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REVIEW

New treatment options for nonmetastatic osteosarcoma: focus on mifamurtide in adolescents

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Abstract: New treatments are needed to improve the clinical outcome for patients with osteosarcoma. Liposomal muramyl tripeptide phosphatidylethanolamine (mifamurtide) is a synthetic peptidoglycan component packaged in multilamellar liposomes. Mifamurtide has been demonstrated to induce recruitment and activation of macrophages and monocytes of the host innate immune system, which leads to antitumoricidal activity. Early clinical trials have demonstrated the safety and tolerability of mifamurtide combined with chemotherapy, and one major study has demonstrated an overall survival benefit in patients with newly diagnosed nonmetastatic osteosarcoma. This review summarizes the mechanism of action, clinical results, and the optimal biologic dose, and raises potential questions for future development of mifamurtide.

Keywords: osteosarcoma, childhood cancer, adolescent, sarcoma, immunotherapy, bone

Introduction

Osteosarcoma is the most common malignant bone tumor occurring in children and adolescents. The peak age of occurrence is during the second decade of life, and in the US the incidence is 4.4 cases per million individuals younger than 25 years.[1] The long bones of the extremities, especially at the metaphyses, are the most common sites involved. The treatment of osteosarcoma is multidisciplinary in nature and involves careful coordination of complete surgical removal of the primary tumor combined with 6–9 months of systemic chemotherapy given before surgery (neoadjuvant) and also after surgery (adjuvant). Currently, there are four chemotherapeutic agents that are considered active standard agents for the treatment of osteosarcoma, ie, doxorubicin, cisplatin, high-dose methotrexate with leucovorin rescue, and ifosfamide. Recent studies evaluating treatment regimens from the 1980s and 1990s using the standard cytotoxic chemotherapeutic agents have shown that the overall survival rates for patients with nonmetastatic osteosarcoma have remained at approximately 60%–70%.[2–5] Thus, the consensus has been that a plateau of efficacy has been reached with traditional cytotoxic chemotherapy regimens and that new agents or therapies are required to improve the survival outcome for patients with osteosarcoma.

Ever since William B Coley's initial description of tumor response in three patients with bone sarcomas following injection of streptococcal organisms in 1891, there has been significant interest in the role of enhancing the innate immune system for solid tumor therapy.[6] A recent study found that among 412 patients with osteosarcoma, those who experienced a postoperative infection within one year of their limb salvage

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surgery had a significantly improved 10-year overall survival outcome compared with those patients without a history of infection (84% versus 62%).[7] This finding is intriguing considering that infections of the extremities had an effect against distant lung metastases. However, another recent clinical study in 31 patients with shorter follow-up did not find a relationship between postoperative infection and improved clinical outcome for patients with osteosarcoma.[8] Other data supporting the potential involvement of the host immune system against tumor cells include the observation of early lymphocyte recovery correlating with improved clinical outcome in patients with bone tumors.[9,10] Previous studies have investigated the potential of immunostimulatory agents, such as interferon and granulocyte-macrophage colony stimulating factor, for patients with osteosarcoma, but the results of these studies have been conflicting at best.[11–14] However, it is likely that a variety of clinical factors, such as extent of disease burden, are involved in determining the potential efficacy of enhanced immunity for tumor therapy. Much investigation is still needed regarding the potential benefit of agents that enhance innate immunity in patients with non-metastatic versus metastatic disease and how to integrate immune modulators with traditional therapies for osteosarcoma.

Muramyl tripeptide phosphatidylethanolamine (MTP-PE) is a synthetic derivative of muramyl dipeptide (MDP), which is a peptidoglycan component found in bacterial cell walls.[15,16] Mifamurtide is a formulation of MTP-PE encapsulated into multilamellar liposomes (L-MTP-PE) and functions as a much more potent activator of macrophages and monocytes than MDP, and L-MTP-PE is also less toxic than MDP.[17–19] Much work has already been done to demonstrate that mifamurtide potentiates the tumoricidal ability of macrophages and monocytes. Given that the lungs harbor a significant population of macrophages and are the most common site of metastatic involvement and recurrence of osteosarcoma, there is a rationale for developing mifamurtide as a new therapeutic agent for osteosarcoma.[3,20,21] The purpose of this review is to provide background on the clinical development of mifamurtide and also to discuss the potential use and safety of mifamurtide in adolescents and young adults with osteosarcoma.

Chemistry, pharmacokinetics, and pharmacodynamics

Mifamurtide is produced by combining the active MTP-PE agent with synthetic phospholipids, ie, dioleoyl phosphatidylserine and palmitoyl oleoyl phosphatidylcholine, and then lyophilized to form concentric multilamellar liposomes measuring 2–3 μm in diameter.[22] The phosphatidylserine component binds to corresponding receptors on macrophages, and the liposomes are ingested via phagocytosis.[23] Thus, the distribution of mifamurtide is biased towards organs with heavy concentrations of macrophages and monocytes, such as the liver, lungs, and spleen. This preferential distribution has been demonstrated in patients given mifamurtide labeled with ^{99}Tc.[24] The drug is rapidly cleared from the serum, with one study of adult patients demonstrating free MTP-PE serum concentrations at less than 1% of the administered dose when measured 30 minutes after infusion.[25] While there have not been any studies specifically evaluating the pharmacokinetics of mifamurtide in children and adolescents, a study by Murray et al included older adolescents at least 18 years of age and young adults, and demonstrated rapid distribution of ^{99}Tc-labeled mifamurtide to the reticuloendothelial system within 6 hours.[24]

As each layer of the liposomes degrades, MTP-PE is released within the cell. The main intracellular mechanism of action is felt to be mediated by binding of MTP-PE to the nucleotide-binding oligomerization domain (Nod) 2 receptor, which is highly expressed in antigen-presenting cells.[26,27] Stimulation of Nod2 has been shown to result in activation of the nuclear factor-κB signaling pathway and also increased secretion of interleukin (IL)-1β, leading to activation of the mitogen-activated protein kinase pathway.[28,29]

Activation of macrophages and monocytes results in the secretion of cytokines and other proinflammatory molecules, including IL-1, IL-6, IL-8, tumor necrosis factor alpha, nitric oxide, and prostaglandins D_2 and E_2.[30–34] Increased expression of intracellular adhesion molecules, such as lymphocyte function-associated antigen-1 and intracellular adhesion molecule-1, have also been demonstrated on MTP-PE exposure to human monocytes.[35] Upregulation of these molecules likely leads to activation of contact-mediated tumoricidal function by innate immune cells. The immune-mediated tumor killing mechanism of mifamurtide is supported by in vivo studies demonstrating that following mifamurtide treatment, osteosarcoma tumors demonstrate a pattern of necrosis similar to that of tuberculous granulomas.[36]

A number of in vitro and in vivo studies have not demonstrated any significant adverse interactions between mifamurtide and a variety of chemotherapy agents.[37–41] Also, addition of chemotherapy agents, such as doxorubicin and ifosfamide, does not negatively affect the ability of MTP-PE to activate macrophages.[37,39] However, high doses of

ibuprofen have been found to inhibit the immunomodulatory effects of mifamurtide.[42] A more comprehensive overview of the immunomodulatory and tumoricidal effects and the pharmacokinetics of mifamurtide has been detailed in several excellent reviews.[22,43,44]

Clinical review

Early clinical trials in adult and pediatric patients in a variety of malignancies have demonstrated that mifamurtide is relatively well tolerated with little evidence of acute or long-term organ toxicity. Fever, chills, headache, and fatigue have been the most commonly reported toxicities, occurring shortly following infusion, and these symptoms correspond to the immune-mediated proinflammatory response generated by mifamurtide (Table 1).[24,45–49] Elevations in IL-6 have correlated with the onset of fever.[47] Also, some of the infusion-related effects appear to be dose-related. Significant malaise and hypotension were more commonly observed in patients receiving doses greater than 2 mg/m^2.[24,49] Most of these symptoms are readily addressed by administration of anti-inflammatory medications, such as acetaminophen or ibuprofen given at standard doses. Other toxicities that were reported included dyspnea, hypertension, anorexia, vertigo, diarrhea, leg cramps, and joint pain.[24,46]

The most significant clinical trial demonstrating the benefit of mifamurtide for osteosarcoma was the randomized, prospective Intergroup 0133 study for patients with newly diagnosed osteosarcoma.[48,50] The trial had two main study questions which were posed using a 2×2 factorial design. The first objective was to determine if addition of ifosfamide to the three-drug combination of doxorubicin, cisplatin, and high-dose methotrexate would improve the outcome. The second

Table 1 Most commonly reported acute infusion-related toxicities for mifamurtide

References	Study population	L-MTP-PE dosing	Summary of infusion-related toxicities
Murray et al[24]	Phase I study of 28 adult patients with metastatic cancer. Most common diagnoses were colorectal (n = 10) and renal cell carcinoma (n = 5).	Starting dose of 0.05 mg/m^2 biweekly for 3 weeks and then escalated up to 12 mg/m^2.	The MTD was 6 mg/m^2. At the MTD, the most common toxicities encountered were chills, fever, malaise, nausea/vomiting, anorexia, headache, myalgia, and cough. Two patients experienced grade 3–4 toxicities at a dose of 3 mg/m^2, ie, one patient with fever, hypotension, and dyspnea and another patient with dyspnea.
Creaven et al[46]	Phase I study of 37 adult patients. Most common diagnoses were renal cell carcinoma (n = 13) and melanoma (n = 6).	Dosing of 0.01–6 mg/m^2 biweekly for 4 weeks.	The MTD was considered to be 2–4 mg/m^2. At the MTD, the most common toxicities encountered were rigors, tachycardia, tachypnea, nausea/vomiting, cyanosis, headache, hypotension, and fatigue.
Urba et al[49]	Phase I study of 27 adult patients. Most common diagnoses were colorectal (n = 15), renal cell carcinoma (n = 4), and melanoma (n = 4).	Dosing of 0.1–2.7 mg/m^2 weekly for 8 weeks.	The MTD was not reached. Most common toxicities reported were fever and rigors. Hypotension noted in two patients treated at 2.7 mg/m^2 dose.
Kleinerman et al[47]	Phase II study of 16 patients with osteosarcoma.	2 mg/m^2 biweekly for 12 weeks and then weekly for 12 weeks.	Fevers, rigors, and headache were the most common toxicities.
Kleinerman et al[45]	Phase IIb study of 9 patients with osteosarcoma.	Combined with ifosfamide. Dose of 2 mg/m^2 biweekly for 12 weeks and then weekly for 12 weeks.	Fever, chills, myalgias, fatigue, and headache were most common toxicities reported. Two patients with pre-existing history of asthma experienced wheezing in first 2 weeks of therapy but resolved with bronchodilators.
Meyers et al[48]	Phase III study of 662 patients with nonmetastatic osteosarcoma.	Combined with MAP ± ifosfamide. Starting at week 12, dosing was 2 mg/m^2 biweekly for 12 weeks and then weekly for 24 weeks.	Fever and chills were most common toxicities reported.

Abbreviations: L-MTP-PE, liposomal muramyl tripeptide phosphatidyl ethanolamine; MTD, maximum tolerated dose; MAP, doxorubicin, cisplatin, and methotrexate.

objective was to determine if addition of mifamurtide with chemotherapy would be of additional benefit. Those patients assigned to receive the drug started receiving mifamurtide after completing neoadjuvant chemotherapy and having their primary tumor surgically resected. The dosing schedule was twice weekly for 12 weeks then weekly for 24 weeks; thus, the total number of doses administered was 48 doses over a 36-week period.

When reviewing the results for patients with nonmetastatic disease, the study determined that addition of ifosfamide did not appear to confer an outcome benefit. However, addition of mifamurtide resulted in a significant improvement in 6-year overall survival (78% with mifamurtide versus 70% without mifamurtide; $P = 0.03$).[50] Interestingly, when also reviewing the outcome in patients with metastatic osteosarcoma, there did appear to be a possible trend toward improvement in 5-year overall survival for those patients who received mifamurtide (53%) versus those who did not receive mifamurtide (40%; $P = 0.19$), but this did not achieve statistical significance, likely due to the small cohort size.[51] A compassionate access trial for patients with high-risk osteosarcoma has recently completed enrollment in the US (n = 200 patients), and the data from this trial are currently being analyzed. At this time, mifamurtide is approved for use as treatment for nonmetastatic osteosarcoma in Europe, while in the US mifamurtide has not yet received approval from the Food and Drug Administration.

Future directions

While mifamurtide has demonstrated some promise in the treatment of osteosarcoma, there remain questions regarding which clinical scenarios will benefit the most from mifamurtide therapy. Although preclinical work has indicated that the best results with enhanced immune therapy are seen in the setting of low disease burden, the best means to achieve minimal disease status rapidly remains to be determined. While the combination of mifamurtide with cytotoxic chemotherapy has been studied, combination of mifamurtide with other therapeutic agents, such as radiopharmaceuticals, has not been evaluated and provides an avenue for additional studies. Samarium-153 ethylene-diamine-tetramethylene phosphonic acid (^{153}Sm-EDTMP) is a bone-seeking radiopharmaceutical agent that has been used in patients with osteosarcoma and bone metastases.[52,53] Thus, one potential scenario for future study may be the addition of mifamurtide following treatment with ^{153}Sm-EDTMP or another bone-seeking radiopharmaceutical, such as radium-223.

Another area for further study is the combination of mifamurtide with other immunomodulatory agents, such as

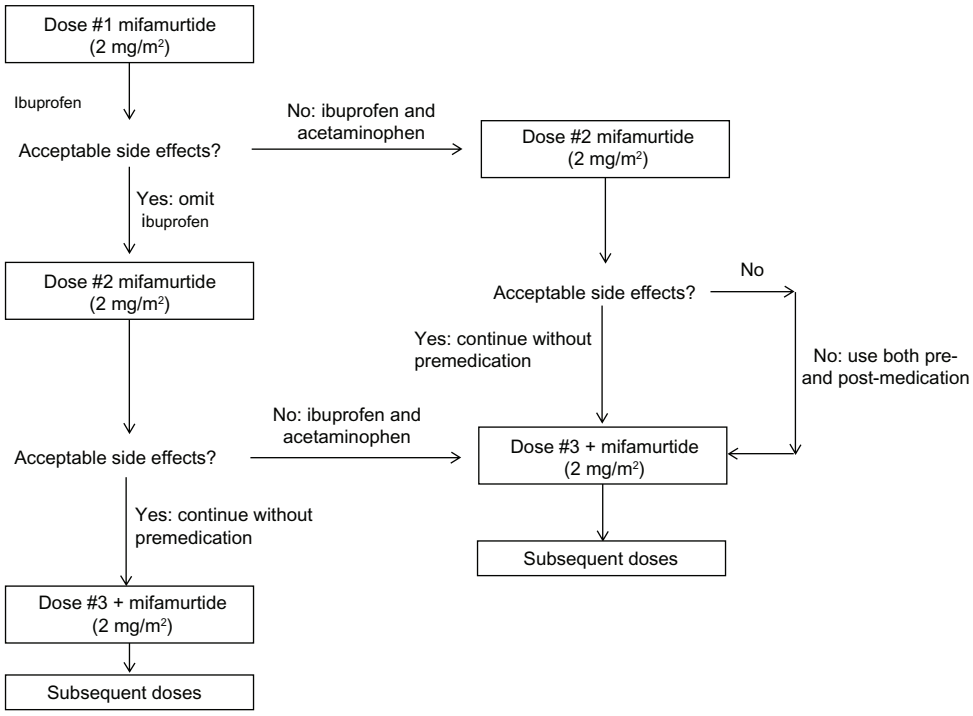

Figure 1 Algorithm for treating infusion-related side effects of mifamurtide.
Notes: Although premedication is often needed to reduce side effects after the first dose, most patients will require less premedication for subsequent doses. Occasional patients will require both before and after medication to reduce infusion-related symptoms.

inhaled granulocyte-macrophage colony stimulating factor.[11] A recent Phase I clinical trial by the Children's Oncology Group evaluating inhaled granulocyte-macrophage colony stimulating factor demonstrated its safety and tolerability in pediatric patients but was considered clinically ineffective.[11] However, the combination of mifamurtide with inhaled immunomodulators or harvested immune cells, such as natural killer cells or chimeric antigen receptor T cells, may provide synergy in the recruitment and activation of macrophages and monocytes. While there may be concern that the combination of such agents could result in an increased likelihood of side effects, such as fever, chills, and myalgias, prompt treatment with nonsteroidal anti-inflammatory medications would likely ameliorate the fever and myalgias, while meperedine would address the chills. Corticosteroids are not recommended because their immunosuppressive properties may suppress the antitumoricidal activity of mifamurtide. An algorithm to address these symptoms is proposed in Figure 1.

Finally, the role for mifamurtide in metastatic or recurrent osteosarcoma has not been fully evaluated. Future study of the use of mifamurtide in patients with newly diagnosed osteosarcoma and metastatic disease is warranted. Although such an endeavor will require a high level of participation from multiple institutions for this orphan indication, this may be the best means to provide a "confirmatory trial" to facilitate future approval of mifamurtide by the Food and Drug Administration.

Disclosure

The authors report no conflicts of interest in this work.

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