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Abstract

Canine mammary tumours are generally treated with surgery alone, despite the fact that 50% of them are malignant and many will eventually lead to recurrence or metastases. A prospective clinical trial in which dogs with aggressive mammary carcinoma of clinical stages IV and V were treated with surgical excision (n = 9) or with surgery and adjuvant weekly gemcitabine (n = 10) for at least four cycles was conducted. Gemcitabine was given as an intravenous infusion at the dose of 800 mg m⁻². Aim of the study was to explore potential beneficial effects of gemcitabine on time to local recurrence (TTR), time to distant metastases (TTM) and overall survival (OS) in canine patients with operated mammary tumours bearing high risk for locoregional failure and distant metastases. Also, factors associated with OS, including neutering status, body weight, age, clinical stage at presentation, tumour size, histological grade and, in dogs receiving chemotherapy, the number of gemcitabine treatments, were investigated. Finally, acute toxicities related to chemotherapy and quality of life were assessed in dogs receiving gemcitabine. Dogs treated with surgery alone or surgery followed by gemcitabine had no difference in TTR, TTM or OS (P > 0.05). In the group of dogs receiving adjuvant chemotherapy, the number of gemcitabine treatments was positively correlated with OS (P = 0.017). Gemcitabine treatment was well tolerated, with no dogs experiencing clinically relevant haematological or gastrointestinal toxicity. Despite being safe at the present dose, gemcitabine chemotherapy as an adjunct treatment to surgical excision may not be recommended in dogs with aggressive mammary carcinoma.
ADJUVANT GEMCITABINE AFTER SURGICAL REMOVAL OF AGGRESSIVE MALIGNANT MAMMARY TUMORS IN DOGS.

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Abstract
Canine mammary tumors are generally treated with surgery alone, despite 50% of them are malignant and many will eventually lead to recurrence or metastases. A prospective clinical trial in which dogs with aggressive mammary carcinoma of clinical stages IV
and V were treated with surgical excision (n=9) or with surgery and adjuvant weekly gemcitabine (n=10) for at least 4 cycles was conducted. Gemcitabine was given as an intravenous infusion at the dose of 800 mg/m². Aim of the study was to explore potential beneficial effects of gemcitabine on time to local recurrence (TTR), time to distant metastases (TTM), and overall survival (OS) in canine patients with operated mammary tumors bearing high-risk for locoregional failure and distant metastases. Also, factors associated with OS, including neutering status, body weight, age, clinical stage at presentation, tumor size, histological grade and, in dogs receiving chemotherapy, the number of gemcitabine treatments, were investigated. Finally, acute toxicities related to chemotherapy and quality of life were assessed in dogs receiving gemcitabine. Dogs treated with surgery alone or surgery followed by gemcitabine had no difference in TTR, TTM or OS (p>0.05). In the group of dogs receiving adjuvant chemotherapy, the number of gemcitabine treatments was positively correlated with OS (p=0.017). Gemcitabine treatment was well tolerated with no dogs experiencing clinically relevant hematologic or gastrointestinal toxicity. Despite safe at the present dose, gemcitabine chemotherapy as an adjunct treatment to surgical excision may have limited beneficial effects in dogs with aggressive mammary carcinoma.

Key words
Mammary carcinoma, chemotherapy, gemcitabine, surgery, dog

Introduction
Mammary gland carcinomas are a very common malignancy in adult bitches. In dogs, approximately 50% of mammary gland tumors are malignant, and 50% of these tend to infiltrate the surrounding tissues and metastasize to regional lymph nodes and lungs. Furthermore, different histological subtypes can be frequently found in different mammary glands, some of which correlate with clinical behavior. Surgical excision is the main treatment option for mammary gland tumors; however, aggressive tumors are rarely cured by surgery alone, and affected dogs may ultimately die or be euthanized because of distant metastases. Due to the poor outcome in many dogs, adjuvant treatments are clearly warranted. Chemotherapy has been rarely attempted in dogs with aggressive malignant mammary tumors and scarce data has been published on post-operative adjuvant treatments. At present, a standard of care for adjuvant chemotherapy has not been established.

In human patients with breast cancer, mastectomy alone is often disappointing. Thus, adjuvant systemic therapy following local treatment is used in order to eradicate micrometastases and ameliorate disease-free interval and overall survival. Currently, adjuvant chemotherapy is offered to patients with node-positive tumors or with high-grade tumors and to those with metastatic disease. Chemotherapeutic drugs that have shown efficacy in the management of breast cancer include anthracyclines (such as doxorubicin or epirubicin), taxanes (such as paclitaxel or docetaxel), and the newly introduced vinorelbine, capecitabine, and gemcitabine in both pretreated and unpretreated patients, either as single agents or in combination regimens. In particular, gemcitabine, a pyrimidine nucleoside antimetabolite, has emerged as one of the most promising new cytotoxic agents, because of its proven antitumor activity in a variety of solid tumors, its good toxicity profile and no apparent multidrug resistance.
Gemcitabine is not widely used in veterinary medicine, and few articles are available in the literature. To the authors’ knowledge, adjuvant gemcitabine chemotherapy for resected mammary cancer care has not been documented in dogs. The aims of the study were i) to verify the potential beneficial effects of gemcitabine and surgery over surgery alone on time to local recurrence (TTR), time to distant metastases (TTM) and overall survival (OS) in dogs with mammary carcinoma; ii) to assess factors contributing to OS in either group of dogs; and iii) to assess the safety of gemcitabine administration and quality of life in dogs receiving adjuvant chemotherapy.

**Materials and methods**

**Patient eligibility**

Following radical unilateral or bilateral mastectomy, depending on tumor distribution in the mammary chains, canine patients were eligible for recruitment only if they had selected malignant histological variants, including simple carcinoma, squamous cell carcinoma, sarcoma and carcinosarcoma with infiltrative growth in the stroma and residual neoplastic cells at the primary surgical site. Inflammatory mammary carcinoma was excluded. In addition, to be included in the study, dogs with mammary tumors had to exhibit one or more of the listed features: (1) vascular and/or lymphatic invasion, (2) metastases to regional lymph nodes, and (3) distant metastases. In cases of multiple mammary tumors, the most malignant tumor was considered. All dogs were staged accordingly to the WHO clinical staging system for canine mammary tumors. For dogs undergoing chemotherapy, additional entry requirements included fully informed,
written consent from the pet owner, the presence of adequate bone marrow function, as evidenced by neutrophil counts of $\geq 3,000/\mu l$, platelet counts of $\geq 120,000/\mu l$, and packed cell volume $\geq 30\%$, and administration of at least 4 doses of gemcitabine. Dogs were enrolled irrespective of hormone receptor status. Dogs affected by coexistent non-mammary malignancies, and those which had received previous chemotherapy or hormonal therapy were not enrolled.

Post-operative chemotherapy was offered to all dogs. If owners rejected adjuvant treatment, dogs were included in Group 1. Dogs whose owners wished to pursue chemotherapy were included in Group 2.

**Pre-treatment evaluation**

Before entering the study, all patients underwent staging work-up including a complete history and physical examination, bidimensional measurement of the tumors, cytologic evaluation of the mammary tumor and of regional lymph nodes, complete blood cell count (CBC), serum biochemical profile, urinalysis, abdominal ultrasound, and thoracic radiographs (right and left lateral, and ventrodorsal views).

Radical unilateral mastectomy was performed if only one mammary chain was involved, and bilateral mastectomy if either mammary chain was involved. For staging purposes, regional lymphadenectomy was performed in all dogs.

**Histological assessment**

Tissue samples were routinely fixed in 10% neutral buffered formalin and embedded in paraffin. Five-micron sections were taken, stained with hematoxylin and eosin (H&E) and examined by light microscopy. Histological assessment of mammary tumors and
draining lymph nodes was performed by two independent pathologists (FA, AR) according to the World Health Organization’s (WHO) classification scheme\textsuperscript{32}. The following data were recorded: histological diagnosis, status of the resection margins, presence of stromal infiltration, vascular-lymphatic invasion, and grade of the tumor determined on the basis of previously described guidelines.\textsuperscript{5} Hormone receptor status was not evaluated.

**Post-operative chemotherapy plan**

Patients were scheduled to start chemotherapy one week after mastectomy. Gemcitabine (Gemzar, Eli Lilly and Company, Sesto Fiorentino, Firenze, Italy) was administered intravenously (IV) at a dose of 800 mg/m\textsuperscript{2} once weekly for at least 4 cycles. The dose used in this study was derived from a phase I clinical trial in 33 dogs with transitional cell carcinoma of the urinary bladder\textsuperscript{33}. After reconstitution of one 200 mg gemcitabine vial with 5 ml of 0.9\% sodium chloride, a concentration of 38 mg/ml was obtained. Reconstituted gemcitabine was then added to a 100 ml 0.9\% saline bag and administered over a period of 30 to 60 minutes via an indwelling catheter through a peripheral vein.

Chemotherapy was prepared within a class II biologic safety cabinet (Cytosafe-N 2003, Faster, Ferrara, Italy). To reduce occupational exposure, chemotherapy was administered by safe handling techniques and personal protection equipment. After chemotherapy administration, all used materials were disposed in special boxes accordingly to safety guidelines.

All patients were given prophylactic 25 mg kg\textsuperscript{-1} BID oral clavulanate-potentiated amoxicillin\textsuperscript{6} (Synulox tablets 500 mg, Pfizer, Rome, Italy) for 7 days after each of the
first 3 treatments, and only if needed thereafter. Standard antiemetic therapy with SQ 0.4 mg kg\(^{-1}\) metoclopramide (Plasil tablets 10 mg, Lepetit, Milan, Italy) was administered if necessary. Additional experimental drugs were not administered during the study.

During the chemotherapeutic period, dogs were seen weekly as outpatients for clinical assessment, to record adverse events and toxicities as reported by the owner, to measure hematological and, when indicated, biochemical parameters, and finally to administer gemcitabine. Furthermore, thorax radiographs were obtained after 2 and 4 gemcitabine treatments, based on the presence or absence of lung metastases at presentation, respectively. Thoracic radiographs were also obtained at the end of treatment in all patients. Chemotherapy was suspended if intolerable toxicity occurred or beneficial effects were not evident on local recurrence or distant metastases. Additional gemcitabine administrations, up to a maximum of 10, were administered to patients exhibiting complete remission or, in cases of pulmonary metastases, if respiratory symptoms had improved. Toxicity resulting from gemcitabine was assessed based on the dog history, physical examination and CBC before the beginning of each next cycle, as stated by the Veterinary Co-operative Oncology Group\(^{34}\). If treatment had to be delayed, the CBC was repeated every 2 days in order to resume chemotherapy as soon as possible. A new cycle at full dose was only started if neutrophils were >3.000/\(\mu\)l, platelet count was >120.000/\(\mu\)l, and the non-hematologic toxicity grade was ≤1. Dose-limiting toxicity was defined as grade 3 or 4 hematologic or gastrointestinal toxicity. The safety analysis was performed on data from all patients who received gemcitabine.

**Follow-up**
After surgical excision (Group 1) or completion of chemotherapy (Group 2), dogs were scheduled to be checked once monthly for three months, and every three months thereafter. Follow-up examination included physical examination with particular attention to local recurrence, CBC, serum biochemical profile, abdominal ultrasound, and thoracic radiographs (three views).

**Statistical analysis**

Data analysis was performed with GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA). For both groups, survival time, TTM (beyond regional lymph nodes), and TTR were explored with the Kaplan-Meier product limit method followed by logrank test. In either group, timing was considered from surgical excision. In the analysis of survival, distant metastasis and local recurrence, dogs were censored if they were alive at the time of data accrual closure. For time to distant metastases and local recurrence dogs were also censored if, by the last examination, distant metastases had not developed or the tumor had not reappeared locally, respectively. In the two groups, the following variables were evaluated for their influence on survival time: neutering status, body weight, age, clinical stage at presentation, tumor size at surgery (measured at its maximum diameter), histological grade and, in dogs receiving chemotherapy, the number of gemcitabine treatments. Correlations were not investigated for TTM and TTR because of the low number of dogs which developed metastasis or local recurrence available for analysis. Spearman’s correlation coefficient was used to measure the strength of relationships. Significance was set at a $p$ value of $\leq 0.05$. 
Results

Patient characteristics

Nineteen dogs with histologically confirmed malignant mammary gland tumors were enrolled. Among these, 9 dogs were treated with surgery alone (Group 1), whereas the remaining 10 received post-operative adjuvant gemcitabine (Group 2). All patients were treated at the Clinica Veterinaria L’Arca between October 2003 and October 2006.

In Group 1, 6 breeds were represented, including crossbreeds (n=4), and one each of the following: shi-tzu, German shepherd, German pointer, poodle, and Yorkshire terrier. Median age at presentation was 12 years (range, 8-15), and median body weight was 18.2 kg (range, 4.2-34.3). Six dogs (66.7%) were intact females, and the remaining 3 had been spayed at the ages of 10, 11 and 13 years, respectively. Unilateral radical mastectomy was performed in 3 dogs (33.3%); whereas bilateral mastectomy was performed in the remaining 6 dogs (66.7%), and 4 out of the 6 intact bitches were ovariohysterectomized at the time of surgery.

In Group 2, 7 breeds of dogs were represented. Mixed dogs were the most common (n=4; 40%), followed by one each of the following: Yorkshire terrier, beagle, German shepherd, shi-tzu, English setter and White West Highland terrier. Median age at diagnosis was 10 years (range, 8-15), and median body weight was 15 kg (range, 4.75-34.95). Five dogs (50%) were intact females and 5 dogs were spayed. All dogs had been spayed between the age of 9 and 15 years. Unilateral radical mastectomy was performed in 6 dogs (60%), whereas surgery consisted in bilateral mastectomy in the remaining 4 bitches (40%). The 5 intact dogs were ovariohysterectomized at the time of surgery.
Tumor characteristics

Group 1

At presentation, the median number of tumors per dog was 3 (range, 1-6) and the median size of the major tumor nodule at its maximum diameter was 4.2 cm (range, 0.9-20). When histological type was considered, all dogs but one (88.9%) had simple carcinoma, 3 of which were tubulopapillary, 2 were tubular, 2 were anaplastic and 1 was solid. One dog (11.1%) had a carcinosarcoma. The tumors were also graded according to the degree of stromal infiltration, vascular-lymphatic invasion and distant metastases, as follows: 8 (88.9%) tumors were grade 2, and one (11.1%) was classified as grade 3. Residual neoplastic cells were noticed at the primary surgical site in all dogs. According to WHO clinical staging system for canine mammary tumors, 7 (77.8%) of the bitches had stage IV disease and 2 (22.2%) had stage V. The distant metastatic site for both dogs with stage V disease was the lungs. Eight dogs (88.9%) had regional lymph node involvement; of these, 7 were inguinal and 1 was axillary. Overall, all 9 bitches had metastatic disease at presentation, and 2 of these had pulmonary involvement.

Group 2

In this group, the median number of tumors per dog was 2 (range, 1-6) and the median size of the major tumor nodule at its maximum diameter was 3.7 cm (range, 1.7-6). Concerning histological type, 8 bitches (80%) had simple carcinoma, 3 of which were anaplastic, 2 were solid, 2 were tubulopapillary and one was tubular. In addition, there was one carcinosarcoma and one adenosquamous carcinoma. The tumors were histologically graded as follows: 6 tumors (60%) were grade 2, and 4 tumors (40%)
were grade 3. Histologic evidence of residual tumor at the primary surgical site was noted in all dogs.

At diagnosis, 6 dogs (60%) had stage IV disease and 4 (40%) had stage V. The metastatic site in the dogs with stage V disease was the lungs, whereas the histologically involved lymph nodes were the inguinal in 7 dogs, the axillary in one dog, and both, the inguinal and the axillary, in one dog. Overall, all 10 dogs had metastatic disease at the beginning of gemcitabine chemotherapy, and 4 of these had pulmonary involvement.

Chemotherapy

Chemotherapy was started between 7 and 14 days after mastectomy in 10 dogs. A total of 61 gemcitabine treatments were administered at the dose of 800 mg/m$^2$. Dogs received from 4 to 10 gemcitabine treatments (median, 6.1). All patients completed the planned four cycles of chemotherapy, and 7 of them (70%) received 1-6 additional treatments.

Response and time-to-event measures

The median follow-up time (from surgery to last visit) was 178 days (range, 17-410) and 203 days (range, 52-659) for Group 1 and 2, respectively.

Overall, 4 (44.4%) out of 9 dogs with no postoperative gemcitabine developed local recurrence (Group 1). Median time to local recurrence was 175 days (range, 150-345). None of the 10 dogs that received adjuvant chemotherapy developed local recurrence (Group 2). Time to local recurrence was not different between groups.

In Group 1, 4 dogs had distant metastases. In particular, 2 dogs showed pulmonary metastases at presentation, and 2 others developed pulmonary metastases after 212 and
216 days, respectively. In Group 2, 5 dogs that received gemcitabine had distant metastases, 4 of which had pulmonary metastases at presentation with signs of respiratory distress, and one developed peritoneal carcinomatosis 52 days after beginning of chemotherapy. One of the dogs with lung metastases developed histologically confirmed bone metastases to the spine 55 days after beginning of chemotherapy. In the 4 dogs with pulmonary involvement and respiratory signs, the owners reported improvement of clinical signs after gemcitabine treatment. Moreover, in one of these dogs a size reduction of the pulmonary metastases was observed for 40 days of gemcitabine treatment, as assessed by measurements of tumoral lesions on chest films (Fig. 2). However, despite initial amelioration in these 4 dogs, all were eventually euthanized due to disease progression, after 55, 70, 195 and 210 days, respectively.

Time to distant metastases was not different between the two groups.

At the time of data analysis closure, 8 dogs were still alive, whereas the remaining 11 had died as a result of their mammary disease. In particular, 6 out of the 9 dogs which had not been treated in an adjuvant setting had died by the end of the study period, and 5 out of 10 dogs died in the group receiving adjuvant gemcitabine. With Kaplan-Meier analysis, in Group 1 a median survival time of 212 days was calculated and in Group 2 median survival was 200 days. The cause of death for animals in Group 1 was local recurrence (n=2), and development or progression of pulmonary metastases (n=4). Two of the 3 dogs that survived had evidence of local recurrence and the last one had no evidence of disease. Among dogs in Group 2, all 5 animals that did not survive had distant metastases. The 5 survivors had no evidence of local recurrence or of distant metastases, at 172, 405, 415, 656, and 659 days. Furthermore, none of the 5 dogs with lymph node metastases at presentation experienced disease progression. OS did not
differ between dog treated with surgery alone or with surgery and adjuvant gemcitabine (Fig. 1).

Results of treatments for Group 1 and 2 are summarized in Table 1 and Table 2, respectively.

Regarding factors associated with OS, a significantly positive correlation was documented for the number of gemcitabine treatments in dogs receiving adjuvant chemotherapy (p=0.017). No correlations were found for other analyzed variables.

**Toxicity**

All 10 patients receiving gemcitabine chemotherapy were evaluated for toxicity. Treatment was well tolerated and no dose reduction was necessary during the study period. No dog required treatment interruption. Most of the treatments (57 of 61, 93%) were administered every week, as planned. In 4 cases treatment delays were necessary due to grade 1 neutropenia, which resolved uneventfully after 3, 4, 4 and 5 days, respectively, by adding oral 5 mg kg$^{-1}$ SID enrofloxacin (Baytril tablets 150 mg, Bayer, Milan, Italy) for one week with no need for hospitalization. All treatment delays occurred after the first 4 courses of chemotherapy. Grade 2 to grade 4 hematologic toxicity was not reported during the study.

Non-hematologic toxicity occurred in very rare cases and was limited to grade 1-2 gastrointestinal adverse events. One dog experienced nausea without alteration in eating habits for 24 hours after each cycle, whereas another dog had one episode of loss of appetite of 36 hours duration and 3-5 episodes of vomiting in 48 hours. Further non-hematologic toxicities were not observed during the study. Chemotherapy did not affect wound healing in any case.
Discussion

Much interest has focused on the treatment of mammary carcinoma in dogs. Until recently, mammary carcinoma in bitches was viewed as a surgical issue and no medical treatment was recommended.\(^1,3\) However, approximately 50% of mammary tumors behave aggressively and will ultimately locally recur and progress to metastatic disease, suggesting that occult metastases are already present when dogs first present with operable mammary cancer.\(^1,2\) As a consequence, surgical excision alone in most instances is not curative and, to improve outcome, adjuvant chemotherapy should be explored in clinical trials. Adjuvant chemotherapy in dogs that have undergone mastectomy may prove effective to treat occult regional or systemic disease (such as lymph node or pulmonary micrometastases), palliate evident distant metastases, and sterilize tumor margins in case of incomplete surgical excision.

Recently, the use of adjuvant chemotherapy for the treatment of resected canine mammary tumors has been reported.\(^7,8\) In an early study, 8 bitches with malignant mammary tumors were treated in an adjuvant post-operative setting with 5-fluorouracil and cyclophosphamide.\(^7\) When compared with bitches treated by surgery alone, the authors found a benefit in survival time if chemotherapy was added.\(^7\) More recently, 12 dogs with invasive mammary gland tumors were treated with doxorubicin or docetaxel after mastectomy; however, outcome was not improved by chemotherapy.\(^8\)

In people, gemcitabine has shown activity in a variety of solid tumors,\(^35-39\) has limited toxicity, and does not exacerbate toxic effects of other chemotherapeutic drugs.\(^40\) Moreover, among the novel chemotherapeutic drugs, gemcitabine has emerged as an
important agent in the treatment of breast cancer in women.\textsuperscript{22,24,26,41-44} The efficacy and tolerability of this chemotherapeutic drug, as well as its lack of cross-resistance with anthracyclines and taxanes, have led to its inclusion in combination regimens.\textsuperscript{45-48}

Motivated by the favorable results in humans with aggressive breast cancer, and the good tolerability shown in canine patients,\textsuperscript{33} we designed a clinical protocol with gemcitabine given as a single agent to dogs with aggressive mammary cancer. In the present study, the analysis of post-operative adjuvant therapy with gemcitabine showed prolongation of the recurrence-free survival over no adjuvant chemotherapy. In particular, none of the dogs receiving adjuvant gemcitabine developed local relapse, whereas 4 out of the 9 untreated dogs experienced local recurrence. Although some increase in local control rate was observed in this study, no clear advantage in terms of TTM and OS was achieved for adjuvant chemotherapy over surgery alone. These results may be partly attributable to the presence of clinically evident pulmonary metastases in 4 out of the 10 treated dogs. According to the Goldie and Coldman model,\textsuperscript{49} the number of resistant tumor cells increases directly with the size of the tumor, thus accounting for the possibility of resistant cell lines if widespread macro-metastatic disease is already present. Another possible reason is that this study had an insufficient follow-up interval such that an OS advantage could not be discerned. In general, the effect of an adjuvant therapy should be considered in terms of both disease-free interval and OS, and the potential benefits of treatment need to be carefully balanced against its potential side effects. We believe that the improvement in disease-free interval achieved in the treated dogs may ultimately translate into a survival benefit as well. This consideration is supported by the fact that none of the node-positive dogs treated with chemotherapy experienced disease progression, suggesting that gemcitabine may have eradicated
micrometastases. Of note, one of the dogs with stage V disease experienced a reduction in size of lung metastases. As a consequence, it can be assumed that systemic therapy given to selected dogs with operable mammary tumors in their early-stage disease may further improve the long-term outlook. At present, it is well known that the extent of disease and several histopathological features partially reflect the biologic behaviour of canine mammary tumors.\textsuperscript{32,50-53} Hopefully, a more detailed understanding of the molecular biology of canine mammary cancer will ultimately lead to the identification of the subset of cancer patients who would most benefit from the use of chemotherapy in an adjuvant setting.

Limitations of this study are the low number of recruited patients in both groups, partly due to the strict enrolment criteria and the relatively short follow-up achieved in some dogs. We hypothesize that the patient number was too low to detect statistical differences among groups, and that longer follow-up would be necessary to better understand the potential beneficial effects of gemcitabine on survival time and time to occurrence of metastases.

Several clinico-pathological prognostic factors have been identified for canine mammary tumors.\textsuperscript{5,50-53} In the present study, several variables were evaluated for their prognostic influence, and the analysis indicated that clinical stage at diagnosis had a statistically significant negative influence on the survival rates of dogs treated by surgery alone. This finding is in agreement with previously reported data.\textsuperscript{53,54} The number of gemcitabine treatments had a significant positive influence on survival time of treated dogs in this study. However, this finding needs to be interpreted with caution, as it may be explained by the fact that dogs living longer received additional cycles of gemcitabine. None of the other analyzed variables proved to be associated in the present
canine population, probably due to the low number of enrolled dogs and to the aggressive nature of the tumor in the selected animals.

In this study high-dose gemcitabine treatment was well tolerated with a low incidence of adverse events. Quality of life was maintained, if not improved in all treated dogs, especially in those presenting with respiratory signs. The planned timing of chemotherapy was respected for most of the cycles and only 4 treatments were delayed as a result of grade 1 neutropenia. In these 4 dogs, neutropenic episodes were uncomplicated and rapidly reversible, and hospitalization was not necessary. It may be possible that prophylactic antibiotic therapy given to all dogs during the first 3 weeks of treatment prevented infection-related events and attendant chemotherapy delays and dose reductions. Moreover, in this study non-hematologic toxicity was very mild and was observed in two dogs only. No grade 2-4 hematologic and grade 3-4 non-hematologic toxicity was observed, and no treatment-related deaths occurred. These findings have important implications, not only for the feasibility of gemcitabine in the adjuvant setting, but also for its potential use in combination regimens.

In conclusion, there are still many unanswered questions about the absolute benefit of adjuvant chemotherapy in canine mammary tumor care, and further studies are needed to better understand the role of gemcitabine and to substantiate its use. In human patients, combination regimens are generally superior to single agents in terms of response rate, duration of response and survival. Indeed, as single agent, gemcitabine yields response rates ranging from 16% to 37%; whereas, if combined with other chemotherapeutic drugs, response rates rise to 50% to 80%. Given these data, future veterinary clinical trials in dogs with resected aggressive mammary carcinoma should
focus on the adjuvant role of gemcitabine alone or in combination with other chemotherapeutic drugs.

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