A Randomized Controlled Trial of Octreotide Pamoate Long-acting Release and Carboplatin versus Carboplatin Alone in Dogs with Naturally Occurring Osteosarcoma: Evaluation of Insulin-like Growth Factor Suppression and Chemotherapy

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ABSTRACT

Purpose: The purpose of this research was to determine whether insulin-like growth factor (IGF) suppression, using a long-acting analogue of somatostatin (OncoLAR, octreotide pamoate long-acting release), will decrease chemotherapy resistance by eliminating an important survival signal to osteosarcoma (OSA) cells in a relevant naturally occurring cancer model.

Experimental Design: We conducted a randomized, blinded, placebo-controlled preclinical study in pet dogs with naturally occurring OSA. The study compared primary tumor necrosis and apoptosis, and survival of pet dogs receiving OncoLAR and carboplatin chemotherapy compared with dogs receiving placebo and carboplatin.

Results: Dogs receiving OncoLAR had suppression of serum IGF levels by ~43% without toxicity. No differences in primary tumor necrosis, apoptosis, tumor IGF mRNA expression, or survival were seen between the dogs receiving OncoLAR plus chemotherapy compared with OncoLAR alone.

Conclusion: The suppression of IGF levels by the extent and/or duration achieved in the trial was not sufficient to improve chemotherapy-related antitumor effects in pet dogs with OSA.

INTRODUCTION

OSA is the most common primary tumor of bone. Despite effective control of the primary tumor (most often at skeletal sites), metastases to the lung result in treatment failure in >30% of patients within 5 years. OSA tends to occur in pediatric patients during the second decade of life. This period of life is associated with the highest GH and IGF-I serum concentrations; furthermore, the risk for OSA development is greatest in large-boned children. These epidemiological observations suggest the potential importance of the GH-IGF-I pathway in the development and biology of OSA.

The pleiotropic effects of IGF-I suggest many possible roles for this growth factor in the biology of cancer, including OSA. These effects include transformation, proliferation, anti-apoptosis (life signal), and prometastasis (motility, invasion, and angiogenesis; Ref. 4). OSA cells express both IGF-I and IGF-I receptors, proliferate in response to IGF-I, and demonstrate an antiapoptotic phenotype in vitro after IGF-I exposure (5, 6).

In mice that have undergone hypophysectomy OSA growth is significantly reduced compared with nonhypophysectomized controls (7). This antitumor effect is hypothesized to be the result of suppressed serum GH and IGF-I in these mice after hypophysectomy. Treatment of mice with agents that result in GH blockade also result in antitumor effects in xenograft models including OSA (8, 9). For these reasons we have considered the inhibition of the IGF-I pathway to be a potentially valuable treatment strategy in OSA patients.

Therapeutic opportunities to block the IGF-I pathway include gene therapy or antisense therapy targeting the IGF-I receptor, antibodies that compete with or block the IGF-I receptor, disruption of IGF-I regulatory proteins (IGF-I binding proteins or other circulating binding proteins), suppression of serum IGF-I (hepatic) through inhibition of GH directly, or GH releasing hormone inhibition (5, 10, 11). We have demonstrated previously the effectiveness of a short-acting somatostatin analogue (Somatuline) in reducing serum IGF-I concentrations in OSA patients. This agent required frequent administration and was associated with moderate gastrointestinal toxicity. A novel and long-acting somatostatin analogue, named OncoLAR, has been developed recently to target tumors expressing somatostatin receptors (12). This long-acting agent held the promise of...
serum IGF-I suppression with more convenient once monthly dosing and a more favorable toxicity profile. We have completed recently a clinical trial that examined the safety and effectiveness OncoLAR in pediatric oncology patients (13). Results of this trial suggest that OncoLAR is well tolerated by pediatric patients and results in significant reductions in circulating serum IGF-I; however, no significant antitumor activity was demonstrated using OncoLAR alone in this Phase I clinical trial.

Chemotherapy-induced DNA damage results in cell death after a tumor cell recognizes DNA damage and then responds by undergoing apoptosis. The decision to enter this death pathway is in part determined by the genetic make up of the cell but also by extracellular signals, including IGF-I. The survival signal of IGF-I has been demonstrated to contribute to chemotherapy resistance in cancers including OSA (14). Therefore, it is possible that the antitumor activity of chemotherapy would be enhanced by the inhibition of IGF-I even if simple reduction in serum IGF-I concentrations was insufficient to provide a therapeutic benefit by itself. To test this hypothesis in a relevant preclinical setting we initiated a trial of OncoLAR plus chemotherapy compared with chemotherapy alone in pet dogs with naturally occurring OSA.

It was expected that the use of this stringent model system would allow rapid translation of results to the future management of pediatric patients. The biology of OSA in pet dogs is identical to that of pediatric patients in many important ways including higher risk in large breed dogs, tumor histology, anatomical distribution of primary tumors, aggressive pattern of metastases (most commonly to the lung), and dependence on the IGF-I pathway (15–17). The preclinical trial described herein was a randomized placebo-blinded trial of OncoLAR plus carboplatin chemotherapy versus carboplatin alone. Results of this trial demonstrated reduction in serum IGF-I to concentrations consistent with the use of OncoLAR in pediatric patients and the absence of toxicity when given in combination with chemotherapy. No differences in primary tumor apoptosis or necrosis were seen in dogs receiving OncoLAR and carboplatin compared with carboplatin alone; furthermore, no differences in survival or metastatic pattern were seen. The lack of treatment benefit suggested that the reduction of serum IGF-I concentrations by levels approaching 50% was not sufficient to increase chemotherapy effectiveness in a relevant model of OSA. Ongoing studies will determine whether more significant or long-lasting IGF-I and GH suppression can be attained, and if such suppression can improve treatment outcomes for OSA patients.

### MATERIALS AND METHODS

#### Study Design and Treatment Protocol

The study was designed as a randomized, single-blind, controlled trial for dogs with appendicular OSA. All of the dogs were entered to this trial at participating veterinary referral hospitals (Veterinary Teaching Hospital, University of Minnesota, St Paul, MN; Veterinary Referral Associates, Gaithersburg, MD; Veterinary Hospital University of Pennsylvania, Philadelphia, PA; SouthPaws Veterinary Referral, Arlington, VA; Beltway Referral, Landover, MD; Gulf Coast Veterinary Specialists, Houston, TX; Friendship Hospital for Animals, Washington, DC; and North Carolina State University Veterinary School, Raleigh, NC) between July 1997 and September 1998. Dogs eligible for the study had appendicular OSA, no evidence of pulmonary metastases on thoracic radiographs, no clinical evidence of metastases to other sites, and were able to undergo amputation of the tumor-bearing limb. In addition, dogs were included only if performance status (based on modified Eastern Cooperative Oncology Group grade) was 0, 1, or 2 (Table 1), hematologic status was grade 0 or 1 (Table 2), serum creatinine concentration was <4.0 mg/dl, chemotherapeutic drugs had not been given before entry into the study, and body weight was >20 kg (44 pounds). Dogs that did not meet all of these criteria were excluded from the study. Owners of all of the dogs included in the study provided written informed consent.

For all of the dogs included in the study, a complete history and physical examination, CBC, platelet count, serum biochemical analyses, urinalysis, and thoracic radiography were performed before treatment. Clinical stage was determined on the basis of established criteria (16). Biopsy samples from tumor-bearing limbs were examined by a single pathologist (D. H.).

#### Randomization

Dogs were randomly assigned to one of two treatment groups: carboplatin plus OncoLAR (OncoLAR group) versus carboplatin plus placebo (placebo group) using a random permuted block method (18). Treatment group assignments were performed by an author (J. P.) not involved directly in the care of the pet dogs. Owners were blinded to treatment group assignment and were not present during treatment administration. If the code for treatment group assignment was broken.

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**Table 1** Performance status (modified Eastern Cooperative Oncology Group grade)

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal activity</td>
</tr>
<tr>
<td>1</td>
<td>Restricted activity; decreased activity from predisease status</td>
</tr>
<tr>
<td>2</td>
<td>Compromised; ambulatory only for vital activities; consistently defecates, and urinates in acceptable areas</td>
</tr>
<tr>
<td>3</td>
<td>Disabled; must be force fed; unable to confine urination and defecation to acceptable areas</td>
</tr>
<tr>
<td>4</td>
<td>Dead</td>
</tr>
</tbody>
</table>

**Table 2** Hematologic grade

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Hb concentration, &gt;10 gm/dl</td>
</tr>
<tr>
<td></td>
<td>Platelet count, &gt;100,000/ul</td>
</tr>
<tr>
<td>1</td>
<td>Hb concentration, 9–9.9 gm/dl</td>
</tr>
<tr>
<td></td>
<td>Platelet count, 50,000–99,999/ul</td>
</tr>
<tr>
<td>2</td>
<td>Hb concentration, 7 to 8.9 gm/dl</td>
</tr>
<tr>
<td></td>
<td>Platelet count, 25,000–49,999/ul</td>
</tr>
<tr>
<td>3</td>
<td>Hb concentration, 5–6.9 gm/dl</td>
</tr>
<tr>
<td></td>
<td>Platelet count, &lt;25,000/ul</td>
</tr>
<tr>
<td>4</td>
<td>Hb concentration, &lt;5 gm/dl</td>
</tr>
<tr>
<td></td>
<td>Platelet count, &lt;25,000/ul</td>
</tr>
</tbody>
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*Hb, hemoglobin.*
for any individual dog, that dog was still eligible to remain in the study, but lack of blinding was recorded.

**Treatment Protocol.** The treatment protocol (Fig. 1) consisted of three cycles of OncoLAR (or placebo consisting of vehicle alone) and carboplatin administered once every 21 days. OncoLAR or placebo was administered on days 0, 21, and 42 of the study at a dose of 60 mg i.m. (fixed dose). Carboplatin was administered on days 7, 28, and 49 of the study at a dose of 300 mg/m^2^ by slow i.v. infusion. Amputation of the tumor-bearing limb was undertaken on day 14 of the study (14 days after OncoLAR and 7 days after carboplatin administration). Before each treatment with carboplatin a complete blood count was examined. If drug-induced neutropenia (i.e., grade 2, 3, or 4 hematologic status) was detected, carboplatin treatment was delayed until the hematologic status was grade 0 or 1. The dosage of carboplatin was reduced by 25% for the remainder of the study. If severe drug-induced gastrointestinal toxicity developed, carboplatin, OncoLAR, or placebo was withheld until signs had resolved for >3 days. If gastrointestinal toxicity was associated with carboplatin the dosage of carboplatin last administered was reduced by 25% for the remainder of the study. Standard supportive and life-saving care was given if warranted and deemed necessary by the attending veterinary clinicians. Serum and plasma samples were collected once weekly through the treatment protocol and then once a month for 3 months. Follow-up examinations were then undertaken once every 3 months. These examinations consisted of physical examination and thoracic radiographs. A necropsy was performed on all of the dogs when possible. Pulmonary metastases were collected and snap frozen in liquid nitrogen when possible. All of the protocols were reviewed and approved by the Animal Care Committee or the head veterinarian of each participating center.

**Sample Size, Study Endpoints, and Analysis.** Power analysis (using InStat; GraphPad, San Diego, CA) indicated that 21 dogs in each group would be sufficient to measure a 3-month difference in disease-free survival between treatment groups, assuming a 4-month SD in the disease-free survival, with a P of 0.05 and power of 0.80. Treatment group comparisons included duration of remission (i.e., number of days from the onset of therapy to the first day of relapse), survival time (i.e., number of days from the onset of treatment to death, regardless of whether death was related or unrelated to OSA), number of episodes of dose-limiting neutropenia during treatment, number of dose-limiting episodes of gastrointestinal toxicity during treatment, and number of dogs in which death was associated with treatment. Two study populations were defined for analysis: an intent-to-treat population, which included all of the dogs assigned to a treatment group and a treatment-received population, which included only those dogs that received three complete cycles of OncoLAR or placebo and carboplatin, and for which complete follow-up information was available. Complete outcome analysis was undertaken in the treatment-received populations only. Duration of first remission and survival time was compared between treatment groups for each of the study populations.

**Assessment of Surrogate Endpoints in Primary Tumor.** The amputated tumor-bearing limb was cleaned of overlying skin and musculature to allow en bloc transverse resection of the tumor with a 3-cm margin of normal bone. A tumor was bisected using a surgical saw in a transverse plane to allow half of the tumor to be fixed in 10% formalin and half of the tumor to be snap frozen in liquid nitrogen, and then stored at −80°C. Tumor samples were then shipped using overnight delivery to the principal laboratory for additional processing. The formalin-fixed tumor section remained in formalin for at least 36 h. Three core samples were collected from the formalin-fixed tumor in standardized planes using a 12-mm trephine biopsy core. Tumor cores were independently labeled and decalcified (Cal-Ex; Fisher Scientific, Pittsburgh, PA) for 24 h. Tumor cores were washed in fresh water for 2 h and then returned to 10% formalin. The three tumor cores were then embedded in the same paraffin block, and sectioned for routine histology and immunohistochemistry.

**OSA Tissue Arrays.** OSA tissue arrays were prepared using paraffin blocks collected in this study (described above). A H&E stained section of each block was reviewed, and the region of greatest tumor cellularity was grouped by one of the authors (S. M. H.). The array was constructed using a manual arrayer (Beecher Instruments, Silver Spring, MD) using 1-mm needles. The construction of the array was essentially identical to the method described by Kononen et al. (19). The design of the array was such that two cores from close to each other were taken from each donor block and placed in the recipient. The recipient block was sectioned using the tape transfer method according to the manufacturer (Instrumedics, Inc., Hackensack, NJ).

**Analysis of Primary Tumor Apoptosis, Necrosis, and Proliferation.** The original paraffin-embedded OSA samples and tissue arrays made from these samples were used to assess apoptosis and necrosis in dogs entered for study, and to define differences in apoptosis and necrosis between treatment groups. Tumor cores from each dog were examined by the same pathologist (D. H.) to confirm the diagnosis of OSA and to estimate percentage of necrosis. Tumor necrosis was defined in three cores collected from each case as described previously without knowledge of treatment group assignment. The mean necrosis from the three cores was used as a measure of necrosis in the...
tumor of the dog. Sections from these standard tumor cores were mounted on Gel Probon slides and assessed for TUNEL reactivity using the Death Detection kit (Roche) with peroxidase (POD) conversion according to manufacturer’s recommendations. OSA tissue arrays (derived from the tissue blocks above) were examined using H&E-stained slides for morphological features of apoptosis and using in situ TUNEL detection (In Situ Death Detection kit, POD; Roche, Indianapolis, IN) in formalin-fixed tissues. For the tissue array TUNEL assay, tissues were permeabilized in ice-cold, nuclease-free water with 0.1% sodium citrate and 0.1% Triton X-100 following standard deparaffinization. The TUNEL reaction was performed according to manufacturer’s recommendation. Positive control slides were prepared by treatment with DNase I before application of the terminal deoxynucleotidyl labeling enzyme, whereas negative controls were not treated with the labeling enzyme. Tissue arrays were also used to examine PCNA in tumors from dogs in both treatment groups. For PCNA detection, a 1:500 dilution of mouse anti-PCNA antibody (Santa Cruz Biotechnologies, Santa Cruz, CA) was used in an antibody diluent from DAKO Corporation (Carpinteria, CA). Control slides were treated with antibody diluent without antibody. Staining development was carried out according to the LSAB+ Peroxidase kit. Tissue was then counterstained with Mayer’s hematoxylin (Lillie’s Modification; DAKO Corp.) and coverslipped with Permount mounting medium (Fisher Scientific). A semiquantitative scoring system was used to define TUNEL and PCNA positivity in the primary tumors (0, no staining; 1, mild intensity staining; 2, moderate intensity staining; 3, marked intensity staining).

Analysis of Plasma IGF-I Concentrations. Plasma samples collected at days 0, 7, 14, 21, 28, 42, and 65 were assayed for IGF-I from selected dogs in both treatment groups (selected dogs include the first 7 cases in which all of the serum samples were available). Analysis was undertaken using a RIA for human IGF-I (Quest Laboratories Inc., San Diego, CA).

Northern Analysis of Primary Tumor for IGF-I Expression. Frozen tumor samples were freeze-fractured and then homogenized in TRIzol using a Polytron PT1200 tissue homogenizer (Kinematica AG, Luzern, Switzerland). Total RNA was extracted using TRIzol according to manufacturer’s specifications. Northern blots consisted of 20 µg of total RNA from primary tumor samples (where available) of dogs in both the OncoLAR and placebo treatment groups. A plasmid probe for canine IGF-I was generated by PCR using the following primers (upstream 5′-TCA CAT CTC TTC TAC CTG GC-3′; downstream 5′-GTA GGT CTT TCC TGC AC-3′). PCR products were ligated into expression plasmids and sequence verified. cDNA inserts were excised by restriction digest and then labeled by nick translation (Amersham Life Science) with [32P]dCTP. Hybridization of Northern blots was carried out using Express Hybe (Clontech, Palo Alto, CA) according to manufacturer’s recommendations. Equal loading of RNA was confirmed by probing membranes with an [32P]dCTP-labeled canine β-actin probe.

Statistical Analysis. Descriptive statistics were calculated, and results for groups were compared by use of nonparametric and alternate (Welch’s) t tests, using computerized software (Prism for Macintosh). A value of $P < 0.05$ was considered significant. Survival time and duration of remission were compared between groups by use of Cox proportional hazard model. Dogs were censored from the analysis of the treatment-received population if follow up was incomplete or if dogs were disease free or alive at the time of the analysis. An alternate Welch’s t test was used to compare baseline serum IGF-I concentrations in dogs to time points following OncoLAR treatment.

RESULTS

Fifty-four dogs were considered for inclusion in the study. Of these, 44 were included in the treatment-received group. Ten were not included in the study for various reasons, including death after amputation (related to surgical complications), death from accidental carboplatin overdose, persistent carboplatin induced neutropenia, owner decision to withdraw from study, and misdiagnosis of primary tumor. Of the 44 dogs included in the study, 23 were assigned to the OncoLAR plus carboplatin treatment group, and 21 were assigned to the placebo plus carboplatin treatment group. These 44 dogs comprised the treatment-received population; median follow-up time for all of the cases was 235.5 days (range, 50–757 days). Clinical characteristics (breed, age, sex, body weight, and tumor location) were similar for dogs in both treatment groups (data not shown).

Serum IGF-I concentrations were assessed in 7 dogs selected from each randomization group within the treatment-received population of dogs. Baseline IGF-I concentrations in both treatment groups were highly variable among individual dogs. No association between body weight and percentage of IGF-I suppression was found (fixed dose of OncoLAR given to all of the dogs 20–40 kg). Serum IGF-I concentrations in dogs treated with OncoLAR were reduced by 43% ($P = 0.14$) from baseline (Fig. 2). Suppressed IGF-I concentrations were first noted 7 days after treatment and persisted for ~21 days after the final dose of OncoLAR. Dogs receiving placebo had no suppression in serum IGF-I concentrations through the observation period.

Reported side effects in the OncoLAR- and placebo-treated
dogs were similar. Side effects included sterile injection site abcessation (both dogs in the OncoLAR treatment group), gastrointestinal toxicity (placebo treatment), and transient weakness (placebo treatment). No differences in carboplatin-associated toxicities (including neutropenia) were seen in dogs receiving OncoLAR and placebo.

For the intent-to-treat population the median survival for dogs randomized to receive OncoLAR was 179 days (95% CI, 139–267 days). The median survival for dogs randomized to the placebo group was 107 days (95% CI, 124–267 days). No significant differences were seen in the median survival of these two groups (P = 0.42). Ten dogs from the intent-to-treat population were not included in the treatment-received population. Median survival for these 10 dogs was 22 days (range, 0–373 days). For the treatment-received population the median disease-free interval and overall survival for dogs in the OncoLAR treatment group was 215 days (95% CI, 179–363 days) and 242 days (95% CI, 210–392 days), respectively. The median disease-free interval and survival for dogs in the placebo treatment group was 196 days (95% CI, 155–361 days) and 230 days (95% CI, 209–407 days), respectively. No significant differences were seen in the median disease-free internal and survival in these populations. Kaplan-Meier analysis of survival for both treatment groups is presented in Fig. 3. At the completion of the study observation period 7 dogs in the OncoLAR treatment group and 5 dogs in the placebo group were still alive. The median survival for dogs still alive in the OncoLAR group was 753 days and was 608 days in the placebo group. Sites of metastases in both treatment groups were similar. The most common site for metastases was the lung (n = 23). Other sites of metastases included bone (n = 7), intra-abdominal (n = 3), retroperitoneal (n = 1), and intraocular (n = 1).

Fig. 4 demonstrates a statistically significant correlation between percentage of primary tumor necrosis (7 days after i.v. carboplatin administration; day 14 of therapy) and survival in all of the dogs included in the treatment-received population. No correlation between primary tumor apoptosis (7 days after i.v. carboplatin administration; day 14 of study) and disease-free interval or survival in dogs was seen in all of the dogs in the treatment-received population. Furthermore, no difference in primary tumor apoptosis was seen in dogs in the OncoLAR treatment group compared with dogs in the placebo groups. Apoptosis was assessed in individual tumor sections and in tissue arrays using the TUNEL assay. Primary tumor proliferation, assessed at day 14 of the study with PCNA staining, correlated with survival in dogs in the treatment-received population of dogs. Dogs with tumors demonstrating higher cellular proliferation (PCNA staining intensity 2 and 3) had poorer survival compared with dogs with none to low cellular proliferation (PCNA staining intensity 0 and 1). Median survival for dogs with none to low PCNA staining was 247 days compared with 117 days in dogs with high PCNA staining (P = 0.042). The hazards ratio for higher expression of PCNA in the primary tumor was 0.52 (95% CI, 0.33–0.98). No differences in PCNA staining intensity were found between dogs in the OncoLAR and placebo treatment groups.

OncoLAR treatment did not influence primary tumor expression of IGF-I compared with dogs treated with placebo. Northern analysis using RNA extracted from the primary tumor (at the time of amputation, day 14 of therapy) demonstrated similar and very low levels of expression of IGF-I in dogs receiving OncoLAR and those receiving placebo (data not shown).

**DISCUSSION**

Canine OSA represents a highly relevant and naturally occurring cancer model that may improve our understanding of the biology and therapy of OSA. The canine model mirrors the
human condition in many important ways, including, histopathology, biological behavior, metastatic pattern, molecular biology, and IGF-I dependence. The management of pet dogs with OSA includes amputation of the tumor-bearing limb in combination with conventional chemotherapy (most often single-agent use of carboplatin, cisplatin, or doxorubicin). With amputation alone the median disease-free interval for pet dogs is relatively short (4 months). The addition of chemotherapy may increase the duration of this disease-free interval to 7–12 months depending on the treatment regimen used (16). In this study, dogs with naturally occurring OSA provided a stringent translational model that allowed an important clinical and biological question to be answered. The results of this preclinical study suggest that short-term suppression of serum IGF-I is not sufficient to increase primary tumor apoptosis or necrosis above that induced by chemotherapy alone and does not improve treatment outcome for dogs with OSA when compared with dogs receiving chemotherapy alone.

The suppression of serum IGF-I seen in pet dogs treated with a fixed dose of 60 mg i.m. of OncoLAR was similar to the 50% suppression seen in pediatric patients in a recently completed clinical trial (13). It is important to note that the use of OncoLAR was well tolerated in dogs and did not exacerbate side effects related to the carboplatin. Baseline IGF-I concentrations were highly variable in all of the dogs, however, suppression of serum IGF-I was seen as early as 7 days after OncoLAR therapy. A similar suppression was not seen in dogs treated with placebo. The fixed dose of OncoLAR given to dogs of all sizes allowed a dose response to be assessed. Size of dog did not appear to predict suppression of IGF-I, suggesting that maximal suppression had been seen. No escalation in the dose of OncoLAR was considered for these reasons.

The lack of differences detected between OncoLAR and placebo groups for all of the endpoints except serum IGF-I levels may suggest that either the extent and/or duration of IGF-I suppression achieved in this study was not sufficient to produce a clinical effect. It is possible that more substantial decreases in serum IGF-I may be attained through combinations of GH blockade with other IGF-I inhibiting strategies (i.e., GH-releasing hormone blockade or GH antagonists). We are currently investigating the impact of more significant IGF-I suppression on OSA biology using Cre-albumin/loxP IGF-I genetically engineered mice that have 70% reductions in serum IGF-I (20). Since the initiation of this preclinical trial a growing list of reports suggest the role of IGF-I in promoting tumor cell motility, adherence, invasion, and angiogenesis, and the potential role of reversing these metastatic activities through IGF-I inhibition (21). For the proposed antimitotic effects of IGF-I suppression to be seen, longer and more sustained IGF-I suppression is likely necessary. Such sustained IGF-I inhibition was not provided by the OncoLAR treatment regimen used in this study.

The use of neoadjuvant chemotherapy allowed the induction of apoptosis and necrosis to be assessed in the primary tumor as a function of the treatment group. The timing of the amputation was based on the need to remove an often-painful primary bone tumor from pet dog within 14 days of diagnosis. The positive correlation between necrosis in the amputated tumor and survival in the intent-to-treat population provides validation for the use of necrosis as an end point at this time point for the comparison of the OncoLAR and placebo treatment groups. Conversely the absence of a correlation between apoptosis and survival in the intent-to-treat group suggests that this end point may not be as valuable in the group comparisons at this time point. The timing of amputation relative to the OncoLAR treatment or the chemotherapy may not have been optimal for the assessment of apoptosis. Time course analysis of biopsy tissue taken from tumors after treatment may have defined a kinetic difference in the induction of apoptosis between treatment groups and potentially an optimal time for the assessment of apoptosis. Experience with our evaluation of the amputated primary tumor suggests that the examination of several sections of tumor is necessary to accurately estimate necrosis and apoptosis. Follow-up studies that include serial incisional biopsies may be feasible on a small subset of cases but would not likely be accepted by most pet owners.

Access to large amounts of primary tumor tissue (amputation samples) in this study allowed the successful extraction of tumor RNA after OncoLAR and placebo treatment. RNA samples were used to determine whether local (i.e., tumor) production of IGF-I was influenced by GH blockade. Northern analysis did not demonstrate any effect on primary tumor IGF-I expression in dogs treated with OncoLAR compared with placebo; however, the low baseline expression of IGF-I may not have allowed detection of subtle changes in expression. Similarly low tumor IGF-I levels have been demonstrated in human OSA samples (5). The maintenance of local IGF-I expression during suppression of serum IGF-I by OncoLAR may suggest that the IGF-I pathway can remain locally active (autocrine or paracrine) in the tumor microenvironment. In future studies the suppression of IGF-I in tumor tissue should be included in the evaluation of therapies that intend to target IGF-I.

This study in pet dogs with OSA has provided important preclinical information for the future use of IGF-I inhibiting strategies for cancer. This negative data has suspended the progression of OncoLAR toward combination treatment with chemotherapy in pediatric patients. We believe that 50% suppression of IGF-I in combination with chemotherapy is insufficient to improve chemotherapy-induced apoptosis and patient outcome. Future clinical and preclinical trials should consider longer term and more significant serum IGF-I suppression, and consider the importance of the local production of IGF-I in the biology of OSA.

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