemotherapy for nonmetastatic OST. However, our results should not preclude further investigation into the potential of maintenance for patients with OST. First, recent preclinical findings suggest that MC may be more potent against metastatic disease.[33] This finding is consistent with the potential role of metronomic maintenance in eradicating metastatic disease in the setting of leukemia or other adult or pediatric solid tumors such as metastatic colorectal cancer.[17,19,34] Therefore, adding metronomic maintenance for patients with metastatic OST should be considered. Second, many anticancer drugs can be used in a metronomic fashion, so the combination tested here, though potent against metastatic breast cancer,[20] may not be optimal for nonmetastatic OST. In addition, adding a targeted-like effect through drug repositioning could allow more potent treatment with the addition of, for instance, valproic acid or β-blockers.[35]

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The authors made no disclosure.

AUTHOR CONTRIBUTIONS

Andreza A. Senerchia: Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, visualization, writing–original draft, and writing–review and editing. **Carla Renata Macedo:** Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, visualization, and writing–review and editing. **Sima Ferman:** Conceptualization, data curation, funding acquisition, investigation, methodology, project administration, resources, visualization, and writing–review and editing. **Marcelo Scopinaro:** Conceptualization, data curation, funding acquisition, investigation, methodology, project administration, resources, visualization, and writing–review and editing. **Walter Cacciavillano:** Conceptualization, data curation, funding acquisition, investigation, methodology, project administration, resources, visualization, and writing–review and editing. **Erica Boldrini:**

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REFERENCES

1. 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;115:1531-1543.
2. Marina N, Bielack S, Whelan J, et al. International collaboration is feasible in trials for rare conditions: the EURAMOS experience. *Cancer Treat Res.* 2009;152:339-353.
3. Whelan JS, Bielack SS, Marina N, et al. EURAMOS-1, an international randomised study for osteosarcoma: results from pre-randomisation treatment. *Ann Oncol.* 2015;26:407-414.
4. Petrilli AS, de Camargo B, Filho VO, et al. Results of the Brazilian Osteosarcoma Treatment Group Studies III and IV: prognostic factors and impact on survival. *J Clin Oncol.* 2006;24:1161-1168.
5. Meyers PA, Schwartz CL, Krailo MD, et al. Osteosarcoma: the addition of muramyl tripeptide to chemotherapy improves overall survival—a report from the Children's Oncology Group. *J Clin Oncol.* 2008;26:633-638.
6. Kreuter M, Bieker R, Bielack SS, et al. Prognostic relevance of increased angiogenesis in osteosarcoma. *Clin Cancer Res.* 2004;10:8531-8537.
7. Pasquier E, Kavallaris M, Andre N. Metronomic chemotherapy: new rationale for new directions. *Nat Rev Clin Oncol.* 2010;7:455-465.
8. Stempak D, Seely D, Baruchel S. Metronomic dosing of chemotherapy: applications in pediatric oncology. *Cancer Invest.* 2006;24:432-443.
9. Bertolini F, Paul S, Mancuso P, et al. Maximum tolerable dose and low-dose metronomic chemotherapy have opposite effects on the mobilization and viability of circulating endothelial progenitor cells. *Cancer Res.* 2003;63:4342-4346.
10. Andre N, Cointe S, Barlogis V, et al. Maintenance chemotherapy in children with ALL exerts metronomic-like thrombospondin-1 associated anti-endothelial effect. *Oncotarget.* 2015;6:23008-23014.
11. Russell HV, Groshen SG, Ara T, et al. A phase I study of zoledronic acid and low-dose cyclophosphamide in recurrent/refractory neuroblastoma: a New Approaches to Neuroblastoma Therapy (NANT) study. *Pediatr Blood Cancer.* 2011;57:275-282.
12. Traore F, Togo B, Pasquier E, Dembele A, Andre N. Preliminary evaluation of children treated with metronomic chemotherapy and valproic acid in a low-income country: Metro-Mali-02. *Indian J Cancer.* 2013;50:250-253.
13. Minturn JE, Janss AJ, Fisher PG, et al. A phase II study of metronomic oral topotecan for recurrent childhood brain tumors. *Pediatr Blood Cancer.* 2011;56:39-44.
14. Andre N, Abed S, Orbach D, et al. Pilot study of a pediatric metronomic 4-drug regimen. *Oncotarget.* 2011;2:960-965.
15. Minard-Colin V, Ichante JL, Nguyen L, et al. Phase II study of vinorelbine and continuous low doses cyclophosphamide in children and young adults with a relapsed or refractory malignant solid tumour: good tolerance profile and efficacy in rhabdomyosarcoma—a report from the Société Française des Cancers et Leucémies de l'Enfant et de l'Adolescent (SFCE). *Eur J Cancer.* 2012;48:2409-2416.
16. Peyrl A, Chocholous M, Kieran MW, et al. Antiangiogenic metronomic therapy for children with recurrent embryonal brain tumors. *Pediatr Blood Cancer.* 2012;59:511-517.
17. Felgenhauer JL, Nieder ML, Krailo MD, et al. A pilot study of low-dose anti-angiogenic chemotherapy in combination with standard multiagent chemotherapy for patients with newly diagnosed metastatic Ewing sarcoma family of tumors: a Children's Oncology Group (COG) phase II study NCT00061893. *Pediatr Blood Cancer.* 2013;60:409-414.
18. Bielack SS, Smeland S, Whelan JS, et al. Methotrexate, doxorubicin, and cisplatin (MAP) plus maintenance pegylated interferon alfa-2b versus MAP alone in patients with resectable high-grade osteosarcoma and good histologic response to preoperative MAP: first results of the EURAMOS-1 good response randomized controlled trial. *J Clin Oncol.* 2015;33:2279-2287.
19. Simkens LH, van Tinteren H, May A, et al. Maintenance treatment with capecitabine and bevacizumab in metastatic colorectal cancer (CAIRO3): a phase 3 randomised controlled trial of the Dutch Colorectal Cancer Group. *Lancet.* 2015;385:1843-1852.
20. Colleoni M, Rocca A, Sandri MT, et al. Low-dose oral methotrexate and cyclophosphamide in metastatic breast cancer: antitumor activity and correlation with vascular endothelial growth factor levels. *Ann Oncol.* 2002;13:73-80.
21. Huvos AG, Rosen G, Marcove RC. Primary osteogenic sarcoma: pathologic aspects in 20 patients after treatment with chemotherapy en bloc resection, and prosthetic bone replacement. *Arch Pathol Lab Med.* 1977;101:14-18.

22. Gravel J, Opatrny L, Shapiro S. The intention-to-treat approach in randomized controlled trials: are authors saying what they do and doing what they say? *Clin Trials.* 2007;4:350-356.
23. Rubin G, Michowitz S, Horev G, et al. Pediatric brain stem gliomas: an update. *Childs Nerv Syst.* 1998;14:167-173.
24. Connell AM. Employing complier average causal effect analytic methods to examine effects of randomized encouragement trials. *Am J Drug Alcohol Abuse.* 2009;35:253-259.
25. Jo B. Statistical power in randomized intervention studies with noncompliance. *Psychol Methods.* 2002;7:178-193.
26. Luetke A, Meyers PA, Lewis I, Juergens H. Osteosarcoma treatment—where do we stand? A state of the art review. *Cancer Treat Rev.* 2014;40:523-532.
27. Petrilli AS, Kechichian R, Broniscer A, et al. Activity of intraarterial carboplatin as a single agent in the treatment of newly diagnosed extremity osteosarcoma. *Med Pediatr Oncol.* 1999;33:71-75.
28. Petrilli S, Penna V, Lopes A, Figueiredo MT, Gentil FC. IIB osteosarcoma. Current management, local control, and survival statistics—São Paulo, Brazil. *Clin Orthop Relat Res.* 1991;270:60-66.
29. Petrilli AS, Gentil FC, Epelman S, et al. Increased survival, limb preservation, and prognostic factors for osteosarcoma. *Cancer.* 1991; 68:733-737.
30. Mathes T, Pieper D, Antoine SL, Eikermann M. Adherence influencing factors in patients taking oral anticancer agents: a systematic review. *Cancer Epidemiol.* 2014;38:214-226.
31. Barillet M, Prevost V, Joly F, Clarisse B. Oral antineoplastic agents: how do we care about adherence? *Br J Clin Pharmacol.* 2015;80: 1289-1302.
32. Bhatia S, Landier W, Hageman L, et al. Systemic exposure to thiopurines and risk of relapse in children with acute lymphoblastic leukemia: a Children's Oncology Group study. *JAMA Oncol.* 2015;1: 287-295.
33. Ebos JM, Mastri M, Lee CR, et al. Neoadjuvant antiangiogenic therapy reveals contrasts in primary and metastatic tumor efficacy. *EMBO Mol Med.* 2014;6:1561-1576.
34. Malik PS, Raina V, Andre N. Metronomics as maintenance treatment in oncology: time for chemo-switch. *Front Oncol.* 2014;4:76.
35. Andre N, Carre M, Pasquier E. Metronomics: towards personalized chemotherapy? *Nat Rev Clin Oncol.* 2014;11:413-431.