Evaluation of a novel immunomodulator composed of human chorionic gonadotropin and bacillus Calmette-Guerin for treatment of canine mast cell tumors in clinically affected dogs

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Objective—To evaluate safety and efficacy of LDI-100, a preparation containing human chorionic gonadotropin (hCG) and bacillus Calmette-Guerin (BCG), in the treatment of dogs with mast cell tumors and to compare results with those from a control group receiving single-agent vinblastine.

Animals—95 dogs with measurable grade II or III mast cell tumors.

Procedures—Dogs were randomized to receive either LDI-100 (1.35 ng of BCG and 2 units of hCG, SC, q 24 h) or vinblastine (2 mg/m^2, IV, q 1 wk) for 6 weeks. Tumors were measured at baseline and day 42, and dogs were monitored for signs of toxicosis. Clinical performance scores were recorded at each visit. Differences in host factors (sex, weight, and age), clinical performance score, tumor response, and adverse events were analyzed.

Results—46 dogs received LDI-100, and 49 dogs received vinblastine. No significant differences were found between the 2 treatment groups with regard to host factors or clinical performance score. Tumor response (≥ 50% reduction) rates were similar between the LDI-100 and vinblastine group (28.6% and 11.7%, respectively). Dogs in the LDI-100 group had significantly less neutropenia than the vinblastine group.

Conclusions and Clinical Relevance—hCG and BCG have immunomodulatory and antitumor effects against a variety of malignancies in humans and dogs. In this study, LDI-100 provided clinical responses comparable to single-agent vinblastine chemotherapy but without myelosuppression. LDI-100 is a promising new agent that should be further investigated for multimodality therapy of mast cell tumors in dogs. (*Am J Vet Res* 2007;68:1246–1251)

In dogs MCTs that are not amenable to surgical resection present a clinical dilemma for practitioners, especially when external beam radiation therapy is not available or feasible. Current medical treatment for nonresectable or recurrent high-grade MCTs generally includes single- or multiple-agent protocols with vinblastine, corticosteroids, and alkylating agents.[1-3] In 1 study[1] in which the use of prednisone and vinblastine combination treatment for dogs with MCTs was investigated, the MST for the entire population was not reached with a median follow-up of 573 days; howev-

Abbreviations

MCT	Mast cell tumor
MST	Median survival time
CCNU	1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea
BRM	Biological response modifier
BCG	Bacillus Calmette-Guerin
hCG	Human chorionic gonadotropin
RECIST	Response evaluation criteria in solid tumors
CPS	Clinical performance score

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er, the MST for dogs with high-grade tumors was 331 days. Results of another study[2] in which the local tumor control achieved with prednisone and vinblastine was evaluated in a population of dogs with intermediate or high-grade cutaneous MCTs indicated a comparable outcome to that achieved with complete surgical excision or surgery followed by radiation therapy. Likewise, CCNU single-agent treatment has been reported to have similar efficacy in treating MCTs.[3] More recently, protocols incorporating CCNU and vinblastine have been developed, with results reported at scientific meetings but, to our knowledge, not yet published in the peer-reviewed literature.

Biological response modifiers have been studied for years in veterinary medicine. These are defined as agents or approaches that modify the relationship between the tumor and the host by modifying the biological response of the host to tumor cells.[4] One such BRM is BCG, a bacterial cell wall derivative that incites an antitumor effect that is thought to be attributed, in part, to increases in interleukin-18 and subsequent increases in T-helper cell type-1 cytokines including interleukin-2 and interferon-γ.[5] Bacillus Calmette-Guerin has been used to treat a variety of malignancies in humans, the most notable of which is bladder cancer, for which BCG is considered standard of care for superficial lesions.[6] In veterinary medicine, BCG has been widely used in the treatment of horses with sarcoids and cattle with ocular squamous carcinomas.[7-9] In addition, BCG has been evaluated as an adjuvant treatment for dogs with osteosarcoma, providing improvement in MST, compared with historical control dogs.[10] To date, the use of BCG in the treatment of dogs with MCTs remains largely unexplored.

As with BCG, hCG has been investigated as an immunomodulator for the treatment of inflammatory and neoplastic diseases in humans.[11-13] Human chorionic gonadotropin is a glycoprotein hormone that is produced and secreted by the trophoblastic cells of the uterus during pregnancy. Human chorionic gonadotropin is a member of a superfamily of structurally related growth factors including transforming growth factor, nerve growth factor, and platelet-derived growth factor. It is also expressed on tumor cells of trophoblastic and nontrophoblastic origin.[14-16] Intralesional or systemic administration of hCG preparations has led to tumor regression in human patients with multiple idiopathic hemorrhagic sarcoma (ie, Kaposi's sarcoma) A proapoptotic effect is believed to be one of the underlying mechanisms leading to such responses.[11,12] A reduction in expression of the antiapoptotic protein, bcl-2, was identified in human patients with acute myelogenous leukemia after treatment with hCG, lending further support to the idea that the antitumor effects of hCG are related to a proapoptotic mechanism.[17] In 1998, Feldman et al[a] presented data at the American Society of Hematology meeting regarding patients with myelodysplastic syndrome that were treated with daily SC injections of 2 units of hCG, along with weekly administration of levamisole chloride (50 mg, PO). They reported hematologic improvement in 5 of 23 patients, with no adverse reactions observed in any patient.[a] The same group subsequently completed a study randomizing 43 human patients with myelodysplastic syndrome to either receive daily SC administration of hCG plus standard supportive care (ie, antimicrobials and transfusions) or standard supportive care alone. The study permitted crossover to the hGG arm after 12 weeks or at the time of disease progression. In total, 31 patients received hGG and 27 were evaluated for response. Durable hematologic responses were reported for > 30% of the patients with myelodysplastic syndrome, with minimal to no adverse effects. The authors proposed that responses to hCG might be attributable to proapoptotic and antiangiogenic activity.

Although hCG and BCG are FDA-approved compounds, to our knowledge, the clinical evaluation of these compounds in combination for cancer treatment has not been previously reported. In the study reported here, we investigated a novel immunomodulator, LDI-100,[b] which is composed of low concentrations of BCG and hCG. The main objective of the study was to evaluate the safety and efficacy of daily LDI-100 administration to dogs with naturally occurring cancer. The MCTs were chosen for study because of the frequency of veterinary hospital admissions for this disease and the rapidity with which response could be evaluated. To provide a clinically relevant comparison group, dogs were randomized to receive LDI-100 or single-agent vinblastine, the chemotherapy agent used most often in the treatment of high-grade MCTs.

Materials and Methods

Animals—This was a prospective, multi-institutional study, enrolling client-owned dogs with naturally occurring, measurable (by use of calipers, ultrasonography, and radiography), nonresectable Patnaik system[18] grade II or III MCTs. All enrolled dogs were required to have an anticipated survival time of ≥ 8 weeks and to be lacking clinical or laboratory evidence of impaired hepatic or renal function. Dogs that had undergone any treatment for MCT within 30 days prior to the screening visit were considered ineligible. Dog owners were fully informed of the investigational nature of the study protocol (including risk and possible adverse effects) and were required to provide a signed consent form. Patient withdrawal from the study was permitted in the event of disease progression, serious adverse events, or decline in overall quality of life as determined by the pet owner. All procedures were conducted in compliance with the appropriate institutional animal care and use committee guidelines. This study was designed with the intent to gain conditional licensure of LDI-100 as a biologic.

Study protocol—At the time of initial screening, a complete medical history was obtained and physical examination, CBC determination, serum biochemical analysis, buffy coat evaluation for circulating mast cells, and urinalysis were performed. Patient sex; age; weight; physical examination findings; and tumor size as determined by RECIST, a unidimensional measurement of the longest diameter of the mass, were recorded.[19] The RECIST was published as a guideline in the *Journal of the National Cancer Institute* at the time of study inception and was considered to be the most accurate method to measure solid tumors.

Ninety-five dogs met study inclusion criteria and were randomly allocated into 2 groups, 49 (51.6%) to

the vinblastine group and 46 (48.4%) to the LDI-100 group, between May 2000 and May 2003. Randomization was via a computer-generated randomization table without stratification or blocking. Each dose of LDI-100[b] contained 0.05 mL of BCG (27 ng/mL) and 2 units of hCG in a total dose volume of 0.2 mL. Dogs assigned to the LDI-100 group were treated with a daily dose of 0.2 mL of LDI-100 administered SC for 6 weeks. Investigators instructed pet owners in the proper technique for SC administration of LDI-100 at home. A daily medication diary form was given to the owner on each visit for completion prior to the next evaluation. Dogs in the vinblastine group received vinblastine (2 mg/m^2, IV) once a week for 6 weeks. Follow-up visits on weeks 2, 4, and 6 were scheduled for dogs in both groups. On weeks 2 and 4, complete physical examination, CBC determination, serum biochemical analysis, and urinalysis results were obtained. Clinical performance scores were documented by use of Karnofsky Performance Score criteria[20] adapted for veterinary patients (**Appendix**). Clinical performance scores were reassessed at each visit. Any adverse effects were also recorded at each visit and scored on a 4-point scale (1 = mild, 2 = moderate, 3 = severe, or 4 = life threatening). Although this study took place prior to the publication of standardized toxicologic criteria for veterinary clinical trials through the Veterinary Cooperative Oncology Group, the scoring system approximated this same system, with the exception that it did not contain a score of 5, designated in the Veterinary Cooperative Oncology Group system as the score for death as an adverse event.[21] At week 6 (day 42), in addition to the same data obtained at weeks 2 and 4, tumors were measured to assess for response, again by use of RECIST.

Statistical analysis—Age, sex, weight, tumor size, PCV, WBC, adverse events, CPS, and tumor grade were analyzed with a statistical software program.[c] A Fisher exact test was used when < 5 observations were recorded per treatment arm. Adverse events including neutropenia (assessed according to laboratory reference range values at each site), gastrointestinal adverse effects (vomiting or diarrhea), change in PCV between week 1 and 6, and development of fever were monitored, recorded, and analyzed by use of a χ^2 test. Adverse events were considered substantial if they altered the administration of treatment, were recorded as such by the investigator on the serious adverse events form, or were of grade III or IV severity.

Clinical performance scores were analyzed by use of a rank sum test because of the lack of normality. Differences in tumor size were analyzed by use of an ANOVA. Either a χ^2 test or an ANOVA were used to analyze the remainder of the variables (age, sex, weight, PCV, WBC, and tumor grade). Values of $P < 0.05$ were considered significant.

Results

Forty-six dogs were randomized to the LDI-100 group, and 49 were randomized to the vinblastine group (**Table 1**). All dogs had histologically confirmed grade II or III MCTs. However, histopathologic grading was recorded only as high grade, without assigning a specific grade as II or III, for 4 dogs (2 dogs in each group). Tumor tissue was not available for review for these 4 dogs; thus, data regarding tumor grade were analyzed for only 91 of the 95 dogs enrolled.

Clinical performance scores at baseline were not significantly ($P = 0.206$) different between the 2 groups. Likewise, when baseline score for each patient was

Table 1—Patient characteristics and outcome for dogs receiving either LDI-100 or vinblastine chemotherapy for treatment of MCTs.

	Dog groups		
Variables	**LDI-100 (n = 46)**	**Vinblastine (n = 49)**	***P* value**
Age (mean ± SD; y)	8.6 ± 3.4	8.8 ± 2.9	0.746
Sex			
Female (No.)	28	24	0.338
Male (No.)	18	25	
Weight (kg)	29.4 ± 10.8	25.5 ± 13.5	0.195
Tumor grade*			
II (No.)	29	34	0.662
III (No.)	15	13	
Tumor size (mm)	35.0 ± 25.07	38.0 ± 23.54	0.583
No. of responders†			
Complete response (100% Dec)	3 (14.3%)	2 (5.8%)	0.859
Partial response (≥ 50% to < 100% Dec)	3 (14.3%)	2 (5.8%)	
Stable disease (1% to < 50% Dec)	5 (23.8%)	17 (50%)	
No response	10 (48%)	13 (38%)	
Change in CPS			
Decrease (No.)	2	6	0.905
No change (No.)	19	27	
No. of adverse events			
0	25	24	0.121
1	15	11	
≥ 2	6	14	
No. of dogs with neutropenia (%)	0 (0)	11 (22.4)	< 0.001

*Histopathologic grading was recorded only as high grade, without assigning a specific grade as II or III, for 2 dogs in each group. †Total number of dogs evaluated in the LDI-100 and vinblastine groups was 21 and 34, respectively.

Table 2—Number of dogs with adverse effects that received LDI-100 or vinblastine chemotherapy for treatment of MCTs.

Variables	Dog groups: LDI-100 (n = 46)	Dog groups: Vinblastine (n = 49)	*P* value
Neutropenia	0	11	< 0.001
No neutropenia	46	38	
Signs of depression	1	5	0.205
No signs of depression	45	44	
Gastrointestinal tract signs	7	6	0.769
No gastrointestinal tract signs	39	43	
Increase in rectal temperature	2	3	1.00
No increase in rectal temperature	44	46	
Severe adverse effects	12	17	0.536
No severe adverse effects	34	32	

compared with the last reported CPS (at either study completion or withdrawal from the study) and differences compared between the 2 groups, there was no significant (P = 0.905) difference between groups. In the LDI-100 group, 25 dogs withdrew from the study before their day 42 evaluation, versus 15 in the vinblastine group. This difference in rate of study withdrawal between the 2 groups could not, however, be accounted for by a difference in CPSs. The range of change from baseline CPSs in the LDI-100 group was from a 20-point improvement to a 40-point decline, and the range for the vinblastine group was from a 40-point improvement to a 70-point decline.

Comparisons between tumor measurements on days 0 and 42 were available for 21 dogs in the LDI-100 group and 34 dogs in the vinblastine group. Analysis of response data for these dogs indicated no significant (P = 0.859) difference in the efficacy of LDI-100 or vinblastine in the treatment of measurable grade II or III MCTs. Both drugs provided some tumor responses in more than half of the dogs completing the protocol, as evidenced by reduction in tumor size during the 6-week study period. Complete responses were observed in 3 of 21 (14.3%) dogs evaluated in the LDI-100 group and 2 of 34 (5.9%) dogs evaluated in the vinblastine group. Tumor volume reduced ≥ 50% in 6 of 21 (28.6%) dogs evaluated in the LDI-100 group and in 4 of 34 (11.8%) dogs evaluated in the vinblastine group. No significant differences were found between the 2 groups with regard to any of the patient variables or tumor response data examined.

The assessment of adverse events between groups included evaluation of number of adverse events, as well as severity of adverse events (**Table 2**). Although both groups had similar numbers of dogs that did not have any adverse events (54% [25/46] in the LDI-100 group and 49% [24/49] in the vinblastine group), only 6 of 46 (13%) dogs in the LDI-100 group had ≥ 2 adverse events, compared with 14 of 49 (29%) dogs in the vinblastine group. Four of the 14 vinblastine group dogs that had adverse events had ≥ 4 episodes, but this difference between the 2 groups was not significant. As expected, the development of neutropenia was significantly (P < 0.001) different between groups. None of the dogs in the LDI-100 group had any episodes of neutropenia, whereas 11 of 49 (22.4%) dogs in the vinblastine group had ≥ 1 episode of neutropenia. No other reported adverse event categories (gastrointestinal tract signs, signs of depression, and increased rectal temperature) were significantly different between groups, in either occurrence or severity. Change in PCV was also compared between groups, and no significant (P = 0.09) difference was observed.

Discussion

On the basis of our results, daily administration of LDI-100 appears to be safe and to possess antitumor activity similar to single-agent vinblastine chemotherapy in dogs with MCTs. Although a long-term toxicologic study was not performed, adverse events in the LDI-100 group were infrequent and of similar or lesser severity to those in the vinblastine group.

To provide a benchmark for comparison of antitumor activity of LDI-100 to that of other chemotherapy, we sought to design this trial with a control group receiving standard-of-care chemotherapy for MCTs. Based on the literature at the time of the study design, chemotherapy with either a vinblastine and prednisone combination protocol or a CCNU single-agent protocol was considered standard of care. Because of the prolonged dosing interval (once every 21 days) with CCNU single-agent treatment, this was not considered an ideal drug for comparison to a daily administered immunomodulator, in that 21 doses of LDI-100 would be given in the same time frame as 1 dose of CCNU. As such, a chemotherapy agent given on a more frequent basis, specifically weekly vinblastine, was considered more appropriate for comparative purposes. Although prednisone is administered in conjunction with vinblastine in the protocol described by Thamm et al,[1] the decision was made to compare 1 single-agent protocol (vinblastine only) to another (LDI-100 only). Given that response rates to prednisone alone are reported to be only ≤ 20% for dogs with MCTs, we chose to compare LDI-100 to vinblastine, believing it to be the more active of the 2 drugs in the vinblastine and prednisone protocol against MCTs.[22] It should, however, be stated that little prospective data exist regarding response rates of MCTs to prednisone alone. Likewise, no prior studies have been conducted to evaluate the efficacy of single-agent vinblastine against MCTs. However, the fact that 62% of the dogs that completed the vinblastine-only protocol in our study had some measurable reduction in tumor size suggests that the drug does, indeed, have antitumor activity against MCTs that is independent of prednisone treatment. A prospective evaluation comparing vinblastine alone to vinblastine and prednisone combination administration for the treatment of dogs with MCTs would be needed to ascertain the comparative efficacy of the single-agent protocol.

A limitation of our study is the large number of dogs that were withdrawn prior to completion of the 6-week protocol. Of the 95 dogs in our study, only 55 had day 42 tumor measurements for comparison to baseline and a greater number of dogs in the LDI-100 group withdrew early, compared with the vinblastine

group (54.3% vs 30.6%). One possibility that we explored as an explanation for differences in withdrawal rates was that a decline in quality of life for the LDI-100 dogs led owners to opt for other treatment courses or euthanasia more often than did owners of the vinblastine group dogs. However, comparison of changes in CPSs between the 2 groups revealed no significant difference. Assuming CPS to be 1 measure of quality of life, these results suggest that early study withdrawal caused by declining quality of life was not the correct explanation for the observed difference. Lack of tumor response is another factor that could have led to a difference between the 2 arms of the study in terms of early withdrawal. Owners with dogs randomized to the non–standard-of-care arm (LDI-100) may have felt a greater willingness to withdraw their pets from the trial quickly to pursue options that are more traditional. Conversely, with vinblastine-based chemotherapy considered the standard of care, owners with dogs in that arm of the study may have had greater patience to complete the treatment regimen than owners with dogs in the LDI-100 experimental group. After all, the standard of care does represent the best prior prognosis for success on the basis of all previous study results. A willingness to abandon the experimental treatment rather quickly may have been especially true for dogs with a high-grade MCT, for which tumor progression can occur quite rapidly. Because LDI-100 required daily SC administration, it is also likely that owners with dogs in this group were more apt to withdraw their dog from the study and seek a more convenient alternative treatment than were owners of dogs treated once weekly with vinblastine. It could, however, be argued that at-home care with SC injections is actually more convenient than the weekly hospital visits required of the vinblastine group dogs. Finally, the advanced stage of disease at the time of enrollment made many dogs poor candidates for treatment. Quality of life was considered the primary factor in any decisions regarding early study withdrawal.

The standard of care for dogs with MCTs is evolving to include multimodality and multidrug protocols that take advantage of general cytotoxic (standard chemotherapy and radiation therapy) and more targeted (receptor tyrosine kinase inhibitors) treatments.[23,24] We envision that LDI-100 will become an adjuvant treatment that will enhance the clinical response to such protocols.

To our knowledge, this is the first report of the clinical use of an hCG-BCG combination for treatment of naturally occurring malignancy. Given the favorable safety profile of LDI-100, as well as its antitumor effects, further prospective clinical evaluation of the role of LDI-100 in veterinary and human cancer treatment is warranted.

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Appendix

Clinical performance scoring system used to evaluate study dogs. On a CPS form, owners were instructed to circle 1 number that corresponded to the description of the condition of their dog.

Clinical performance scoring system description	Score
Alert, normal appetite, overweight or ideal weight, normal or no change in body condition, no evidence of dehydration, no secondary infections	100
Normal activity and exercise tolerance, normal elimination behavior	90
Signs of mild depression or dull, slow to respond to surroundings, but normal response to physical stimuli; responds to name	80
Mild weight loss, mild decrease in activity and exercise tolerance, wants to exercise but tires more easily, occasional inappropriate elimination with apparent awareness, mildly compromised body condition, mild dehydration	70
Signs of moderate depression; dull, poor response to surroundings; fair response to physical stimuli; does not respond to name; moderate anorexia; eats only special flavored foods	60
Moderate weight loss, mild compromise of physical condition, moderate decrease in activity and exercise tolerance, willing to exercise but tires easily, some inappropriate eliminations with no awareness, secondary infection present	50
Signs of severe depression, stuporous, slow and poor response to surroundings and physical stimuli, severe anorexia, eats special food only when coaxed or hand fed, substantially compromised body condition, moderate dehydration	40
Severe loss of weight and physical condition, severe decrease in activity and exercise tolerance, no desire to exercise, unaware of frequent inappropriate eliminations	30
Semicomatose or comatose, complete anorexia, skin and bones, grossly compromised body condition, severe dehydration	20
Cachexia, skin and bones, no voluntary or involuntary exercise, total incontinence	10
Dead	0