11 Muramyl Tripeptide-Phosphatidyl Ethanolamine Encapsulated in Liposomes (L-MTP-PE) in the Treatment of Osteosarcoma

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Abstract

The recruitment of autologous macrophages to attack osteosarcoma represents a novel immunotherapy approach to the treatment of osteosarcoma. Muramyl tripeptide-phosphatidyl ethanolamine encapsulated in liposomes (L-MTP-PE) was derived as a compound with the ability to stimulate macrophages to destroy autologous osteosarcoma tumor cells. Preclinical studies including studies in dogs with spontaneously arising osteosarcoma showed the ability of L-MTP-PE to control microscopic metastatic disease in osteosarcoma. A pivotal clinical trial led to the approval of L-MTP-PE for the treatment of newly diagnosed osteosarcoma in over 40 countries.

Keywords

Osteosarcoma · Muramyl tripeptide · Immunotherapy · Macrophages · Adjuvant therapy

Introduction

The idea that the immune system could be activated to attack cancer is an old one. In 1891, Coley reported his experience at the Memorial Sloan Kettering Cancer Center (MSKCC). He used direct injections of bacteria into tumors to cause infection which in some cases led to regression of sarcomas [1]. In the ensuing century, a variety of immune effector cells have been tested for their anticancer properties including tumor-infiltrating lymphocytes, lymphokine-activated killer cells, and genetically modified T cells. Immune stimulating agents such as interferon have been used to treat melanoma. There was less attention paid to the macrophage as a potentially active antitumor immune effector cell. Liposomal muramyl tripeptide phosphatidyl ethanolamine (L-MTP-PE) was developed to stimulate monocytes and macrophages to become tumoricidal against autologous tumor cells and has undergone extensive testing in preclinical, phase I, phase II, and phase III trials and was ultimately approved as adjuvant therapy for the treatment of osteosarcoma.

Background

Bacille Calmette-Guerin (BCG) is a bacterium that was derived from the tuberculosis bacterium by repeated passage to obtain an isolate of

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attenuated virulence that could be used as a vaccine against tuberculosis. In the early decades of the twentieth century, BCG was used as an adjuvant to stimulate the immune system in patients with cancer. BCG is part of the armamentarium of modern cancer therapy. In the treatment of superficial cancer of the urinary bladder, injection of BCG into surface malignancies of the bladder leads to spontaneous regression [2].

Zwilling and Campolito showed that BCG could stimulate pulmonary macrophages to become tumoricidal in an autologous model [3]. Namba et al. showed that this tumoricidal activity resided in a component of the BCG cell wall [4]. Ellouz et al. isolated peptidoglycans from the BCG cell wall and reported that a synthetic analogue, N-acetyl-muramyl-L-alanine-D-isoglutamine, or muramyl dipeptide (MDP) preserved the activity of the intact cell wall [5]. Benacerraf et al. reported that MDP was an effective immune adjuvant [6]. Fidler and colleagues reported that packaging lymphokines in liposomes resulted in improved activation of immune effector cells [7]. They also reported that MDP encapsulated in liposomes could lead to macrophage destruction of autologous tumor cells [8]. Fidler's group reported that intravenous administration of MDP encapsulated in liposomes could prevent the development of pulmonary metastases in a murine model [9].

MDP is a small molecule and disappeared rapidly from the circulation following intravenous administration [10]. Small molecules like MDP leak rapidly from liposomes. Fidler's group modified MDP by adding a third peptide to created muramyl tripeptide (MTP). They also linked MTP to phosphatidyl ethanolamine so that the resulting liposomes incorporated the MTP into multilamellar membranes [11]. Kleinerman and Fidler used the resulting agent liposomal muramyl tripeptide phosphatidyl ethanolamine (L-MTP-PE) to demonstrate autologous tumoricidal activity in human models [12].

Clinical Trials

The first trials of L-MTP-PE in humans were carried out at the MD Anderson Cancer Center (MDACC). The first phase I trial reported mild to moderate side effects, including chills, fever, nausea, and malaise [13]. The maximum tolerated dose (MTD) was reported to be 6 mg/m^2. Radiolabeled L-MTP-PE was taken up by the reticuloendothelial system including the liver, spleen, lungs, and nasopharynx. Kleinerman studied the peripheral blood monocytes from the patients who participated in the phase I trial and reported activation of tumoricidal activity in monocytes in 24 of 28 subjects [14]. The dose of MTP which achieved the best immune stimulation was 0.5–2.0 mg/m^2, lower than the MTD of 6 mg/m^2.

When patients with osteosarcoma are initially diagnosed, most of them do not have clinically detectable metastatic disease. In the absence of systemic therapy, 80–90% of them will go on to develop metastatic disease, and the great majority of the metastases are pulmonary [15]. L-MTP-PE had been shown to induce autologous tumoricidal activity in human monocytes and macrophages . L-MTP-PE had been shown to prevent the development of pulmonary metastases following intravenous injection of tumor cells in murine models. This suggested that L-MTP-PE might be a useful adjunct in the treatment of osteosarcoma.

Most anticancer drugs are treated in models in which human tumor cell lines are grown in mice with a compromised immune system. These models, called heterotopic xenografts, are imperfect models of human disease. The cell lines have often undergone mutation so that they no longer recapitulate the human tumor. The tumors are grown in compartments that do not recapitulate the tumor microenvironment in which they arose. The lack of a competent immune system in the mice, necessary to establish the xenograft, precludes testing therapies that involve immune effector cells. Osteosarcoma arises in dogs

spontaneously and largely recapitulates human disease. Tumors arise in long bones and metastasize to the lung, and death results from pulmonary failure. Osteosarcoma in dogs represents an excellent model in which to test potential new treatments for human osteosarcoma.

MacEwen performed a prospective, randomized, double-blind, placebo-controlled trial of L-MTP-PE in dogs with osteosarcoma [16]. All the dogs underwent amputation. They were then randomly assigned either to receive L-MTP-PE or placebo. 100% of the dogs that received placebo developed metastatic disease and went on to die with a median survival of 77 days. The dogs treated with L-MTP-PE had a statistically significant improved median survival of 222 days, and 4 of 14 dogs remained alive and free of recurrence 1 year following treatment. These results supported subsequent trials in human patients including phase II trials and ultimately the phase III randomized trial.

Investigators at MDACC performed a phase II trial of L-MTP-PE in patients with osteosarcoma who developed recurrent pulmonary metastases after frontline therapy including surgery and multi-agent chemotherapy [17]. All patients had surgical removal of the pulmonary metastases. One group of patients received L-MTP-PE twice weekly for 12 weeks. A second group of patients received L-MTP-PE for 24 weeks. Progression-free survival (PFS) for the two groups was compared to a comparable group of patients treated at MDACC without L-MTP-PE (historical control). The median time to progression for the second group of patients treated for 24 weeks was 9 months, significantly longer than the median PFS of 4.5 months for historical control group. Median PFS for the second group was better than for the first group, suggesting that longer duration of therapy was beneficial. Among the patients who went on to develop pulmonary recurrence despite the administration of L-MMTP-PE, some had surgical resection of these new pulmonary nodules. Nodules resected after administration of L-MTP-PE demonstrated infiltration by monocytes and macrophages and a rim of fibrosis, supporting the conclusion that L-MTP-PE provoked an immune inflammatory response in the metastatic nodules [18].

Treatment of osteosarcoma always includes the use of systemic chemotherapy. Kleinerman investigated the interaction between chemotherapy and L-MTP-PE. She reported that doxorubicin had no effect on cytokine release or induction of tumoricidal activity in monocytes by L-MTP-PE [19, 20]. She retrieved circulating monocytes from patients before, during and after administration of chemotherapy and demonstrated no difference in the response to L-MTP-PE [21].

Investigators at MDACC and MSKCC performed a phase II study in patients with osteosarcoma which recurred after initial therapy with surgery and multi-agent chemotherapy which did not include ifosfamide [17]. Patients were treated with concurrent ifosfamide and L-MTP-PE. They reported the usual and customary toxicity with ifosfamide; there was no increased toxicity seen with concurrent administration. Administration of L-MTP-PE was associated with similar increases in circulating cytokines to that seen when L-MTP-PE was administered without concurrent ifosfamide. Some patients underwent resection of metastatic pulmonary nodules after administration of ifosfamide and L-MTP-PE. Pathologic review of the resected nodules showed tumor necrosis similar to that seen after administration of chemotherapy without L-MTP-PE; it also showed inflammatory infiltrates and surrounding fibrosis similar to that seen when L-MTP-PE was administered without concurrent chemotherapy. This study showed that chemotherapy did not interfere with L-MTP-PE activity.

Randomized Phase III Trial

L-MTP-PE had a very favorable safety profile. A phase II trial in recurrent osteosarcoma suggested that prolonged administration of L-MTP-PE was associated with decreased risk for recurrence. A prospective, randomized, double-blind study of L-MTP-PE in dogs with osteosarcoma showed a statically significant improvement in progression-

free survival and apparent cures. All of this evidence justified a phase III trial of L-MTP-PE in patients with osteosarcoma.

As the North American pediatric cooperative groups began consideration of the design of the phase III trial in osteosarcoma, there was an additional prominent question. Ifosfamide had shown activity in metastatic recurrent osteosarcoma with reports of 30–50% objective responses [22, 23]. The phase III clinical trial was designed to answer two questions:

1. The trial would be a comparison of a three-drug chemotherapy regimen with cisplatin, doxorubicin, and high-dose methotrexate to a four-drug chemotherapy regimen with cisplatin, doxorubicin, high-dose methotrexate, and ifosfamide. Would adding a fourth chemotherapy agent improve outcome?
2. Would the addition of L-MTP-PE to systemic chemotherapy improve outcome?

Osteosarcoma is a rare disease. In order to answer both questions in a reasonable period of time, we decided to use a factorial design. In factorial design, patients are randomly assigned to each intervention, but each intervention is analyzed for its effect on the entire population. All patients who received four-drug chemotherapy would be compared to all patients who received three-drug chemotherapy, ignoring whether or not they had been assigned to receive L-MTP-PE. All patients assigned to receive L-MTP-PE would be compared to all patients assigned not to receive L-MTP-PE, without considering whether they had been assigned to receive three- or four-drug chemotherapy. These marginal analyses can only be performed if there is no interaction between the two study interventions. No preclinical or clinical evidence suggested that there would be an interaction between the two study interventions, and there was no plausible biological basis to suggest an interaction [21]. The final analysis at the completion of the randomized prospective phase III trial detected no interaction [24].

The design for the chemotherapy question was an addition study. Patients assigned to treatment arm A received cisplatin, doxorubicin, and high-dose methotrexate. Patients assigned to treatment arm B received the same agents with the addition of ifosfamide. As had become widespread practice for the treatment of osteosarcoma, patients received an initial period of chemotherapy followed by definitive surgical resection of the primary tumor followed by additional adjuvant chemotherapy. Assessment of necrosis in the primary tumor after the initial period of systemic chemotherapy was performed as there is a strong correlation between the degree of necrosis in the primary tumor following initial therapy and outcome [25]. Longer periods of chemotherapy prior to definitive surgery can be associated with higher degrees of necrosis at the time of definitive surgery, so it was important to maintain an identical duration of initial chemotherapy in both arms of the study [26].

We relied on preclinical and early clinical data to decide when to introduce L-MTP-PE. All of the available evidence suggested that L-MTP-PE was more likely to provide benefit in the setting of minimal tumor burden, i.e., after definitive resection of the primary tumor and any macroscopic metastatic disease [9, 17]. Since L-MTP-PE has its maximum effect against minimal residual disease, L-MTP-PE therapy was initiated after surgical resection of the primary tumor. There were four treatment arms: A, A+, B, and B+. Patients assigned to regimen A received chemotherapy with cisplatin, doxorubicin, and high-dose methotrexate. Patients assigned to regimen B received chemotherapy with the same three drugs with the addition of ifosfamide. Patients assigned to receive L-MTP-PE were designated with the addition of a plus sign to the chemotherapy regimen; 677 patients were randomly assigned to one of the four treatment regimens at the time of study enrollment. In retrospect, this was an error in study design, because it allowed for an imbalance in the number of patients with poor necrosis after initial therapy, which is associated with worse prognosis, to one arm. This design flaw ultimately masked the treatment success of L-MTP-PE in the three-drug plus L-MTP-PE group (A+) as discussed below.

The frequency of more favorable and less favorable necrosis following initial chemother-

apy was the same when we compared patients treated with regimen A and B. Toxicities on all four arms of the study were very similar. There was no increased toxicity among the patients assigned to receive L-MTP-PE (regimens A+ and B+).

Analysis of the results of the study approximately 9 years after the last patient was enrolled (13 years after enrollment of the first patient) was reported in 2008 [24]:

1. Treatment with three chemotherapy drugs (regimen A) and four chemotherapy drugs (regimen B) achieved the same probability for both event-free and overall survival.
2. All patients assigned to receive L-MTP (with three- or four-drug chemotherapy) showed an improvement in event-free survival compared to those that received three- or four-drug chemotherapy alone. The probability for event-free survival 6 years from study entry was 67% with L-MTP-PE and 61% without. The *p* value for this difference was 0.08.
3. The same comparison showed a statistically significant improvement in overall survival. The probability for overall survival 6 years from study entry was 78% with L-MTP-PE and 70% without. The *p* value for this difference was 0.03.
4. The hazard ratio for death from osteosarcoma comparing treatment with L-MTP-PE to treatment without was 0.7.

Necrosis following initial chemotherapy in the randomized prospective trial was analyzed according to the method described by Huvos [25]. Less necrosis (Huvos grade 1 and 2 necrosis) was associated with a higher probability of recurrence and death than more necrosis (Huvos grades 3 and 4). When we analyzed the frequency of greater and lesser necrosis among the patients assigned to receive each of the four possible randomized therapies, we observed an excess of patients with less necrosis assigned to receive three-drug chemotherapy in combination with L-MTP-PE (regimen A+). Since the observation of less necrosis strongly correlates with a higher probability for recurrence, this imbalance could explain the apparent failure to observe an improved outcome for event-free survival among the patients receiving three-drug chemotherapy who were assigned to receive L-MTP-PE.

Further analysis of the imbalance in necrosis revealed that by chance most of the imbalance took place in patients older than 16 at study entry. For patients aged less than 16 at study entry, there was better balance among the study arms in the frequency of patients with greater and lesser necrosis following initial chemotherapy. This allowed us to examine the effect of the addition of L-MTP-PE to chemotherapy in 496 patients free from the confounding effect of an excess of patients with poor necrosis in one study arm. For this group of 496 children, the addition of L-MTP-PE to chemotherapy resulted in improved event-free survival. The improvement was seen with both chemotherapy regimens to the same degree. There was no interaction between the two study questions. For this group, the addition of L-MTP-PE to chemotherapy resulted in improved overall survival. The improvement was exactly the same for both chemotherapy regimens.

The hazard ration for death associated with the addition of L-MTP-PE was 0.5 ($p = 0.001$). This analysis of 496 children in a prospective randomized trial represents one of the largest experiences ever reported for osteosarcoma and demonstrates a clinically and statistically significant improvement for both event-free and overall survival when L-MTP-PE is added to chemotherapy. The benefit was independent of the chemotherapy regimen to which the patients were assigned.

Phase III Randomized Trial for Patients with Metastatic Disease at Initial Presentation

The phase III randomized trial allowed enrollment of patients with newly diagnosed osteosarcoma who presented with clinically detectable metastatic disease if the clinical assessment indicated the possibility of surgical resection of all sites of metastatic disease as well as the primary tumor. Most patients who present with metastatic

disease have metastasis limited to the lungs and resection of pulmonary nodules is feasible. The protocol specified that patients would be randomized to the same four treatment arms as the patients with localized disease. Patients would undergo resection of the primary tumor and all sites of metastatic disease prior to the initiation of L-MTP-PE. The total number of patients with metastasis who participated in the prospective randomized trial was only 91 patients which greatly decreased the ability to make statistical comparisons between the 2 interventions. We reported the results of this stratum in 2009 [27]:

1. We observed no interaction between the two study interventions, that is, addition of ifosfamide to three drug chemotherapy and addition of L-MTP-PE.
2. Both event-free and overall survival were the same for patients treated with three-drug and four-drug chemotherapy regimens.
3. Both event-free and overall survival were better for the patients who received L-MTP-PE than for those who did not. Neither of these improvements reach a conventional level of statistical significance.
4. The hazard ratio associated with the risk of death when patients who received L-MTP-PE were compared to patients who did not was 0.7, which with the same as the hazard ratio we observed for patients with localized osteosarcoma.

Compassionate Access Trial

We conducted a compassionate access clinical trial of L-MTP-PE from 2008 to 2012 [28]. Eligibility included patients who presented either with osteosarcoma with metastatic disease at initial presentation or metastatic recurrent osteosarcoma after initial therapy with surgery and multi-agent chemotherapy. Trial design called for all patients to receive L-MTP-PE, either as a single agent or in combination with chemotherapy if the treating clinician felt that chemotherapy was appropriate. We enrolled 40 patients with initially metastatic disease and 165 patients with recurrent osteosarcoma. Among the 50 patients for whom it was possible to resect all sites of clinically detectable tumor, overall survival at 2 years following study enrollment was greater than 50%. Many of these patients were treated following two or more recurrences following their initial therapy for osteosarcoma.

Regulatory Status of L-MTP-PE

The sponsor presented L-MTP-PE to the Oncology Drugs Advisory Committee of the United States Food and Drug Administration (FDA) in May, 2007. Data from the pivotal phase III randomized trial was analyzed at two time points. The first analysis with data truncated in 2003 was reported in 2005 [29]. The sponsor recognized that follow-up at the first data point was poor and worked with the Children's Oncology Group to improve ascertainment of patient status for all study participants. The second analysis, with data truncated in 2006, was reported in 2008 [24]. Although the updated data set was provided to the FDA prior to the hearing, the FDA chose to analyze and present only the earlier data set. Based on that analysis, the FDA did not grant an indication for the use of L-MTP-PE in osteosarcoma. In 2008, the sponsor presented the updated data set to the European Medicines Agency. L-MPT-PE, marketed at MEPACT (mifamurtide), was approved for treatment of osteosarcoma in patients between the ages of 2 and 30 when administered in conjunction with multiagent chemotherapy [30]. As of 2019, L-MTP-PE is licensed and approved for that indication in 45 countries.

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