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Mifamurtide for the treatment of non-metastatic osteosarcoma

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Mifamurtide for the treatment of non-metastatic osteosarcoma

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Abstract

Importance of the field

Osteosarcoma belongs to the orphan diseases but is the most common primary malignant tumor of bone. Currently, the standard treatment for osteosarcoma requires both macroscopic surgical wide resection and postoperative multi-drug chemotherapy in neo-adjuvant and adjuvant settings. However, the 5-year event-free survival remains at a plateau of 60-70% of patients with non-metastatic osteosarcoma for over 30 years.

Areas covered in this review

Mifamurtide [liposomal muramyl tripeptide phosphatidylethanolamine (L-MTP-PE)] is a new agent. L-MTP-PE is a nonspecific immunomodulator, which is a synthetic analog of a component of bacterial cell walls. L-MTP-PE activates macrophages and monocytes as a potent activator of immune response. The addition of L-MTP-PE to standard chemotherapy improves the overall survival from 70 % to 78% and results in a one-third reduction in the risk of death from osteosarcoma.

What the reader will gain

Recently, L-MTP-PE has been approved in Europe for the treatment of non-metastatic osteosarcoma with chemotherapy. According to preliminary clinical report, L-MTP-PE is well-tolerated and has little severe side effects.

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7 ***Take home message***
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10 L-MTP-PE in combination with traditional treatment is expected to go mainstream and to
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12 be beneficial for patients with osteosarcoma.
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16 **Key words** osteosarcoma, liposomal muramyl tripeptide
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18 phosphatidylethanolamine, macrophage, chemotherapy, innate immunity
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1. Introduction

Osteosarcoma is the most common primary malignant bone tumor. It usually arises in the metaphyses of long bone in children and adolescents [1, 2]. Approximately 1000 new patients are seen per year in North America and a similar number in Europe [3]. **The standard treatment of the primary osteosarcoma consists of microscopically complete surgical resection and multi-agent chemotherapy in neo-adjuvant and adjuvant settings.** Surgical techniques for osteosarcoma have improved and built up from amputations to limb-salvage surgery during the last decades [4]. However, it did not contribute to the improvement of both EFS and overall survival.

On the other hand, the value of chemotherapy for treatment of osteosarcoma is well established [5, 6]. There are currently four chemotherapeutic agents, which consist of doxorubicin, cisplatin, high-dose methotrexate with leucovorin rescue, and ifosfamide [7-11]. Vigorous chemotherapy treatment with these agents has significantly improved survival of the osteosarcoma patients over the past several decades [12]. It has been reported that EFS of osteosarcoma patients at 3-5 years has reached 60-70% in the non-metastatic condition. **However, in the metastatic relapse or recurrent conditions, EFS of osteosarcoma patients at 3-5 years remained at 10-30% since early 1980s [4, 12-14].** None of the various chemotherapy agents and regimens has demonstrated any

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7 significant superiority yet [15]. No new anti-osteosarcoma agents have been developed in
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10 the intervening years [16]. Recently, Children's Oncology Group carried out long-term
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12 follow-up of the key trial of chemotherapy with or without mifamurtide (liposomal
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14 muramyl tripeptide phosphatidyl ethanolamine [L-MTP-PE]). This group demonstrated
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16 that addition of L-MTP-PE to chemotherapy significantly improved overall survival at 6
17
18 years from 70% with chemotherapy alone to 78 % with chemotherapy and L-MTP-PE (p
19
20 = 0.03) [17]. These data demonstrate that L-MTP-PE may have a potential role in the
21
22 improvement of survival of the osteosarcoma patients remained on a plateau for over two
23
24 decades [16, 18-20]. **The proprietary product name of mifamurtide is Mepact[®]**
25
26 **(Takeda).** L-MTP-PE, a nonspecific immunomodulator, is a synthetic analog of a
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28 component of bacterial cell walls [21]. L-MTP-PE activates macrophages and monocytes
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30 as a potent activator of immune response [22, 23].
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44 In this review, we will summarize the most recent findings about L-MTP-PE and
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46 its therapeutic application for non-metastatic osteosarcoma.
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53 **2. Pharmacology**

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55 MTP-PE is a stimulator of innate immunity and a synthetic molecule derived from
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57 muramyl dipeptide (MDP). MTP-PE results from the covalent addition of alanin and
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7 dipalmitoyl phosphatidyl ethanolamine to MDP (Figure 1), which is a peptidoglycan
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10 found in Gram-positive and Gram-negative bacterial cell walls [21].
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13 ***In vitro and in vivo*** monocytes/macrophages activation by MTP-PE is related to
14
15 the upregulation of tumoricidal activity and secretion of pro-inflammatory cytokines
16
17 including tumor necrosis factor (TNF)- α , interleukin (IL)-1, IL-6, IL-8, nitric oxide (NO),
18
19 prostaglandin E2 (PGE₂), and PGD₂ [24-29]. NO, PGE₂, and PGD₂ are synthesized and
20
21 released by murine Kupffer cells (liver macrophages) after *in vitro* MTP-PE exposure
22
23 [30]. Moreover, L-MTP-PE induces the expression of adhesion molecules including
24
25 lymphocyte function-associated antigen (LFA)-1, intracellular adhesion molecule
26
27 (ICAM)-1, and human leukocyte antigen (HLA)-DR. These molecules could be closely
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29 related to interaction with tumor cells [21, 31].
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41 MTP-PE is superior to MDP in the activation of human monocytes [22]. The
42
43 lipophilic MTP-PE could be efficiently incorporated in the lipid bilayer of liposomal
44
45 structures and distributed primarily in the liver, spleen, and lungs after intravenous
46
47 MTP-PE administration [22, 23, 32]. Thus, the intravenous MTP-PE encapsulated in
48
49 liposomes has been developed to target delivery of the drug selectively to monocytes and
50
51 macrophages, such as those in liver, spleen, and lungs [13, 16, 21]. **The lipid**
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53 **composition of mifamurtide also facilitates macrophage signaling via phosphatidyl**
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7 **serine recognition by macrophages [33].** The particle nature of liposomes converts the
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10 parent drug into “pro-drug”. Liposomes are concentric multi-lamellar vesicles with the
11
12 lipid bilayers resembling an onion of particle size approximately 2-3 μm . Formulation of
13
14 MTP-PE into these phospholipid vesicles enhances the activation of
15
16 macrophages/monocytes tumoricidal properties and extends its existence in the lungs [22,
17
18 32]. In fact, an advantage of MTP-PE over MDP in the activation of human monocytes
19
20 was demonstrated [22]. This advantage was ascribed to the benefit of the lipophilic
21
22 properties of MTP-PE described earlier. In addition, the liposomal formulation
23
24 (L-MTP-PE) has improved the safety profile of several drugs by modifying parent drug or
25
26 solubilization agent toxicity [34]. Due to rapid mononuclear phagocytosis of the
27
28 liposome transporter, L-MTP-PE has very rapid clearance from the blood only
29
30 approximately 0.5% of L-MTP-PE remains in the plasma at the 5-min time point
31
32 compared with 93% when administrated as the free form [35]. In humans, there is no
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34 evidence of accumulation of either liposomes or free MTP-PE after L-MTP-PE 4-mg
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36 treatment twice a week for 9 week [36]. The half-life of free MTP-PE can be estimated as
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38 3-6 h from dog and rat studies. Additionally, no accumulation of phospholipids after
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40 repeated administration has been confirmed [32]. Due to such the rapid clearance,
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42 L-MTP-PE shows ten times lesser adverse event level than free MTP-PE in rabbits and
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7 dogs [21].
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10 MTP-PE can bind to Toll-like receptor (TLR) 4 and activate extracellular-signal
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12 regulated kinase 1/2 (ERK 1/2), nuclear factor-kappa B (NF-κB) and adaptor protein
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14 (AP)-1 [29, 37]. However, the other receptor for MTP-PE may be intracellular [22].
15
16 Recently, MTP-PE has been reported as a specific ligand of nucleotide-binding
17
18 oligomerization domain (Nod) 2 receptor. Nod2, an intracellular MDP sensor, is strongly
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20 expressed in monocytes, granulocytes, myeloid dendritic cells, and macrophages [38]. It
21
22 induces NF-κB and influences the innate immune response [39]. L-MTP-PE is
23
24 selectively phagocytosed by monocytes and macrophages after intravenous
25
26 administration [40]. The phagocytic cells gradually degrade the liposomal vesicles. Then,
27
28 **MTP-PE is released into the cytosol and is degraded to MDP where it interacts with**
29
30 **Nod2 and activates the cells [22, 41].** Thus, monocytes/macrophages activation by
31
32 L-MTP-PE is considered to be mediated *via* Nod2 [16, 21] (Figure 2). In sum, preclinical
33
34 studies showed that L-MTP-PE, in comparison with free MTP-PE, was more effective in
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36 terms of activating monocytes [22], was longer held in target organs [32], and was
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38 10-fold less toxic [42].
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56 Monocytes activated by L-MTP-PE had no effect on non-tumorigenic cells [24,
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58 26, 43] and even under conditions of co-cultivation with tumorigenic cells [24, 43].
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7 Furthermore, peripheral blood monocytes from patients with cancer could be activated to
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10 similar levels of tumoricidal activity as monocytes from healthy volunteers [26, 39, 43,
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12 44]. L-MTP-PE interacts with interferon (IFN)- γ to up-regulate tumoricidal activity and
13
14 to induce secretion of cytokines such as TNF- α and IL-1 β [21, 45-50]. The mechanism of
15
16 this potentiation remains to be elucidated, but a 2-fold increase in liposome phagocytosis
17
18 was observed after treatment of human monocytes with IFN- γ [50].
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25 Activation of monocytes-mediated tumoricidal activity was investigated
26
27 following *in vivo* treatment with L-MTP-PE in phase I and II clinical trials [36, 51, 52]. In
28
29 the phase I study, 28 patients with metastatic cancer received increasing doses (1h
30
31 intravenous administration) of L-MTP-PE (0.05-12.0 mg/m²) twice a week [36, 51].
32
33 Peripheral blood monocytes were harvested and examined *ex vivo* for cytotoxic activity
34
35 against human A375 melanoma cells before L-MTP-PE therapy and at various time
36
37 points during the 9-week treatment period. Activation of monocytes-mediated cytotoxic
38
39 activity was seen in 24 (86%) of the 28 patients at some time points during the treatment
40
41 period [51]. Monocytes-mediated tumoricidal activity remained for up to 96 hours after
42
43 initial mifamurtide infusion [36]. In the phase II study, 16 patients with relapsed
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45 osteosarcoma received a 1h intravenous infusion of L-MTP-PE (2 mg/m²) twice a week
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47 for 12 weeks [52]. These patients had histologically confirmed osteosarcoma and
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7 pulmonary metastases that had developed during adjuvant chemotherapy or that were
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10 present at diagnosis and had persisted despite chemotherapy. The patients were
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13 pre-treated with surgical resection of visible and palpable lung metastases before
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16 participating in the study. Peripheral blood monocytes were harvested and examined *ex*
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18
19 *vivo* for cytotoxic activity against human A375 melanoma cells before L-MTP-PE
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22 therapy and during the 4-week treatment period. Monocytes-mediated tumoricidal
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24
25 activity was significantly increased in 80% of the patients evaluated. The peak cytotoxic
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27
28 activity was observed at 24 (n = 3), 72 (n = 4), and 96 (n = 1) hours post-infusion [52].
29
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31 Similarly, monocytes and macrophages from non-human species demonstrated increased
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34 tumoricidal activity after *in vitro* [25, 28] and *in vivo* [28, 46, 53-55] treatment with
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37 L-MTP-PE or MTP-PE.
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41 Although various chemotherapeutic agents and regimens have been examined
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44 for osteosarcoma, none of them demonstrates any clear superiority [15, 56]. Since
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47 L-MTP-PE was intended to be used **in combination with both adjuvant chemotherapy**
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50 **and surgery** in the treatment of osteosarcoma, some researchers reported the effects of
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53 cytotoxic agents on the monocytes-mediated tumoricidal activity of L-MTP-PE [39, 57,
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56 58]. These results showed that most chemotherapeutic drugs including the treatment of
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59 osteosarcoma (*i.e.*, doxorubicin, cisplatin, high-dose methotrexate with leucovorin rescue,
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6 and ifosfamide) did not influence on macrophage activation by L-MTP-PE [39, 57, 58].
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10 For example, doxorubicin [39, 57], cisplatin [39], methotrexate [39], or
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12 cyclophosphamide [39] administration to patients with osteosarcoma did not interfere
13
14 with the ability of L-MTP-PE to enhance the monocytes-mediated tumoricidal activity
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16 from these patients *in vitro*. Furthermore, combination chemotherapy of doxorubicin and
17
18 cyclophosphamide strongly suppressed monocytes-mediated tumoricidal activity by
19
20 L-MTP-PE [39]. Similarly, simultaneous administration of ifosfamide and L-MTP-PE
21
22 did not interfere with the ability of L-MTP-PE to activate an *in vivo* immune response
23
24 according to the results of a phase IIb clinical study in patients with relapsed
25
26 osteosarcoma (n = 9) [58]. The trial participants had histologically confirmed
27
28 osteosarcoma and pulmonary metastases that had developed during adjuvant
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30 chemotherapy or that were present at diagnosis and had persisted despite chemotherapy.
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32 They were treated neoadjuvantly and/or adjuvantly with a combination of ifosfamide (up
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34 to 8 cycles in total; each cycle consisted of 1.8 g/m² for 5 days every 21 days) and
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36 L-MTP-PE (2 mg/m² twice a week for 12 weeks and then once a week for 12 weeks).
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38 Up-regulations of serum TNF- α , IL-6, and IL-8 in patients treated with the combination
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40 therapy was not different from those in patients treated with L-MTP-PE alone [52]. In
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42 addition, monocytes-mediated tumoricidal activity was elevated at 24 and 72 hours after
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7 the initial of combination therapy, similar to the following L-MTP-PE alone [51]. In
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10 particular, combination treatment of ifosfamide and L-MTP-PE did not increase the
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12 toxicity of ifosfamide [58]. L-MTP-PE did not decrease the tumoricidal activity of
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14 ifosfamide or doxorubicin in three synergistic murine tumor models [59]. Furthermore,
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16 the suggested course of L-MTP-PE therapy did not worsen the identified renal (*i.e.*,
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18 cisplatin, ifosfamide) or hepatic (*i.e.*, methotrexate, ifosfamide) toxicities of the
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20 concurrently administered chemotherapies in phase III study in patients with
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22 osteosarcoma [60].
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34 3. Clinical therapeutic efficacy

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37 A phase III randomized prospective trial was conducted by the Children's Cancer and
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39 Pediatric Oncology Groups (now collectively known as the Children's Oncology Group)
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41 from 1993 to 1997 [12]. **This central randomized trial in osteosarcoma was known as**
42
43 **the Intergroup Study 0133 (INT study 0133). A total of 662 eligible patients aged ≤**
44
45 **30 years with non-metastatic resectable osteosarcoma were employed in this study**
46
47 **[17].** This study was designed to assess whether the addition of L-MTP-PE and/or
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49 ifosfamide to a standard chemotherapeutic regimen composed of three agents (*i.e.*,
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51 doxorubicin, cisplatin and high-dose methotrexate with leucovorin rescue) would
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7 increase both EFS and overall survival in newly diagnosed patients with high-grade
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10 osteosarcoma. No significant improvement in EFS and overall survival was observed by
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13 the addition of ifosfamide in the dose and schedule used in this study ($p = 0.934$ and 0.992
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15
16 respectively). However, significant improvement in EFS and overall survival were
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18
19 observed in patients randomized to receive L-MTP-PE ($p = 0.030$ and 0.039 respectively).
20
21
22 These findings correspond to a 25% reduction in the risk of recurrence and a 30%
23
24
25 reduction in the risk of death [21, 61]. Most notably, the 6-year overall survival improved
26
27
28 from 70% without L-MTP-PE to 78% with it [17].
29
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31
32 In the INT study 0133, all patients were intended to receive a similar backbone
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34 treatment called MAP (*i.e.*, high-dose methotrexate with leucovorin rescue,
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36 doxorubicin/adriamycin, and cisplatin) with identical cumulative doses of high-dose
37
38 methotrexate (12 times at doses of 12 g/m^2), doxorubicin (6 times at doses of 75 mg/m^2),
39
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41 and cisplatin (4 times at doses of 120 mg/m^2). The randomized prospective study was
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43
44 conducted with a 2×2 factorial design with 4 treatment groups: (1) MAP, (2) MAP +
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47 L-MTP-PE, (3) MAP + ifosfamide, and (4) MAP + ifosfamide + L-MTP-PE. Ifosfamide
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49
50 was administered 5 times at a dose of 9 g/m^2 per course. L-MTP-PE was administered at
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52
53 a dose of 2 mg/m^2 **in the adjuvant setting**. L-MTP-PE was administered intravenously
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59 twice a week for 12 weeks starting at week 12, and then weekly for additional 24 weeks
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7 starting at week 24. The duration of treatment was 20 weeks for patients randomly
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10 assigned to 'MAP' group, 27 weeks for patients randomly assigned to 'MAP +
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12 ifosfamide' group, and 36 weeks for patients randomly assigned to 'MAP + L-MTP-PE'
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14
15 group and 'MAP + ifosfamide + L-MTP-PE' [12, 17]. The results of INT study 0133
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17
18 were analyzed and published in 2005 and 2008 [12, 17]. In 2005, Meyers *et al.* [12]
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20
21 reported their initial analysis of EFS without overall survival. According to their findings,
22
23
24 there was no significant evidence of L-MTP-PE on EFS [12]. Later, this initial report was
25
26
27 cited as inappropriate analyses. **In 2008, both overall survival and EFS were renewed**
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29
30 **as the result of subsequent analysis of this study with longer follow-up [17].** This
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32
33 re-analysis reported improved overall survival with the addition of L-MTP-PE from 70%
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35
36 to 78% 6-year overall survival ($p = 0.03$) [17]. By contrast, in the analysis of EFS, there
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38
39 was no sufficient evidence of the interaction ($p = 0.102$) [17]. This makes the
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41
42 interpretation of INT 0133 study very complicated [62, 63] because the effect of
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45 treatment on overall survival is expected to be mediated through EFS. There were no
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47
48 differences in the frequency of favorable tumor necrosis, which is strongly associated
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51 with EFS and determined by modified Huvos grading [64], among those treatment groups.
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54 Therefore, the authors concluded that ifosfamide and the other chemotherapy agents are
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57 equivalent in their ability to contribute to favorable tumor necrosis. Furthermore, another
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7 study demonstrated that patients with metastatic osteosarcoma were given a good benefit
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10 by administration of a higher dose of ifosfamide (14 mg/m²) [65, 66] . Thus, the most
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13 effective combination of ifosfamide with L-MTP-PE may be a dose question. At any rate,
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15
16 many additional questions including the best way to combine chemotherapy and
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18
19 L-MTP-PE still remain, **but the INT study 0133 demonstrated the possible benefit**
20
21
22 **associated with L-MTP-PE in the treatment of osteosarcoma.**
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28 **4. Dosage and Side effects after premedication**

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30
31 **In European Union, L-MTP-PE is indicated in patients with high-grade,**
32
33
34 **resectable, non-metastatic osteosarcoma after the complete surgical wide resection**
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36
37 **aged between 2 and 30 years [60].** It is currently recommended that L-MTP-PE is
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39
40 intravenously-administered over 1 hour twice weekly for an initial 12 weeks, followed by
41
42
43 once weekly for an additional 24 weeks (total 48 infusions in 36 weeks) [17]. Since all
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45
46 patients recently receiving L-MTP-PE on a compassionate basis have had evidence of
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48
49 biologic activity at 2 mg/m², premedication and use of the fixed 2 mg/m² dose is
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52 suggested. The preparation and infusion of L-MTP-PE has recently been validated in
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55 numerous processes, and these are reproducible and easily performed in the outpatient
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58 setting.
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7 L-MTP-PE therapy was generally well tolerated [36, 67]. The most commonly
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9
10 reported side effects are listed in Figure 3. **Most of those side effects were severe [60].**
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12 In the phase I and II studies, there was no evidence of dose-dependent [68], cumulative
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14 [36], and organ-related [68] toxicity in association with L-MTP-PE. Furthermore, the
15
16 majority of patients experienced side effects with the initial administration of the agent
17
18 [36, 69]. The major side effects of L-MTP-PE administration are fever and chills. **They**
19
20
21 **are typically transient and generally respond to the treatment [60].** The possibility of
22
23 anti-inflammatory drugs relieving these adverse effects has been investigated.
24
25
26 Premedication such as ibuprofen [70] can help prevent the severity of fever and chills.
27
28
29 However, high-dose ibuprofen (> 40 µg/ml) with L-MTP-PE down-regulates the
30
31 antitumor effect and the production of IL-1 and TNF- α in monocytes [70]. The antitumor
32
33 effect of L-MTP-PE was lost in a murine fibrosarcoma model using diclofenac [71].
34
35
36 These findings suggest that anti-inflammatory agents such as cyclooxygenase inhibitors
37
38 can down-regulate the antitumor effect of L-MTP-PE. For ibuprofen-resistant cases,
39
40 acetaminophen and/or meperidine are recommended as alternative agents [13].
41
42
43 Specifically, ibuprofen 200 or 400 mg is given as a premedication, If fever and/or chills
44
45 occur, acetaminophen (15 mg/kg; up to 1000mg) may be given. If needed more, both
46
47 acetaminophen and ibuprofen may be additionally utilized to improve the symptoms [17,
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12 **5. Expert Opinion**

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16 The orphan disease limits the number of patients available to study.
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19 Osteosarcoma is an orphan disease with fewer than 1500-2000 new cases per year
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22 diagnosed in the USA similar to Europe. Indeed, except L-MTP-PE, only IFN- α study are
23
24
25 undergoing as international cooperative trial for osteosarcoma adjuvant chemotherapy.
26
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28 The efficacy of IFN- α , which is initiated after postoperative chemotherapy, is
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31 investigated by EURAMOS 1 [40]. Unfortunately, this trial is still under the recruitment
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34 phase [19]. Therefore, L-MTP-PE, the first new agent approval for the treatment of
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37 osteosarcoma in over 20 years, is strongly expected to become 'routine' for both
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39
40 oncologists and patients with osteosarcoma. Information about potential benefit
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42
43 regarding mifamurtide use in the neoadjuvant setting (*i.e.* before surgery) and/or utility of
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45
46 L-MTP-PE in metastatic in relapsed and metastatic osteosarcoma will require analysis of
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49 expanded access and/or future clinical trials of L-MTP-PE in high burden and low burden
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52 situations.
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Figure legends

Figure 1: The molecular structure of liposomal muramyl tripeptide phosphatidyl ethanolamine (L-MTP-PE).

Figure 2: Nod2 is an intracellular MDP sensor. Monocytes/macrophages activation by L-MTP-PE is mediated *via* Nod2. L-MTP-PE is selectively phagocytosed by monocytes and macrophages after intravenous administration. L-MTP-PE is released into the cytosol and degraded to MDP. Nod2 binding to MDP activates NF- κ B and influences the innate immune response [41]. Abbreviations: Nod2; nucleotide-binding oligomerization domain 2, MDP; muramyl dipeptide, L-MTP-PE; liposomal muramyl tripeptide phosphatidyl ethanolamine, NF- κ B; nuclear factor-kappa B.

Figure 3: Tolerability of mifamurtide. Major side effects in 248 patients with advanced cancer including 51 patients with osteosarcoma [36, 59, 66-68].

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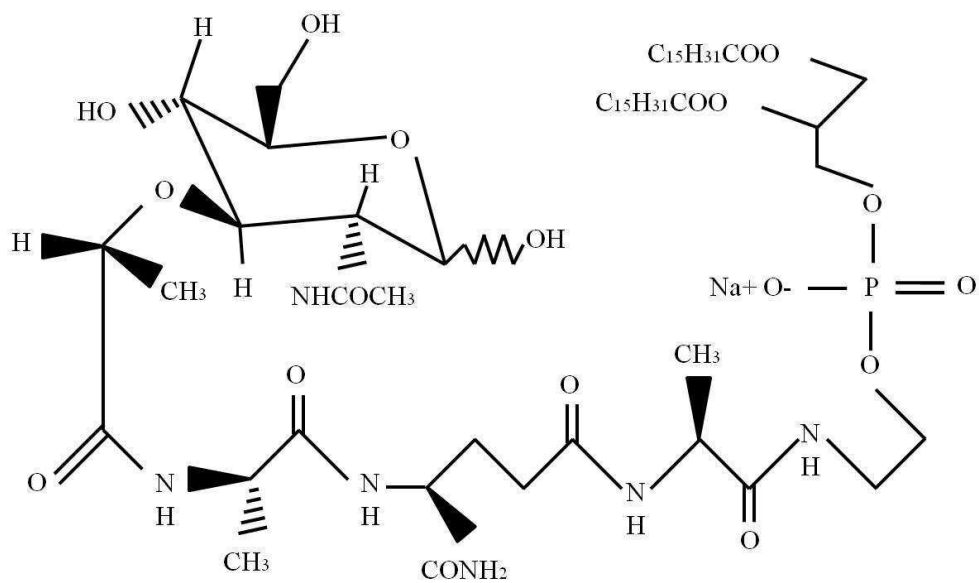
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Drug Summary Box

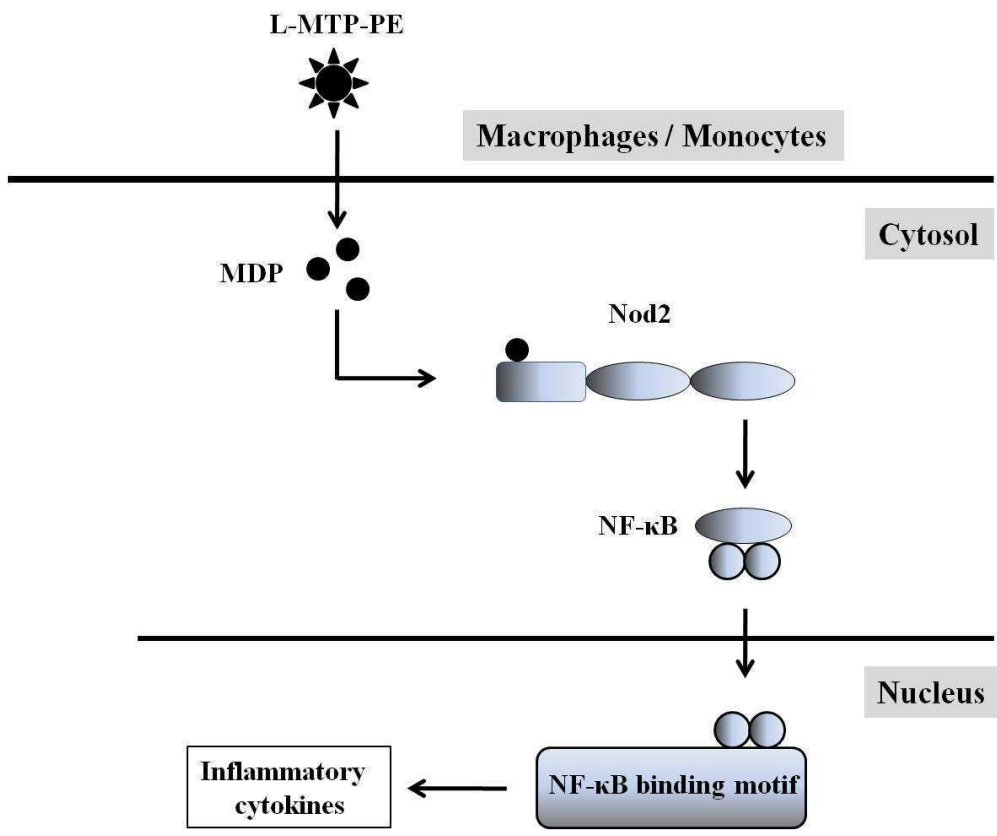
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|--------------------------|---|
| Drug name | Mifamurtide |
| Phase | Launched |
| Launched indication | osteosarcoma |
| Pharmacology description | Macrophage stimulant |
| Route of administration | Parenteral, intravenous |
| Chemical structure | C ₅₉ H ₁₀₉ N ₆ O ₁₉ P |
| Pivotal trial(s) | Phase III |

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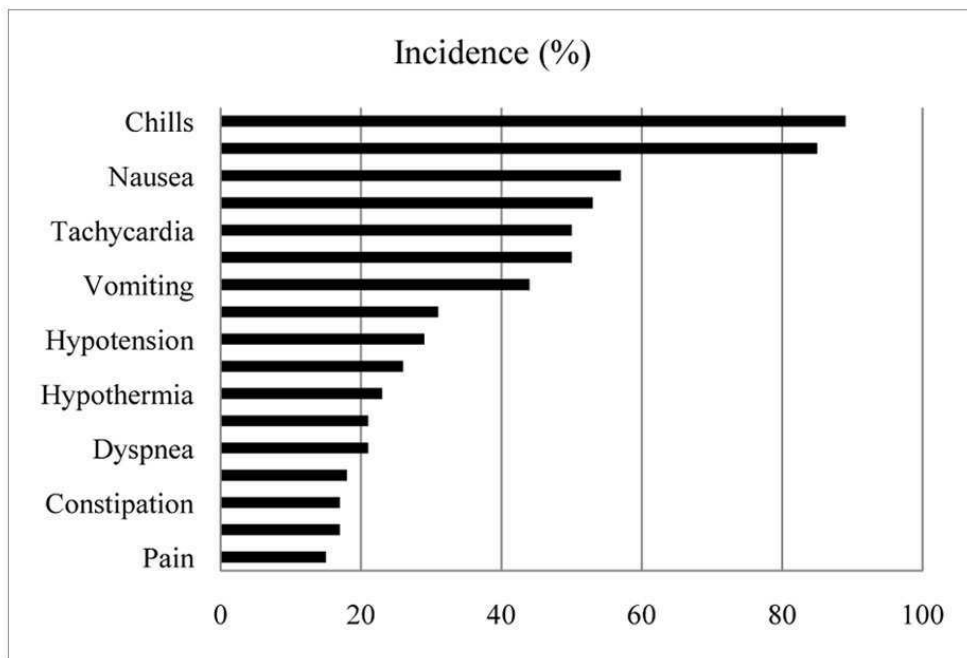


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Declaration of interest:

The authors state no conflict of interest and have received no payment in preparation of this manuscript

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