Adjuvant Therapy for Melanoma in Dogs: Results of Randomized Clinical Trials Using Surgery, Liposome-encapsulated Muramyl Tripeptide, and Granulocyte Macrophage Colony-stimulating Factor


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ABSTRACT

Spontaneous canine oral melanoma (COM) is a highly metastatic cancer, resistant to chemotherapy, and can serve as a model for cancer immunotherapy. Liposome-encapsulated muramyl tripeptide-phosphatidylethanolamine (L-MTP-PE) can activate the tumoricidal activity of the monocyte-macrophage system following i.v. injection. The objective of these studies was to evaluate the therapeutic effectiveness of L-MTP-PE administered alone and combined with recombinant canine granulocyte macrophage colony-stimulating factor (rGM-CSF) in dogs undergoing surgery for oral melanoma.

Ninety-eight dogs with histologically confirmed, clinically staged, oral melanoma were entered into two randomized, double-blind, surgical adjuvant trials. In trial 1, 50 dogs were stratified based on clinical stage and randomized to once a week L-MTP-PE or lipid equivalent (control). When all of the clinical stages were combined, no difference in disease-free survival or in survival time (ST) were detected. However, within stage I, dogs receiving L-MTP-PE had a significant increase in ST compared with control, with 80% of the dogs treated with L-MTP-PE still alive at >2 years. Within each stage II and stage III, there was no difference detected between the treatment groups. In trial 2, 48 dogs were stratified on the basis of clinical stage and extent of surgery (simple resection or radical excision), treated with L-MTP-PE two times a week, and randomized to rGM-CSF or saline (placebo) given s.c. daily for 9 weeks. Within each stage and when all of the stages were combined, there was no difference between the treatment groups. In both studies, stage I COM is associated with a better prognosis. No effect on survival was observed with regard to tumor location in the oral cavity, sex, type/extent of surgery, or age. In a subset of dogs tested, pulmonary alveolar macrophage cytotoxicity was enhanced with combined rGM-CSF and L-MTP-PE but not in dogs treated with L-MTP-PE alone.

The present study indicates that after surgery, L-MTP-PE administered alone or combined with rGM-CSF showed no significant antitumor activity in treating advanced stage COM. In early stage COM, L-MTP-PE was shown to result in a prolongation of ST. Furthermore, this study provides additional rationale for the use of the dog model for human malignant melanoma.

INTRODUCTION

There is ample evidence that the immune system can modulate the progression and metastasis of malignant melanoma. Several biological agents and approaches have demonstrated antitumor activity (1–10). The most widely used treatments include IFN-α (5, 6), IL-25 and adoptive immunotherapy

6 The abbreviations used are: IL, interleukin; DFS, disease-free survival; ST, survival time; PAM, pulmonary alveolar macrophage; MTP-PE, muramyl tripeptide-phosphatidylethanolamine; L-MTP-PE, liposome-encapsulated MTP-PE; MLV, multimellar vesicle; OSA, osteosarcoma; GM-CSF, granulocyte macrophage colony-stimulating factor; rGM-CSF, recombinant canine GM-CSF; ADCC, antibody-dependent

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Monocyte cytotoxicity was detected in 6 of the 9 patients still receiving L-MTP-PE. After the administration of L-MTP-PE, enhanced monocyte cytotoxicity was increased (32). In the other study, 18 stage III melanoma patients with resectable tumors were treated, and, although no antitumor activity was detected, 4 remain free of disease more than 5 years after surgical resection and L-MTP-PE therapy (40). Undoubtedly, the greatest antitumor activity of L-MTP-PE has been demonstrated in children with metastatic OSA leading to a Phase III randomized trial presently underway (41, 42).

Our group has focused on spontaneous canine tumors as models for immunotherapy (43). We have previously reported the antimetastatic activity of L-MTP-PE, administered alone or in combination with chemotherapy, in surgical adjuvant randomized clinical trials in dogs with spontaneous OSA (44, 45) and hemangiosarcoma (46). In over 125 dogs with OSA, L-MTP-PE was found to prolong metastasis-free and overall STs when given alone or after systemic chemotherapy. In dogs with hemangiosarcoma, L-MTP-PE administered concurrently with doxorubicin and cyclophosphamide, resulted in prolonged metastasis-free and overall STs compared with dogs receiving chemotherapy alone (46).

GM-CSF is a cytokine with a unique ability to stimulate differentiation of hematopoietic progenitor cells into dendritic cells, a potent antigen-presenting cell (47, 48). GM-CSF has received particular attention for enhancing the antitumor activity of transduced tumor cell vaccines (14, 15, 49). In vitro, GM-CSF has been shown to slightly enhance cytotoxic activity of peripheral blood monocytes and lymphocytes and to markedly increase ADCC (50, 51) and enhanced monocyte cytotoxicity against a melanoma cell line (52). In vivo administration of GM-CSF in human cancer patients enhanced monocyte ADCC (53) and increased secretion of both TNF-α and IFN (54). In another study in cancer patients, GM-CSF enhanced monocyte cytotoxicity against colon HT29 tumor cells, although no antitumor responses were seen (55).

This study was designed in a randomized manner to evaluate the efficacy of L-MTP-PE when used in a surgical adjuvant setting and administered alone or in combination with GM-CSF.

MATERIALS AND METHODS

Patient Population. Ninety-eight dogs with previously untreated, histologically confirmed spontaneous oral melanoma, without radiographic evidence of distant metastasis, were studied. The pretreatment evaluation included a complete physical examination, three-dimensional measurements of the primary tumor, a complete blood count, serum chemistry profile, and urinalysis. For evaluation of metastatic disease, regional lymph nodes were evaluated with fine needle aspiration or biopsy and radiographs of the thorax. Only dogs without evidence of overt lung metastasis, in overall good health, and a tumor (including regional lymph nodes) amenable to complete surgical removal were eligible for entry into this study. Depending on the location of the primary tumor, radiographs of the mandible or maxilla were performed under general anesthesia to determine the extent of local invasion and to assist in surgical planning. Written consent for entry into these trials was obtained from each dog’s owner before entry into this study.

Treatment. Primary treatment was surgical removal of the primary tumor (stage I and II) and regional lymph nodes (stage III). For tumors involving the gingival region—

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cellular cytotoxicity; TNF, tumor necrosis factor; COM, canine oral melanoma; BAL, bronchoalveolar lavage.
especially if bone involvement was detected in the mandible or maxilla—a partial or complete mandibulectomy or partial maxillec- tomy was performed as described previously (56, 57). The decision to perform this procedure was at the discretion of the surgeon. All of the dogs entered were considered free of clinically detectable tumor after surgery. After surgery, all of the dogs were clinically staged, according to the WHO staging system, into stage I (tumors <2-cm diameter, node negative), stage II (2- to 5-cm diameter, node negative), or stage III (>5-cm diameter and/or node positive). This was a multicenter trial in which dogs from nine veterinary facilities were entered. One investigator (I. D. K.) performed all of the randomization. Drugs for each dog entered were prepared at the University of Wisconsin-Madison and sent by overnight express mail to the participating investigator. A schematic diagram of the protocols for the two trials is shown in Fig. 1.

**Trial 1.** After surgery and stratification based on clinical stage, dogs were treated in a double-blind fashion with L-MTP-PE or lipid equivalent (MLV) once a week for 8 weeks. The liposomes were given via a slow i.v. infusion over 5–8 min. The first dose of L-MTP-PE was 1 mg/m²; all of the subsequent doses were scaled according to the weight of the dog (1 mg/m² for dogs <5 kg, 1.5 mg/m² for dogs 5–10 kg, and 2 mg/m² for dogs >10 kg). This dose of L-MTP-PE was similar to that used in previous studies (41–43).

**Trial 2.** After surgery, dogs were stratified by clinical stage. The extent of surgery was left to the discretion of the surgeon. To minimize potential bias related to the extent of surgery, dogs were further stratified on the basis of type/extent of surgery. Surgery was either radical excision (mandibulectomy or maxillec- tomy) versus simple excision (mandibulectomy or maxillec- tomy) or radical excision (mandibulectomy or maxillec- tomy). All of the dogs entered were considered free of clinically detectable tumor after surgery, although no attempt was made to remove bone. After stratification, the dogs were randomized to receive rcGM-CSF (initial dose 15 μg/kg) or saline, s.c. daily for 9 weeks. The frequency of administration of L-MTP-PE was increased from once a week (in trial 1) to two times a week using the same dosage as described for trial 1. L-MTP-PE was increased from once a week to two times a week because of the lack of therapeutic efficacy detected in stage II and stage III dogs in trial 1.

**Liposome Preparation.** Lyophilized liposomes with or without MTP-PE (CGP 19835A lipid) were provided by Ciba-Geigy, Ltd. (Basel, Switzerland). Liposomes were prepared from a freeze-dried preparation by the addition of buffered saline, without calcium or magnesium, to vials containing dioleoyl-phosphatidylserine and 1-palmitoyl-2-oleoyl-phosphatidylcholine at a 3:7 molar ratio, with (L-MTP-PE) or without (MLV) MTP-PE. The ratio of active ingredient (MTP-PE) to phospholipid was 1:250. After 1 min, the vials contents were agitated on a vortex mixer or vigorously shaken by hand for 1 min, and then the contents were diluted with buffered saline to a concentration of 25 mg lipid/ml.

**GM-CSF.** rcGM-CSF was kindly provided by Amgen, Inc. (Thousand Oaks, CA). Dogs were initially administered 15 μg/kg s.c. daily for 9 weeks, a dose that we found to enhance monocyte tumor cytostasis (58) and is well tolerated in normal experimental dogs. Dogs were started on rcGM-CSF or saline for 1 week prior to L-MTP-PE treatment and continued concurrently with L-MTP-PE for 8 weeks. The rationale to begin rcGM-CSF 1 week before L-MTP-PE was to allow for an increase in absolute monocyte count before the administration of L-MTP-PE. Because of toxicity in 10 dogs in the early phase of trial 2, the dose of rcGM-CSF was reduced to 5 μg/kg/day.

**Follow-Up Evaluation.** In both trials, dogs were evaluated with routine physical examination and thoracic radiographs at 3-month intervals. Physical and historical abnormalities were investigated as clinically indicated at each recheck visit. Patient evaluations continued as long as necessary to determine DFS and overall ST for each dog. The DFS was defined as the time from surgery to evidence of clinical recurrence or metastasis. ST was defined as the time from surgery to death or euthanasia due to advanced disease. Euthanasia was performed when requested by the dog’s owner because the animal’s quality of life was considered severely limited due to advanced disease.

**Cytotoxicity Assays.** A subset of dogs in trial 2 was tested to determine PAM cytotoxic activity after L-MTP-PE with or without rcGM-CSF. PAM cytotoxic activity was evaluated before rcGM-CSF or saline was administered, immediately prior to the first L-MTP-PE treatment and two days after the second L-MTP-PE treatment. Dogs were placed under general anesthesia and PAMs were collected via BAL, using a cold-sterilized fiberoptic bronchoscope and sterile saline according to the procedure described previously (59).

**Table 1 Patient characteristics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>L-MTP-PE</th>
<th>MLV</th>
<th>L-MTP-PE + rcGM-CSF</th>
<th>L-MTP-PE + saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>11.8 (11.6); 4.5–20</td>
<td>10 (10.2); 0.7–16</td>
<td>9.5 (9.7); 5–16</td>
<td>8.5 (9.2); 5–14</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>18 (21.5); 5–43.7</td>
<td>24.7 (23.5); 3.7–47</td>
<td>22 (24.4); 1.9–53</td>
<td>26.8 (28.4); 5.8–50</td>
</tr>
<tr>
<td>Clinical stage, n</td>
<td>I 11</td>
<td>9</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>II 8</td>
<td>11</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>III 6</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Sex, n</td>
<td>Male-intact 5</td>
<td>5</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Male-neutered 10</td>
<td>9</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Female-intact 2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Female-spayed 8</td>
<td>9</td>
<td>11</td>
<td>7</td>
</tr>
</tbody>
</table>

*Median (mean); range.*
PAMs were isolated from lavage fluid using sterile, endotoxin-free density gradient separation (Ficoll-Hypaque, specific gravity 1.077, Sigma Chemical Company, St. Louis, MO). The coculture cytotoxicity assay used has been recently described in dogs (60). Briefly, PAMs were added to wells of a 96-well microtiter plate and allowed to adhere for 90 min at 37°C in 5% CO2 humidified atmosphere. PAMs were cultured in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) supplemented with 10% heat-inactivated FCS (Intergen, Purchase, NY), and 2 mM sodium pyruvate, 2 mM l-glutamine, 10 mM HEPES buffer, 100 units/ml penicillin, and 100 μg/ml streptomycin (Sigma Chemical Company). After adherence, nonadherent PAMs were gently removed. To determine purity of the adherent cell population, cytospin preparations (Cytospin-2 Shandon Inc. Pittsburgh, PA) were made of the cells. The cytospin preparations were stained with Diff-Quick (American Scientific Products, McGaw Park, IL) and examined microscopically. After a 24-h incubation, 3H-labeled CML-6M-C2 canine melanoma cells (kindly provided by L. Wolfe, Auburn University, Auburn, AL) were added to each well in E:T ratios of 2.5:1, 5:1, and 10:1. Tumor cells were added to additional wells for the determination of spontaneous and maximal release of 3H. Cells were cocultured for 72 h, and then, the radioactivity (cpm) of 50 μl

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Analysis of DFS and overall STs</th>
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<tbody>
<tr>
<td></td>
<td>No. of dogs</td>
</tr>
<tr>
<td>Trial 1: L-MTP-PE or MLV</td>
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</tr>
<tr>
<td>All stages combined</td>
<td>25</td>
</tr>
<tr>
<td>L-MTP-PE</td>
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<tr>
<td>Stage I</td>
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<td>L-MTP-PE</td>
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<tr>
<td>MLV</td>
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<tr>
<td>Stage II</td>
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<td>L-MTP-PE</td>
<td>8</td>
</tr>
<tr>
<td>MLV</td>
<td>11</td>
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<tr>
<td>Stage III</td>
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<tr>
<td>MLV</td>
<td>5</td>
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<tr>
<td>Treatment groups combined</td>
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</tr>
<tr>
<td>Stage I</td>
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<td>L-MTP-PE</td>
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<tr>
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<tr>
<td>L-MTP-PE</td>
<td>11</td>
</tr>
<tr>
<td>MLV</td>
<td></td>
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<tr>
<td>Trial 2: L-MTP-PE or rcGM-CSF</td>
<td></td>
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<tr>
<td>All stages combined</td>
<td>24</td>
</tr>
<tr>
<td>rcGM-CSF</td>
<td>24</td>
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<tr>
<td>Stage I</td>
<td></td>
</tr>
<tr>
<td>rcGM-CSF</td>
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<tr>
<td>Saline</td>
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<tr>
<td>Stage II</td>
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<tr>
<td>rcGM-CSF</td>
<td>7</td>
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<tr>
<td>Saline</td>
<td>8</td>
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<tr>
<td>Stage III</td>
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<tr>
<td>rcGM-CSF</td>
<td>4</td>
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<td>Saline</td>
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<tr>
<td>Treatment groups combined</td>
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<td>Stage I</td>
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<tr>
<td>rcGM-CSF</td>
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<td>Saline</td>
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<td>Stage II</td>
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<tr>
<td>L-MTP-PE</td>
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<tr>
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<tr>
<td>L-MTP-PE</td>
<td>2/wk</td>
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<tr>
<td>L-MTP-PE</td>
<td>1/wk</td>
</tr>
<tr>
<td>Effect of surgery</td>
<td></td>
</tr>
<tr>
<td>Simple resection</td>
<td>62</td>
</tr>
<tr>
<td>Radical surgery</td>
<td>36</td>
</tr>
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</table>

*Median not yet reached.*
of supernatant from each well was determined. Percent cytotoxicity of CML-6M-C2 cells was calculated by the following formula:

\[
\frac{T - S}{M - S} \times 100
\]

where \( T \) = mean cpm of supernatant from wells containing tumor and effector cells, \( S \) (spontaneous release) = mean cpm of supernatant from wells containing tumor cells alone, and \( M \) (maximal release) = mean cpm of lysate from wells containing tumor cells alone.

**Anesthesia for the BAL.** Dogs were premedicated with midazolam (0.2 mg/kg, maximum of 5 mg, s.c.) and butorphanol (0.2 mg/kg, maximum of 10 mg, s.c.) 30 min before anesthesia. Anesthesia was induced by injectable thiopental sodium (12–15 mg/kg) and maintained on isoflurane and 100% oxygen, using an endotracheal tube. A bronchoscope was passed through the larynx, and the BAL was performed as described previously (59).

**Statistical Analysis.** In designing trial 1 in dogs with oral melanoma, the expected median ST for surgery alone (all of the stages combined) is 8 months (18, 23); we anticipated a 40% increase in the median survival in dogs treated with L-MTP-PE. With 25 dogs per arm, we should be able to detect that difference with an 80% power at the \( P = 0.05 \) level. For trial 2, we expected a median ST of 11 months for surgery and L-MTP-PE, and we anticipated a 40% increase in the median survival with the addition of rcGM-CSF. With 25 dogs per arm, we would be able to detect that difference with an 80% power at the \( P = 0.05 \) level.

DFS and overall ST were compared between the treatment groups of both trials. STs were also compared between dogs receiving L-MTP-PE once a week in trial 1 and dogs receiving L-MTP-PE two times a week in trial 2. Survival curves were generated by the Kaplan-Meier method and were compared using the Breslow and Mantel-Cox tests of significance between survival curves (61–63). These statistical tests adjust for dogs still alive or lost to follow-up at the time of analysis. A \( P \) of less than 0.05 was considered significant.

Differences in percent cytotoxicity were assessed using paired \( t \) tests. For results covering a range of E:T ratios, the highest \( P \) is reported.

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**Fig. 2** DFS (A) and ST (B) for dogs in trial 1 treated once weekly with L-MTP-PE or MLV. There was no difference in survival between the treatment groups.

**Fig. 3** DFS (A) and ST (B) for dogs in trial 1 with stage I oral melanoma, treated once weekly with L-MTP-PE or MLV. Dogs that received L-MTP-PE had significantly prolonged ST compared with dogs that received MLV; \( P < 0.045 \). There was no significant difference between treatment groups with regard to DFS.
RESULTS

Patient Characteristics. Patient characteristics for all of the dogs entered into both trials are given in Table 1.

Treatment and Survival Data. The L-MTP-PE treatments were well tolerated by the dogs. The only consistent side effect was an elevation in body temperature (1–2°C), lasting 1–4 h after the liposome treatment. The temperature elevation was more pronounced in the L-MTP-PE groups. Body temperature returned to normal within 6 h after injection. The fever response was most apparent on the first few treatments and then subsided on subsequent treatments. In trial 2, the initial dosage for rcGM-CSF was 15 mg/kg s.c. daily. However, 10 dogs experienced adverse side effects that included thrombocytopenia (n = 4), anterior uveitis (n = 2), lethargy and mild diarrhea (n = 2), gastritis (n = 1), and polydipsia/polyuria (n = 1). After documentation of these adverse side effects, all of the subsequent dogs were treated at a dose of 5 μg/kg. Table 2 summarizes the median DFS and ST for dogs according to clinical stage and treatment group for both trial 1 and 2.

Trial 1. Fifty dogs were entered into this clinical trial. Twenty-five were randomized to L-MTP-PE and 25 to MLV. When all of the stages were combined, there was no difference between dogs treated with L-MTP-PE versus placebo (Fig. 2). However, within stage I, dogs receiving L-MTP-PE had significantly prolonged ST (P < 0.05) compared with those receiving MLV (Fig. 3). In the L-MTP-PE group, 80% of the dogs were alive at 2 years compared with 25% in the MLV group. The statistical difference detected is based on 11 dogs treated with L-MTP-PE and 9 dogs treated with MLV (control). Increasing the patient population with stage I COM would increase the power of this observation. However, the 25% 2-year ST for the stage I dogs treated with MLV is similar to previous ST for dogs treated by surgery alone (23).

When treatment groups were combined, stage I (n = 20) had significantly prolonged (P < 0.022) DFS and ST compared with stage II (n = 19) and stage III (n = 11). The prognostic significance of the clinical staging system confirms similar findings in a previously published study (23). No effect on DFS or ST was observed with regard to the location of the primary tumor, type of surgery, sex, or age (data not shown).

Trial 2. Forty-eight dogs were entered into this clinical trial. All of the dogs received L-MTP-PE two times a week, and 24 were randomized to receive rcGM-CSF and 24 to receive saline. Within each stage and with all of the stages combined,
PAM cytotoxicity was detected (Fig. 6).

Cytotoxic activity against CML-6M-C2 was a mean of 13% and increased to 68% after rcGM-CSF and L-MTP-PE administration. In the group of dogs receiving L-MTP-PE and saline (B, n = 2), PAM cytotoxic activity was assessed before treatment (Pretreatment), at day 7 of rcGM-CSF or saline treatment (before L-MTP-PE), and two days after the second L-MTP-PE treatment (Post L-MTP-PE). Cytotoxic activity was significantly enhanced by L-MTP-PE in the dogs that received rcGM-CSF. This effect was not observed in the dogs that received saline.

There was no difference in DFS or ST when dogs receiving L-MTP-PE and rcGM-CSF were compared with dogs receiving L-MTP-PE and saline (Fig. 4). No effect on DFS or ST was observed with regard to the location of primary tumor, type of surgery, sex, or age (data not shown).

**Trials 1 and 2 Combined.** Comparison of dogs receiving L-MTP-PE once a week (trial 1) or two times a week (trial 2) showed no difference in DFS or ST with regard to frequency of L-MTP-PE (Fig. 5). Further analysis on type of surgery (simple versus radical), revealed no significant difference in DFS or ST (Table 2).

**Cytotoxicity Assays.** In 5 dogs, PAMs were collected by BAL and were assayed for cytotoxic activity against the canine melanoma cell line, CML-6M-C2, before and after rcGM-CSF (n = 3) or saline (n = 2) and L-MTP-PE. Mean (±SE) levels for percent cytotoxicity are shown in Fig. 6. Cytotoxic activity was markedly increased (P = 0.053) in the rcGM-CSF group after receiving L-MTP-PE. At a 10:1 Et ratio for PAMs:tumor target, the pretreatment cytotoxicity against CML-6M-C2 was a mean of 13% and increased to 68% after rcGM-CSF and L-MTP-PE administration. In the group of dogs receiving L-MTP-PE plus saline, no increase in PAM cytotoxicity was detected (Fig. 6).

**DISCUSSION**

The present surgical adjuvant therapy for stages I, II, and III (American Joint Committee on Cancer) melanoma is the use of high-dose IFN-α (10). Before the approval of IFN-α, a large number of randomized trials evaluated the use of nonspecific bacteria immunostimulants in the surgical adjuvant setting. Bacille Calmette-Guerin, the bovine mycobacterium used as a tuberculosis vaccine, has been extensively tested (2, 64, 65). A total of 13 published trials involving 1152 patients have been reported (10). None of these randomized controlled studies have shown any significant impact on relapse-free or overall survival in the treated patients. A heat-killed preparation of Corynebacterium parvum (Propionobacterium acnes) has been shown to provide a slight survival advantage (3); however, other trials did not show any significant reduction in metastasis as evidenced by an increase in ST, with 80% having long-term survival (>2 years). However, this difference was seen only in stage I, and the number of dogs randomized within this stage was small (n = 20). Although only 9 dogs were treated with MLV (surgery alone), our previous studies have shown that stage I dogs with COM that are treated with surgery alone have a similar median ST (23). In trial 2, all of the dogs were treated with L-MTP-PE, and the median ST for stage I dogs was 622 days, which suggests a greater therapeutic efficacy than other studies using surgery alone (18, 23).

An important change made between trial 1 and trial 2 was in increasing the administration of L-MTP-PE from once a week to two times a week. This change was made to determine whether two-times-a-week treatment would increase the therapeutic response in stage II and III dogs. Results from trial 2 demonstrated that increasing the treatment frequency to two times a week did not enhance the therapeutic activity of L-MTP-PE. In previous studies using rodent models, two-times-a-week treatments were selected because L-MTP-PE was shown to activate macrophage tumoricidal function for 48–72 h, and two-times-a-week treatment would maintain that activation (25, 69). In comparing the results of trials 1 and 2, no difference between once- or twice-weekly L-MTP-PE administration was seen in dogs with stage II or stage III disease.

Another interesting finding from this study was the demonstration that the extent of surgery (simple resection versus radical resection) had no significant influence on DFS and ST (Table 2). Other reported studies in COM using radical surgery.
Adjuvant Immunotherapy for Canine Melanoma

The effect reported is thrombocytopenia due to activation of the immune system. However, it has also been reported that GM-CSF is important in the maturation of bone marrow-derived dendritic cells (48, 76). Our rationale for combining systemic GM-CSF with the GM-CSF gene is to increase the monocyte/macrophage population; (b) to enhance monocyte/macrophage tumoricidal activity; and (c) to increase antigen presentation by dendritic cells, the most potent antigen-presenting cells in the immune system. However, it has also been reported that GM-CSF may activate suppressor mechanisms to regulate the antitumor response negatively (77, 78) or enhance the tumorigenicity of some tumor cells (79, 80). Our study did not demonstrate any therapeutic advantage of GM-CSF over L-MTP-PE alone. However, the total number of dogs entered (n = 48) into trial 2 may have been too small to detect a difference between each treatment group.

In experimental dogs, doses of 50 μg/kg/day have been studied for hematological activity, and the most common side effect reported is thrombocytopenia due to activation of the monocyte/macrophage system resulting in a decrease in platelet survival (81, 82). In this study, we selected a dose of 5 μg/kg (150 μg/m² s.c. daily) because of toxicity detected at 15 μg/kg (450 μg/m²). In human clinical trials, the doses tested for GM-CSF (when used systemically to activate monocytes or to enhance ADCC) have ranged from 15 to 500 μg/m²/day (48, 54, 55, 83) with 150–250 μg/m² being the most effective dose.

In summary, L-MTP-PE administered alone or combined with GM-CSF has minimal antitumor activity when administered in a surgical adjuvant setting. However, there is suggestive evidence that L-MTP-PE results in the prolongation of survival in dogs with early (stage I) malignant COM. Furthermore, this study helps to strengthen the rationale for the use of the dog as a model for human spontaneous malignant melanoma.

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