Is There a Role for Mifamurtide in Nonmetastatic High-Grade Osteosarcoma? Results From the Italian Sarcoma Group (ISG/OS-2) and Spanish Sarcoma Group (GEIS-33) Trials

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ABSTRACT

PURPOSE Outcome of patients with localized osteosarcoma is challenging. The role of mifamurtide is still a matter of debate. Two prospective trials were carried out in Italy (ISG/OS-2) and Spain (GEIS-33) with mifamurtide in ABCB1/P-glycoprotein (Pgp)–positive patients.

PATIENTS AND METHODS Patients age ≤40 years with localized extremity high-grade osteosarcoma were eligible. Analysis of Pgp expression from diagnostic biopsy was centralized. Patients received two cycles of preoperative methotrexate, doxorubicin, and cisplatinum (MAP) before surgery. Postoperatively, in case of Pgp overexpression (Pgp-positive), mifamurtide was added, combined with doxorubicin (one cycle) and four consecutive cycles of high-dose ifosfamide (HDIFO) for patients with poor histologic response, or with MAP in case of good response. Patients who were Pgp-negative received MAP postoperatively. We present the merged analysis of ISG/OS-2 and GEIS-33 trial, an observational study with same inclusion criteria and treatment of ISG/OS-2. The primary endpoint was 5-year event-free survival (EFS) according to the use of mifamurtide. Secondary endpoint was overall survival (OS).

RESULTS From March 2013 to April 2018, 398 patients were analyzed. The median age was 14 years (range, 4-40), male/female: 238/160 (1.48/1.0); 211 of 398 (53%) tumors were Pgp-positive, and 204 of 398 (51.3%) patients received mifamurtide. With a median follow-up of 70 months (IQR, 49-90 months), the 5-year EFS and OS were 65.2% (95% CI, 60.1 to 69.8) and 74.8% (95% CI, 69.8 to 79.0), respectively, with superior EFS for patients undergoing mifamurtide and chemotherapy as compared with EFS of patients undergoing chemotherapy alone (5-year EFS 71.4% *v* 58.3%; $P = .0139$) not confirmed at multivariable analysis ($P = .0593$).

CONCLUSION In this merged analysis with a risk-adapted strategy for nonmetastatic osteosarcoma, the group with unfavorable prognoses, identified by Pgp expression, performed well when mifamurtide, combined with HDIFO in case of poor response, was administered after surgery.

ACCOMPANYING CONTENT

- Appendix
- Data Sharing Statement
- Data Supplement
- Protocol

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INTRODUCTION

High-grade osteosarcoma is the most frequent primary malignant bone tumor among children and adolescents and young adults (AYAs).[1-3] In the past 50 years, survival has not improved, and novel therapeutic strategies are urgently needed.[4,5]

Histologic response to neoadjuvant chemotherapy according to Huvos has consistently shown a strong correlation with survival in patients with localized osteosarcoma.[6] In addition, high levels of ABCB1/P-glycoprotein (Pgp), an efflux pump that reduces the intracellular concentration of doxorubicin, demonstrated an unfavorable prognostic factor in osteosarcoma in several published series, but controversy exists on its

CONTEXT

Key Objective
Is there a role for mifamurtide in patients with nonmetastatic extremity high-grade osteosarcoma?

Knowledge Generated
This analysis on about 400 patients with nonmetastatic osteosarcoma treated within two European prospective trials shows that high-risk patients, here defined by expression of P-glycoprotein, particularly in case of poor response to adjuvant chemotherapy, when ifosfamide was added, might benefit most from adjuvant mifamurtide.

Relevance *(R.G. Maki)*
Although it is unavailable in the United States, the post-chemotherapy period appears to be an opportune moment to impose other novel immunotherapeutic strategies in this patient population.*

*Relevance section written by *JCO* Associate Editor Robert G. Maki, MD, PhD, FACP, FASCO.

prognostic role.[7-24] Pgp mediates drug efflux through the plasma membrane and increases drug trapping within lysosomal drug safe houses[13,20,24,25] (Appendix Fig A1, online only).

The fine interplay between tumor and immune cells is not fully understood, but M2-polarized macrophages have shown enhanced expression of Pgp.[26] It can be hypothesized that mifamurtide reverses the negative impact of M2 macrophage polarization in tumors overexpressing Pgp. Besides, COX2+ tumor-associated macrophages promote cancer cell proliferation and survival through different mechanisms, including by increasing Pgp.[27]

Mifamurtide is a nonspecific immunomodulator binding to the extracellular Toll-like receptor-4 thus activating monocytes and macrophages, promoting antitumor activity.[4,28,29] In 2009, the European Medicines Agency granted a centralized marketing authorization for muramiltripeptide (mifamurtide; MTP) for patients age between 2 and 30 years presenting a high-grade nonmetastatic resected osteosarcoma (EU/1/08/502/001). However, it is unclear which patients could potentially benefit from mifamurtide the most, and no approval has been obtained from the Food and Drug Administration. Moreover, in several European countries, the use of mifamurtide is still limited as the results of the INT-0133 trial have been a matter of debate between key-opinion leaders.[29]

The ISG/OS-2 and GEIS-33 studies are prospective trials evaluating the role of a risk-modulated approach in patients with localized high-grade osteosarcoma of the extremities, stratifying patients on the basis of PgP expression and tumor response to neoadjuvant chemotherapy.[30,31]

The results of the ISG/OS2 study were recently published,[30] showing that adjuvant mifamurtide, combined with high-dose ifosfamide (HDIFO) in case of poor responder (PR) to induction chemotherapy, could improve event-free survival (EFS) in Pgp-positive patients. This clinical trial also reported improved survival in comparison with previous studies.[10,32]

This study is a secondary analysis of patients included in both ISG/OS-2 and GEIS-33 trial. All patients received methotrexate, doxorubicin, and cisplatinum (MAP) for induction chemotherapy, but those with Pgp expression also received adjuvant mifamurtide. Moreover, in Pgp-positive patients with poor response to preoperative treatment, mifamurtide was combined with a cycle of doxorubicin + four consecutive cycles of HDIFO. Pgp expression might be measured by RNA and immunohistochemistry. Because differences in immunohistochemical Pgp assessment techniques might jeopardize the interpretation of results, Pgp assessment was performed in one institution for all of the patients.

The aim of this study was to analyze the outcome of patients in both studies, thus addressing the role of mifamurtide in osteosarcoma treatment in a larger series of patients.

PATIENTS AND METHODS

Study Design

This study was carried out on patients treated within the ISG/OS-2 and GEIS-33. ISG/OS2 (ClinicalTrials.gov identifier: NCT01459484) is an Italian, multicenter, uncontrolled phase II trial. GEIS-33 (ClinicalTrials.gov identifier: NCT04383288) is a Spanish phase IV, postauthorization, observational, multicenter study in patients age between 2 and 30 years, diagnosed with nonmetastatic high-grade osteosarcoma of the extremities.

For both trials, data collection was performed on electronic case report form and centralized.

Both studies evaluated the efficacy of risk-adapted chemotherapy regimens, with adjuvant mifamurtide and HDIFO in Pgp-positive patients PR to induction chemotherapy. A written informed consent was obtained from the adult patients or from the guardians in case of pediatric patients. The local Ethics Committees approved the protocols.

Patients' inclusion and exclusion criteria were presented elsewhere.[30] Main inclusion criteria were a diagnosis of primary, central, high-grade localized osteosarcoma of the extremities; age ≤40 years; and no prior surgery or chemotherapy for osteosarcoma; main exclusion criterion was metastatic disease at diagnosis (Data Supplement, online only).

Immunohistochemical Detecion of Pgp

For ISG/OS2 and GEIS-33 studies, assessment of Pgp was centralized at the Istituto Rizzoli and the expression of Pgp was evaluated by immunohistochemistry as described.[7,33]

Briefly, only formalin-fixed, paraffin-embedded biopsies were used for immunohistochemical analysis of Pgp. Expression of Pgp was assessed with three monoclonal antibodies, which react with different, mutually exclusive epitopes of this protein: JSB-1 (Sanbio, Uden, the Netherlands), MRK16 (Kamiya Biomedical, Thousand Oaks, CA), and C494 (Signet Laboratories, Dedham, MA). Immunohistochemistry was performed by using an avidin-biotin peroxidase complex method (Vectastain ABC kit, Vector Laboratories, Burlinghame, CA), and the final reaction product was revealed by incubation with diaminobenzidine (Sigma, St Louis, MO). For each specimen, a negative control was carried out by replacing the primary antibody with normal horse serum, whereas to check the antigenicity of the sample, one additional section was incubated with the V9 anti-vimentin monoclonal antibody (Roche Molecular Biochemicals, Mannheim, Germany). Sections of normal human kidney were used as reference control for Pgp immunostaining procedure because of its reported overexpression in proximal tubuli. Only specimens with a diffused immunostaining for Pgp were classified as positive.

From here onward, because the Pgp+ group is superimposable with the mifamurtide-receiving group, in this analysis, we will only refer to the Pgp+ group as the mifamurtide group.

Study Description

Methotrexate is given once a day, 4 hour infusion, while adriamycin and cisplatin are administered as a continuous infusion. Frequency of administration is illustrated in Figure 1. After surgery, chemotherapy-induced necrosis was expressed as a percentage. When the percentage of tumor necrosis was 90% or more, patients were classified as good responders (GRs); when the percentage of tumor necrosis was lower, patients were defined as PRs. In

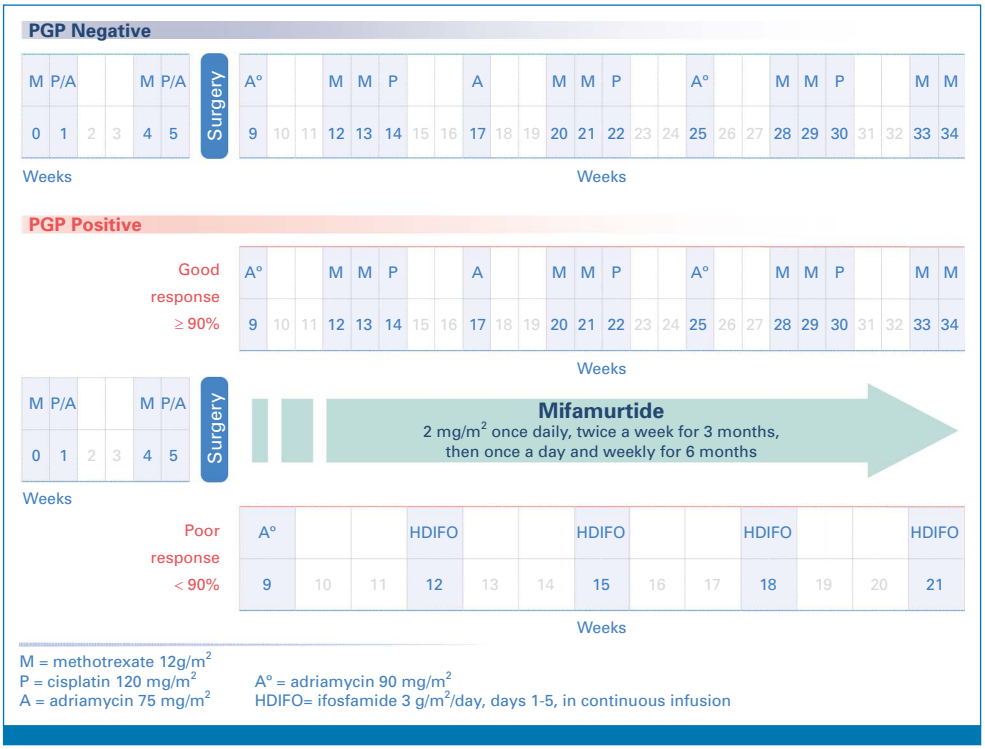

FIG 1. Treatment scheme of the ISG/OS-2 study by Pgp expression. HDIFO, high-dose ifosfamide; Pgp, P-glycoprotein.

Pgp-positive patients, postoperative chemotherapy consisted of MAP and mifamurtide for GRs or one cycle of A 90 mg/m² intravenous (IV) continuous infusion (24h), every 3 weeks followed by four consecutive cycles of HDIFO (3 g/m²/d, day 1-5, continuous infusion) and mifamurtide (2 mg/m²) for PRs. MTP was administered twice a week for 3 months and then weekly for 6 months at the dose of 2 mg/m² (total duration 48 weeks). Patients who were Pgp-negative continued MAP postoperatively, regardless of the response to induction chemotherapy. The total cumulative dose of A was 420 mg/m² (75 mg/m²/day × two cycles in the neoadjuvant setting, combined with P, plus 90 mg/m² × three cycles when administered in monotherapy, in the adjuvant setting).

Statistical Analysis

Continuous data were summarized as median with interquartile range and categorical data as frequencies with percentage. Alkaline phosphatase (ALP) and lactate dehydrogenase levels were classified as high when they exceed the upper limit of the normal range according to laboratories ranges, whereas levels within the ranges are deemed low.

TABLE 1. Baseline Demographic and Clinical Characteristics According to Mifamurtide Treatment

Characteristic	All Sample (N = 398), No. (%)	Mifamurtide Yes (n = 204), No. (%)	Mifamurtide No (n = 194), No. (%)	*P*
Age, years				
0-14	218 (54.8)	126 (61.8)	92 (47.4)	.0041
15-40	180 (45.2)	78 (38.2)	102 (52.6)	
Sex				
Male	238 (59.8)	117 (57.4)	121 (62.4)	.3074
Female	160 (40.2)	87 (42.6)	73 (37.6)	
Serum ALP				
High	133 (33.4)	62 (30.4)	71 (36.6)	.0288
Normal	233 (58.6)	131 (64.2)	102 (52.6)	
Unknown	32 (8.0)	11 (5.4)	21 (10.8)	
LDH				
Normal	258 (64.8)	134 (65.7)	124 (63.9)	.5080
High	115 (28.9)	55 (27.0)	60 (30.9)	
Unknown	25 (6.3)	15 (7.3)	10 (5.2)	
Histologic response				
GR	164 (41.2)	87 (42.6)	77 (39.7)	.4301
PR	227 (57.0)	115 (56.4)	112 (57.7)	
Unknown	7 (1.8)	2 (1.0)	5 (2.6)	
Surgery				
Resection	375 (94.2)	195 (95.6)	180 (92.8)	.3495
Amputation	20 (5.0)	9 (4.4)	11 (5.7)	
Rotationplasty	1 (0.3)		1 (0.5)	
Unknown	2 (0.5)		2 (1.0)	
Margins				
Adequate	361 (90.7)	192 (94.1)	169 (87.1)	.0181
Inadequate	34 (8.5)	12 (5.9)	22 (11.3)	
Unknown	3 (0.8)		3 (1.6)	
Histotype				
Osteoblastic	287 (72.1)	147 (72.1)	140 (72.2)	.8652
Other	110 (27.6)	57 (27.9)	53 (27.3)	
Chondroblastic	45 (40.9)	19 (33.3)	26 (49.0)	
Fibroblastic	21 (19.1)	14 (24.6)	7 (13.2)	
Hemorragic	21 (19.1)	14 (24.6)	7 (13.2)	
Small cell	16 (14.5)	6 (10.5)	10 (18.9)	
Unspecified	7 (6.4)	4 (7.0)	3 (5.7)	
Unknown	1 (0.3)		1 (0.5)	

Abbreviations: ALP, alkaline phosphatase; GR, good response; LDH, lactate dehydrogenase; PR, poor response.

Baseline characteristics according to mifamurtide treatment were compared using the chi-square test or Fisher exact test.

EFS was calculated from the first day of chemotherapy to recurrence (local or distant), death from all causes, the appearance of secondary tumors, or the last follow-up examination. Overall survival (OS) was calculated from the first day of chemotherapy to death or the last follow-up examination. Survival curves were calculated using the Kaplan-Meier method and compared using the log-rank test. Univariable and multivariable hazard ratios with 95% CIs were estimated using the Cox proportional hazards model. The multivariable model was computed including covariates statistically significant in univariable analysis. The proportional hazards assumption was tested using Schoenfeld residuals.

Tests were performed two-sided, and results were reported with 95% CI. We considered a $P \leq .05$ as statistically significant. Statistical analyses were performed using SAS 9.4 (SAS Institute Inc, Cary, NC).

RESULTS

A total of N = 401 patients (from ISG/OS n = 279, and from GEIS n = 122) were included in this study. Three patients did not have survival data and therefore were excluded (Appendix Fig A2). Overall, 398 patients were analyzed: 204 (51.3%) treated with mifamurtide and 194 not treated with mifamurtide (48.7%).

The median age was 14 years (range, 4-40). Clinical characteristics according to mifamurtide use are reported in Table 1. As shown in Table 1, there was an imbalance at

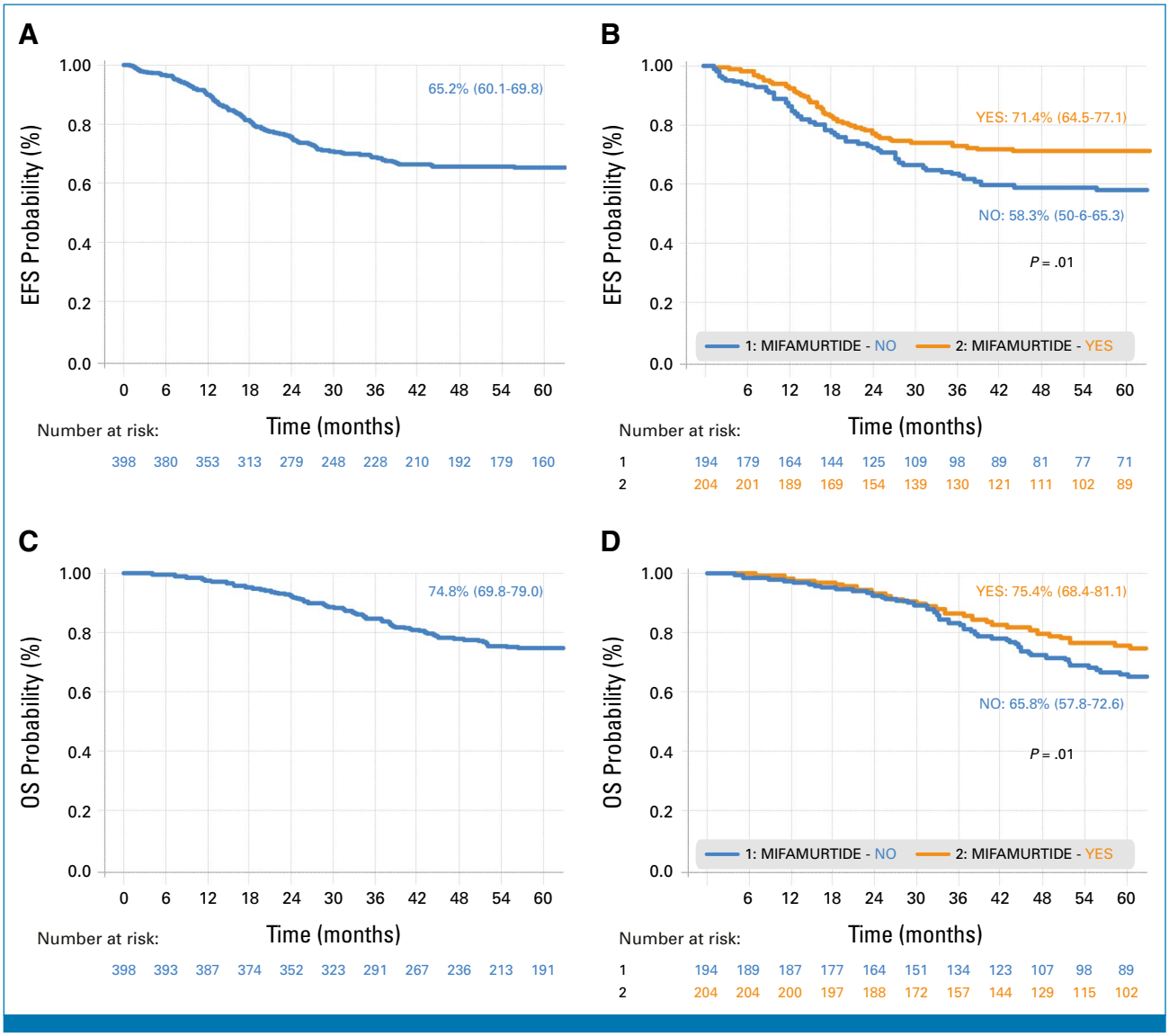

FIG 2. Probability of EFS for (A) the entire cohort and (B) by mifamurtide administration. Probability of OS for (C) the entire cohort and (D) by mifamurtide administration. EFS, event-free survival; OS, overall survival.

baseline for some clinical potential prognostic factors favoring the mifamurtide arm. Toxicities and compliance to therapy were reported elsewhere.[30,31]

Outcomes

EFS

Overall, the median follow-up was 70 months (IQR, 49-90 months). The 5-year EFS was 65.2% (95% CI, 60.1 to 69.8; Fig 2A).

Five-year EFS was 71.4% (95% CI, 64.5 to 77.1) for patients undergoing mifamurtide and MAP (HDIFO in case of PR) and 58.3% (95% CI, 50.6 to 65.3) for patients undergoing MAP only (P = .0131; Table 2; Fig 2B). This difference was also significant in the subgroup of patients with poor response (P = .0359), whereas no significant difference in the GR patients was detected (Table 3; Appendix Fig A3).

Poor histological response to induction chemotherapy and high baseline ALP levels were associated with significant inferior EFS (Table 2), whereas age ≥15 years was not a negative prognostic factor in this series.

In the multivariable analysis, poor response to induction chemotherapy and high baseline ALP level were both confirmed significant prognostic factors for EFS (Table 4).

OS

The 5-year OS was 74.8% overall (95% CI, 69.8 to 79.0; Figs 2A and 2C). The 5-year OS was 65.8% (95% CI, 57.8 to 72.6) for MAP only and 75.4% (95% CI, 68.4 to 81.1) in case of chemotherapy plus mifamurtide (P = .0457; Table 2; Figs 2B and 2D). Poor histologic response to induction chemotherapy and high ALP levels at diagnosis were associated with significant inferior OS (Table 2).

In the multivariable analysis, poor response to induction chemotherapy and high baseline ALP level were both confirmed significant prognostic factors for OS (Table 4).

Survival Analysis on the Basis of Histologic Necrosis and Pgp

A subgroup of EFS and OS analysis on the basis of patients stratification according to histologic response in the Pgp− and Pgp+ subgroups was performed (Appendix Fig A4): Chemotherapy-induced necrosis after neoadjuvant chemotherapy was confirmed as a strong prognostic factor, in both Pgp− and Pgp+ subgroups, supporting the evidence reported in Table 2.

TABLE 2. Univariable Analysis of EFS and OS in Patients With Nonmetastatic Extremity Osteosarcoma Treated in ISG/OS-2 and GEIS-33 Trials

Characteristic	5-Year EFS, % (95% CI)	HR (95% CI)	*P*	5-Year OS, % (95% CI)	HR (95% CI)	*P*
Age, years						
0-14	66.8 (60.0 to 72.8)			74.3 (67.4 to 79.9)		
15-40	63.3 (55.4 to 70.2)	1.0 (0.7 to 1.5)	.8650	75.4 (67.9 to 81.5)	0.9 (0.6 to 1.4)	.6233
Sex						
Male	65.8 (59.1 to 71.7)			76.0 (69.5 to 81.3)		
Female	64.3 (56.2 to 71.4)	1.0 (0.7 to 1.5)	.7972	73.2 (65.0 to 79.7)	1.1 (0.7 to 1.7)	.6423
Serum ALP						
Normal	70.6 (64.0 to 76.2)			80.4 (74.2 to 85.3)		
High	56.3 (47.2 to 64.4)	1.8 (1.2 to 2.5)	.0017	63.7 (54.5 to 71.6)	2.1 (1.3 to 3.2)	.0008
LDH						
Normal	65.8 (59.4 to 71.5)			75.2 (69.0 to 80.3)		
High	62.6 (52.8 to 71.0)	1.2 (0.8 to 1.7)	.4168	69.9 (59.9 to 77.9)	1.2 (0.8 to 1.9)	.3286
Histologic response						
GR	79.0 (71.7 to 84.6)			85.8 (79.0 to 90.6)		
PR	54.9 (47.9 to 61.4)	2.5 (1.7 to 3.7)	<.0001	67.1 (60.0 to 73.2)	2.5 (1.5 to 4.1)	.0002
Mifamurtide						
Yes	71.4 (64.5 to 77.1)			75.4 (68.4 to 81.1)		
No	58.3 (50.6 to 65.3)	1.5 (1.1 to 2.2)	.0139	65.8 (57.8 to 72.6)	1.5 (1.0 to 2.3)	.0457
Histotype						
Osteoblastic	66.2 (60.2 to 71.5)			75.8 (70.1 to 80.7)		
Other	62.5 (52.2 to 71.1)	1.1 (0.8 to 1.7)	.4721	71.9 (61.5 to 78.0)	1.2 (0.8 to 1.9)	.4755

Abbreviations: ALP, alkaline phosphatase; EFS, event-free survival; GR, good response; HR, hazard ratio; LDH, lactate dehydrogenase; OS, overall survival; PR, poor response.

TABLE 3. Univariable EFS According to Histologic Response to Induction Chemotherapy and Mifamurtide

Characteristic	No.	5-Year EFS, % (95% CI)	HR (95% CI)	*P*
Good responder				
MAP + mifamurtide (Pgp+)	87	83.5 (73.7 to 89.9)		.1238
MAP (Pgp−)	77	73.5 (61.5 to 82.3)	1.7 (0.9 to 3.4)	
Poor responder				
MAP + HDIFO + mifamurtide (Pgp+)	115	62.5 (52.7 to 70.8)		.0454
MAP (Pgp−)	112	47.0 (36.9 to 56.4)	1.5 (1.0 to 2.2)	

NOTE. Seven patients with unknown necrosis were excluded from this analysis.
Abbreviations: EFS, event-free survival; HDIFO, high-dose ifosfamide; HR, hazard ratio; MAP, methotrexate, doxorubicin, and cisplatinum; Pgp, P-glycoprotein.

DISCUSSION

This study demonstrated a significant superior EFS and OS in patients undergoing mifamurtide as compared with chemotherapy alone at univariable analysis. Despite the unbalance for age and ALP, the multivariable analysis showed that among these factors, only ALP had an independent prognostic role, whereas the *P* value for mifamurtide administration was .059.

Our study suggests that the anticipated poor prognosis for patients with Pgp+ tumors may have been mitigated by mifamurtide. Despite conflicting evidence regarding the prognostic effect of Pgp expression, we maintain that it holds significant prognostic value.

We believe that there are two different ways to interpret these findings, according with the potential role of Pgp as a prognostic factor.

If we assume that Pgp selects two groups with different prognosis, these results demonstrate that mifamurtide was able to improve EFS in the high-risk patient group. Intriguingly, Pgp-positive patients with PR to neoadjuvant chemotherapy had a statistically significant EFS improvement with this experimental approach (mifamurtide and HDIFO postoperatively), as compared with the Pgp-negative PR cohort (MAP only), suggesting that Pgp prognostic value might be outplayed by an integrated treatment and reinforces the hypothesis that ifosfamide might act synergistically with mifamurtide. It is plausible that ifosfamide and mifamurtide are potentially synergistic. Two decades ago, it was reported that ifosfamide could be an immunomodulator upregulating the expansion of Th1 cells.[34]

Unfortunately, because the Pgp-negative population did not receive mifamurtide, our study cannot exclude the potential benefit of mifamurtide for this subgroup.

On the other hand, if we are skeptical on the prognostic role of Pgp, these analysis shows that, in a cohort of unselected patients with localized osteosarcoma age ≤40 years, a strategy based on mifamurtide and chemotherapy with HDIFO in case of PRs is superior to chemotherapy alone in terms of EFS.

Particularly, INT0133 did not find a correlation between Pgp expression and worse EFS or OS. However, Pgp expression analysis was possible in only 139 (for C494 antibody) of 685 patients (20.3%), using tissues from either diagnostic biopsies or surgical specimens, and the staining technique differed from ours.[10]

In both cases, these data would support, 17 years later, the findings of the controlled study by Meyer et al[35]. The former controlled study from the Children's Oncology Group, INT0133 trial, using a randomized two-by-two factorial design, already hypothesized an interaction between mifamurtide and ifosfamide. This study reported a 3-year EFS of 68% in the group that received a combination of mifamurtide and MAP and 78% for those who received

TABLE 4. Multivariable Analysis of EFS and OS in Patients With Nonmetastatic Extremity Osteosarcoma Treated in ISG/OS-2 and GEIS-33 Trials

		5-Year EFS		5-Year OS	
Characteristic	Variable	HR (95% CI)	*P*	HR (95% CI)	*P*
Serum ALP	High *v* normal	1.9 (1.3 to 2.7)	.0006	2.0 (1.3 to 3.2)	.0013
Histologic response	PRs *v* GRs	2.5 (1.7 to 3.8)	<.0001	2.6 (1.6 to 4.3)	.0002
Mifamurtide	No *v* yes	1.4 (1.0 to 2.0)	.0593	1.4 (0.9 to 2.1)	.1480

NOTE. Models were performed on patients with completed data (n = 359).
Abbreviations: ALP, alkaline phosphatase; EFS, event-free survival; GRs, good responses; HR, hazard ratio; OS, overall survival; PRs, poor responses.

mifamurtide combined with MAP and ifosfamide (9 g/m² continuous infusion per cycle). In a follow-up study, mifamurtide was associated with an OS benefit and with a positive EFS trend.[35] On the basis of these results, concerns whether INT0133's results met generally accepted standards for practice-changing conclusions were raised.

Is the improvement observed in our study because of mifamurtide? Or is it the HDIFO salvage therapy in case of poor response? The EURAMOS study showed that the addition of ifosfamide (2.8 g/m² per day combined with etoposide 100 mg/m² per day, on days 1-5, every 21 days), to MAP does not improve survival, increases toxicity, and should not be considered as a salvage therapy for high-risk patients.[36,37] A recent Japanese phase II study has come to a similar conclusion, failing to demonstrate a survival benefit for ifosfamide given to patients with poor response to MAP.[38] As the most important benefit in terms of EFS in this study was obtained for Pgp-positive patients with PR to induction chemotherapy, treated with both mifamurtide and HDIFO, a positive interaction between these drugs could be hypothesized, and this combination might be proposed as salvage treatment of patients with unresponsive osteosarcoma.

Also, our study confirmed that poor histologic response to induction chemotherapy and high ALP at diagnosis predict worse outcome, as confirmed at multivariable analysis.

Interestingly, no relationship was found between the level of Pgp expression and histologic response after preoperative chemotherapy, as previously reported.[9,10] Most likely, the two parameters identify two phenomena related to resistance to chemotherapy, only partly overlapping. In fact, although poor histologic response might be the result of a cumulative resistance to all drugs administered preoperatively, Pgp mediates a specific resistance to doxorubicin and might also be related to other tumor features, such as differential biological aggressiveness of tumor cells.

We observed that survival for pediatric patients and AYA was not different when treated within the same protocol. This conflicts with other studies[29] and reinforce the suggestion of the AYA Working Group of the European Society for Medical Oncology and the European Society for Pediatric Oncology, that multicenter cooperation, including pediatric and adult cooperation, should be encouraged.[3]

This is a retrospective analysis of data collected prospectively. The major limitation of these clinical trials is the lack of a control group because of inherent difficulties to run a randomized trial in the adjuvant setting of a rare disease. Unfortunately, this is a limitation that will remain unaddressed for now because the design of most of the trials in osteosarcoma is not controlled (Appendix Table A1). Results of the UNIFRA study, which will test the hypothesis that high-risk patients, defined by metastases at diagnosis or poor response to induction chemotherapy, might benefit from mifamurtide, are awaited.[39]

An observational study is ongoing in Italy since 2021 (ISG/AIEOP OS2 Oss) to collect real-word data on osteosarcoma; on the basis of these findings, the recommended approach, as per national guidelines, is to use mifamurtide in patients younger than 30 years, because of regulatory constraints, with a PR after neoadjuvant chemotherapy, and in combination with HDIFO. Similarly in Spain, this study facilitated the prescription of mifamurtide within its framework, promoting its rational use. The results of this study will be critical in establishing the use of high-dose ifosfamide in conjunction with mifamurtide as a national protocol, especially for patients younger than 30 years exhibiting a poor pathologic response to induction therapy with MAP.

Mifamurtide use will also be allowed according to investigator preferences in the future European FOSTER-Cabos trial. This study will randomize maintenance cabozantinib in patients with localized and metastatic osteosarcoma nonprogressing after up-front chemotherapy. A run-in phase will also be undertaken to assess the feasibility of mifamurtide in combination with cabozantinib.

Identifying predictive factors to select patients and stratify treatment on the basis of their likelihood of responding to mifamurtide could lead to increased cost-effectiveness, particularly relevant considering reimbursement and cost saving concerns.

A translational genomic sequencing study on pretreatment sample, to identify subgroup of responders, is ongoing (ClinicalTrials.gov identifier: NCT03737435).

In conclusion, key finding from this study including almost 400 patients with nonmetastatic osteosarcoma is the potential role of HDIFO combined with mifamurtide as salvage treatment for Pgp-positive patients with poor histologic response to neoadjuvant chemotherapy. These results might pave the path for future treatment strategies in selected high-risk osteosarcoma groups. No specific indications on the generalized use of mifamurtide should be derived from this study. Nonetheless, the results of this trial, in the absence of a randomized controlled trial to further explore the role of mifamurtide, might have a prescriptive role for mifamurtide along with HDIFO in patients exhibiting a poor response to neoadjuvant treatment with MAP.

AFFILIATIONS

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Is There a Role for Mifamurtide in Nonmetastatic High-Grade Osteosarcoma? Results From the Italian Sarcoma Group (ISG/OS-2) and Spanish Sarcoma Group (GEIS-33) Trials

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APPENDIX

TABLE A1. Ongoing Studies With Mifamurtide in Patients With High-Grade Osteosarcoma

NCT Number	Study Title	Sponsor	Other ID
NCT00631631	Mifamurtide (L-MTP-PE) for High-Risk Osteosarcoma	Millennium Pharmaceuticals, Inc	MTP-OS-403
NCT01194284	Surveillance Study of Patients With Newly Diagnosed Osteosarcoma	Millennium Pharmaceuticals, Inc	C23003 2009-017204-89 (EudraCT Number)
NCT02441309	A Eurosarc Study of Mifamurtide in Advanced Osteosarcoma (MEMOS)	University of Oxford	OCTO_039
NCT04571229	Expanded Access Use of L-MTP-PE for the Treatment of Osteosarcoma	Memorial Sloan Kettering Cancer Center	20-324
NCT03643133	Mifamurtide Combined With Postoperative Chemotherapy for Newly Diagnosed High Risk Osteosarcoma Patients (SARCOME13)	UNICANCER	UC-0150/1704 2017-001165-24 (EudraCT_Number)
NCT01459484	ABCB1/P-glycoprotein Expression as Biologic Stratification Factor for Patients With Non Metastatic Osteosarcoma	Italian Sarcoma Group	ISG-OS2 2011-001659-36 (EudraCT_Number)
NCT04383288	ABCB1/P-glycoprotein Expression Influence on Nonmetastatic Osteosarcoma of the Extremities	Grupo Espanol de Investigacion en Sarcomas	Geis-33
NCT04890067	Observational Study in Localized Osteosarcoma	AIEOP/Italian Sarcoma Group	AIEOP/ISG OS2 Oss

Abbreviations: ID, identifier; L-MTP-PE, liposomal muramyl tripeptide phosphatidyl ethanolamine; NCT, noncontrolled trial.

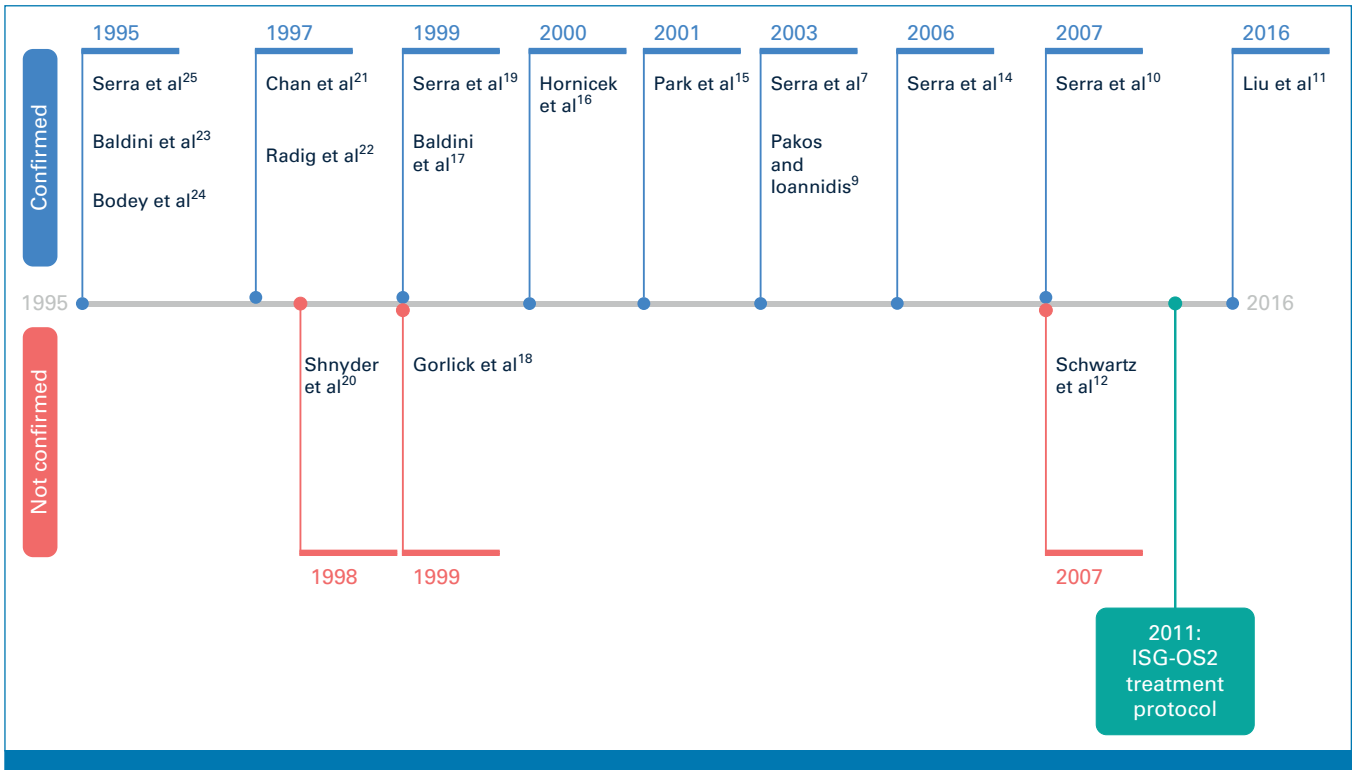


FIG A1. A chronological roadmap for original manuscripts on P-glycoprotein role in osteosarcoma.

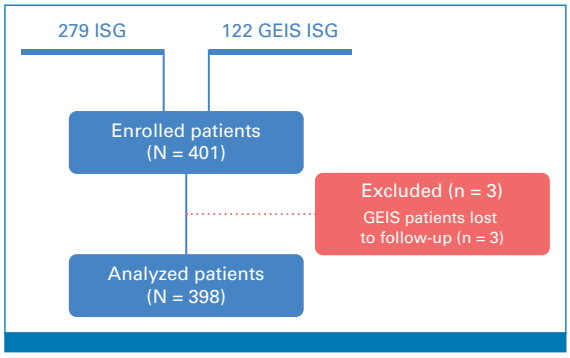
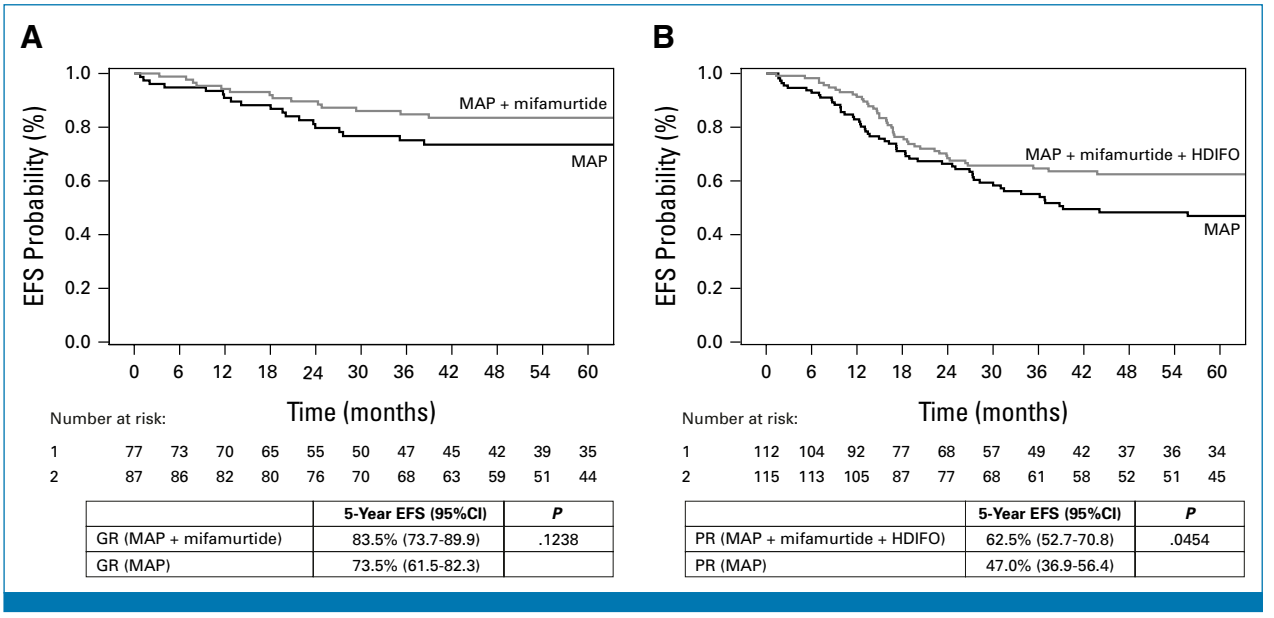


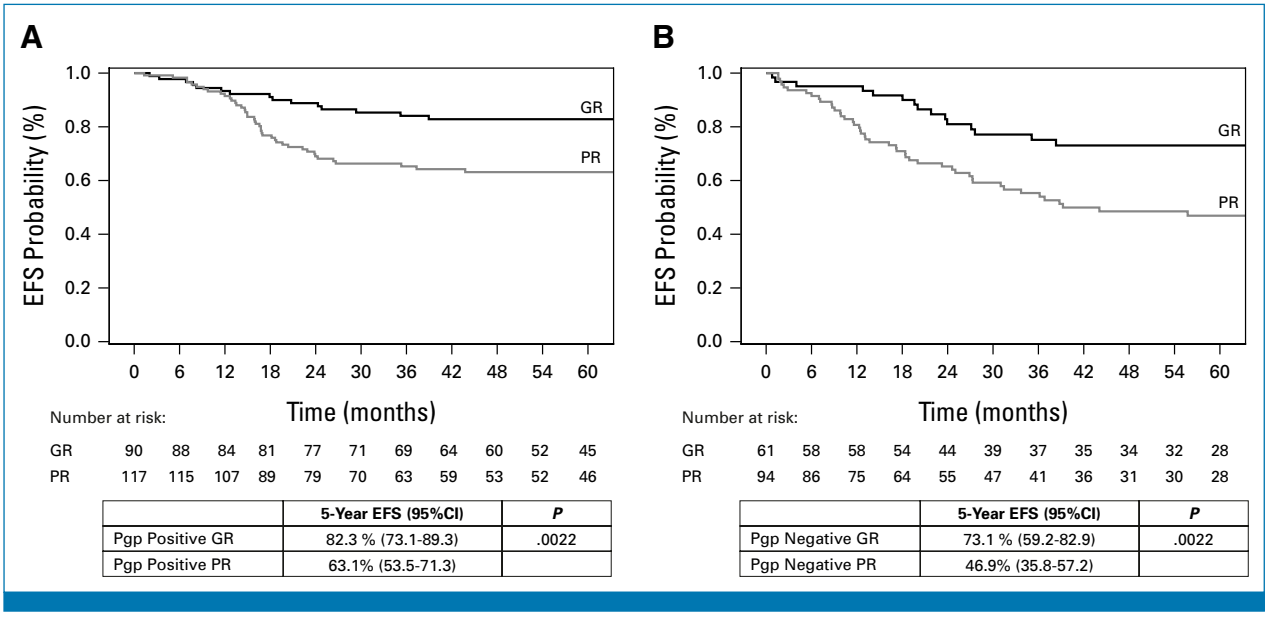
FIG A2. Study flow diagram of the combined analysis of the Italian ISG/OS-2 and Spanish GEIS-33 studies.



	5-Year EFS (95%CI)	P
GR (MAP + mifamurtide)	83.5% (73.7-89.9)	.1238
GR (MAP)	73.5% (61.5-82.3)	

	5-Year EFS (95%CI)	P
PR (MAP + mifamurtide + HDIFO)	62.5% (52.7-70.8)	.0454
PR (MAP)	47.0% (36.9-56.4)	

FIG A3. EFS according to induction chemotherapy and mifamurtide in (A) GRs and (B) PRs. EFS, event-free survival; GRs, good responses; HDIFO, high-dose ifosfamide; MAP, methotrexate, doxorubicin, and cisplatinum; PRs, poor responses.



	5-Year EFS (95%CI)	P
Pgp Positive GR	82.3 % (73.1-89.3)	.0022
Pgp Positive PR	63.1% (53.5-71.3)	

	5-Year EFS (95%CI)	P
Pgp Negative GR	73.1 % (59.2-82.9)	.0022
Pgp Negative PR	46.9% (35.8-57.2)	

FIG A4. (A) EFS curves of Pgp-positive patients stratified according to tumor necrosis, (B) EFS curves of Pgp-negative patients stratified according to tumor necrosis. EFS, event-free survival; GR, good response; Pgp, P-glycoprotein; PR, poor response.