Treatment of Feline Gastrointestinal Small-Cell Lymphoma With Chlorambucil and Glucocorticoids

Gastrointestinal (GI) lymphoma is the most frequently diagnosed form of lymphoma in the cat and is categorized into two distinct forms based on the size of neoplastic lymphocytes. Treatments for both large- and small-cell GI lymphoma have been described previously; however, multiple chemotherapy protocols were used, a minimal amount of histopathological characterization was provided, and, in most studies, the majority of diagnoses were obtained via endoscopic pinch biopsies. Twenty-eight cats (24 with full-thickness intestinal biopsies) were diagnosed with small-cell GI lymphoma and treated with a combination of chlorambucil and glucocorticoids. The majority of cases were strongly CD3+, and many displayed epitheliotropism. The overall clinical response rate was 96%, with a median clinical remission duration of 786 days. Follow-up identified seven cats with relapsed disease—all of which were treated with a rescue protocol of cyclophosphamide and glucocorticoids; the response rate was 100%, and four of the 28 cats were diagnosed with a second malignancy.


Introduction
Feline lymphoma presents in a multitude of anatomical forms, with gastrointestinal (GI) lymphoma being the most frequent form of presentation.1-3 Although the GI form of the disease is most frequently encountered, few reports exist that focus solely on treatment of the GI form.4-7 Often the treatment and outcome of GI lymphoma are included with other forms of the disease, making information regarding treatment of feline GI lymphoma difficult to interpret and compare.

Feline GI lymphoma appears to occur as one of two major types, with a portion of cats being affected by a more indolent, small-cell (lymphocytic) form of lymphoma and others having a more aggressive, large-cell (lymphoblastic) form of lymphoma.4,8 Treatment of large-cell feline GI lymphoma with multiagent chemotherapy protocols has led to median remission durations of 140 to 213 days.6,7 Longer disease-free intervals (DFIs) of 365 and 510 days were reported by Fondacaro et al, although only two (18%) of 11 cats in this study achieved a complete response.4

The purpose of this study is to describe the treatment of feline GI small-cell lymphoma with chlorambucil and glucocorticoids within a well-defined case population.

Materials and Methods
University of Wisconsin-Madison Veterinary Medical Teaching Hospital (UWVMTH) medical records were searched for cats histologically diagnosed with small-cell GI lymphoma and treated with chlorambucil and glucocorticoids between 2001 and 2008. Information recorded from the records included signalment, clinical pathology abnormalities at time of...
Table

Grading of Hematological Toxicities Resulting in Treatment Delays for Cats With Small-Cell Gastrointestinal Lymphoma Treated With Chlorambucil and Glucocorticoids

<table>
<thead>
<tr>
<th>Hematological Toxicity</th>
<th>Grade I</th>
<th>Grade II</th>
<th>Grade III</th>
<th>Grade IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutropenia</td>
<td>&lt;LLN* to 1500/μL</td>
<td>1499 to 1000/μL</td>
<td>999 to 500/μL</td>
<td>&lt;500/μL</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>&lt;LLN to 100,000/μL</td>
<td>99,999 to 50,000/μL</td>
<td>49,999 to 25,000/μL</td>
<td>&lt;25,000/μL</td>
</tr>
</tbody>
</table>

Note: Table modified from VCOG-CTCAE9
* LLN=lower limits of normal

Results
Twenty-eight cats met the entry criteria for this study. Fifteen (54%) cats were castrated males, and 13 (46%) cats were female (one intact, 12 spayed). Of the 28 cats, 19 (68%) were domestic shorthair, five (18%) were domestic longhair, two (7%) were domestic medium hair, one (3.5%) was Siamese, and one (3.5%) was Bengal. The median age at diagnosis was 13 years (range 4 to 20 years). The median weight at time of diagnosis was 4.15 kg (range 2.7 to 6.6 kg).

Available for review were the results of hematological testing in 24 cats from the date of diagnosis; 20 of the cats had abnormalities present. No hematological abnormalities were consistent between cats, and none were severe enough to warrant changes to the proposed therapeutic regimen.

Diagnostic imaging was performed on 27 of the 28 cats. Thoracic radiographs were available for 19 cats, with reported abnormalities being mild enlargement of the cardiac silhouette in eight cats, sternal lymphadenomegaly in four cats, and bronchial disease in two cats. Abdominal ultrasonography was performed on 27 (96%) of the 28 cats prior to diagnosis, with the most frequent finding being intestinal thickening in 22 (81%) of the cats. Details of the remaining five ultrasounds either gave no description of intestinal thickness or were unavailable for review.

Gastrointestinal small-cell lymphoma was diagnosed via histological examination of full-thickness surgical biopsy in 24 (86%) cases and by endoscopic biopsy in two cases. The remaining two biopsies were obtained by the referring veterinarian, and the sampling method was not recorded. Of the 28 cases, 18 (64%) had histopathology specimens available for review at the UWVMTH. After review of the 18 available slides, all cases were consistent with small-cell, or low-grade lymphocytic, lymphoma. The histological description of epitheliotrophic lymphoma was given for 12 (67%) cases, equivocal epitheliotropism was noted in two (11%) cases, and no observable epitheliotropism was seen in the remaining four (22%) cases. Immunohistochemical analysis was performed on 18 cases, with 17 (94%) staining positive for diagnosis, results of pretreatment thoracic radiographs and abdominal ultrasound, date of diagnosis, biopsy method, date of treatment initiation, hematological abnormalities on follow-up clinical laboratory reports, duration of first response, duration of follow-up, and duration of survival. Hematological toxicities were graded according to the criteria established by the Veterinary Cooperative Oncology Group9 [see Table]. Results of postmortem evaluations were included when available. When possible, biopsy specimens were reviewed by one pathologist (Steinberg) to confirm the diagnosis of intestinal small-cell lymphoma. In addition, when an adequate amount of biopsy tissue remained, immunophenotyping was performed using antibodies against CD3, CD20, and CD79a.

All cats were treated with chlorambucil administered at 20 mg/m² orally once every 2 weeks. The chlorambucil dose calculated for each cat was rounded to the nearest whole tablet formulation (2-mg tablets). The type, dose, and frequency of glucocorticoid administered were determined according to the treating clinician’s preference, although 17 (60%) of the 28 cats were started on an immunosuppressive dose of glucocorticoid and gradually tapered to every-other-day therapy.

Cats were considered to be in clinical remission if their clinical signs (i.e., vomiting, diarrhea, weight loss) had resolved. Repeat abdominal sonograms were reviewed when available to document clinical remission or relapse of disease. In cats responding to treatment, the duration of first clinical remission was defined as the time between the first treatment and disease progression or return of clinical signs. For cats considered to be out of clinical remission and receiving a second therapy, the second clinical remission time was calculated as the number of days between the date of rescue chemotherapy initiation and the date they were considered to be out of a second clinical remission. For statistical analyses, cats still in clinical remission at the time of data analysis or cats lost to follow-up before disease relapse were censored at the time of the last known contact.

The Kaplan-Meier product-limit method was used to estimate clinical remission duration.
the CD3 T-cell marker and one staining positive for the CD20 B-cell marker. Fourteen (82%) of the 17 CD3 (+) cases were described as having epitheliotropism.

All cats initially received chlorambucil at a dosage of 20 mg/m² orally once every 2 weeks. Because of client preference, two cats were switched to 20 mg/m² chlorambucil orally once every 3 weeks. Seventeen (60%) of the 28 cats received prednisone or prednisolone at 2 mg/kg orally once daily initially for 1 week; two cats received approximately 1.5 mg/kg orally once daily for 1 week; five cats were started at 1 mg/kg orally once daily for 1 week; one cat received 1.5 mg/kg orally every other day; and one cat received 1 mg/kg orally every other day. All cats had their prednisone/prednisolone dosages tapered to 1 mg/kg orally every other day until disease relapse or progression of disease. Two cats received dexamethasone, initially at immunosuppressive dosages and then at dosages that were gradually tapered over the course of 3 weeks.

Treatment with chlorambucil and a glucocorticoid resulted in clinical remission in 27 (96%) of 28 cats, with a median duration of 786 days for the first clinical response [Figure 1].

The median number of chlorambucil doses received per cat was 23 (range 5 to 110). Three treatment delays were reported as a result of hematological toxicities in cats treated with chlorambucil, one episode of a grade II thrombocytopenia, one episode of a grade II neutropenia, and one episode of a grade III neutropenia. None of the recorded toxicities required any additional therapy, and all resolved with treatment delay. Four (14%) of the 28 cats developed a second malignancy during the study period. Diagnoses included a mammary carcinoma, gastric mast cell tumor, intraabdominal carcinomatosis, and poorly differentiated oral squamous cell carcinoma with metastasis to regional lymph nodes. The total numbers of chlorambucil doses received by cats developing a second malignancy were 34, 35, 38, and 74, respectively. The time lengths between diagnosis of small-cell GI lymphoma and the secondary malignancies were approximately 520, 542, 864, and 1456 days, respectively.

Of the 28 cats in the study, seven (25%) died, 10 (36%) were alive, and 11 (39%) were lost to follow-up at the time of analysis. Of the seven cats that died, two had no evidence of lymphoma at the time of death (based on necropsy results for one and a lack of clinical signs in the other), one cat died of septic cholangiohepatitis with GI lymphoma present on postmortem, and four cats had unknown causes of death. Nine (32%) of the 28 cats were documented with disease recurrence based on the return of clinical signs and/or ultrasonographic changes consistent with relapse. Seven of the nine cats with relapsed disease were treated with oral cyclophosphamide at a calculated dosage of 200 to 250 mg/m² given on days 1 and 3 out of every 2 weeks (25 mg given Monday and Wednesday every other week) and prednisolone (5 mg every other day). All seven of the cats rescued with cyclophosphamide responded based on resolution of clinical signs and normal abdominal palpation. Of the seven cats with relapsed small-cell GI lymphoma, three were in clinical remission at the time of death due to unrelated disease, three were in clinical remission at the time they were lost to follow-up, and one had disease recurrence based on return of clinical signs. The durations of second clinical remission for the three cats that had died of unrelated disease were 239, 283, and 418 days. Three cats were lost to follow-up at 116, 162, and 946 days. The final cat relapsed 241 days after starting cyclophosphamide and prednisolone.

**Discussion**

This retrospective study supports prior findings that chlorambucil and a glucocorticoid result in a long duration of clinical remission for cats with GI small-cell lymphoma. Kiselow et al reported a duration of 897 days for first clinical remission in cats achieving a complete response, which compares favorably with the DFI reported herein of 786 days. Similarily, the study by Fondacaro et al reported a median DFI of 615 days.

In the current study, chlorambucil was administered at 20 mg/m² every 2 weeks compared to 2 mg orally every 2 to 3 days. The administration of chlorambucil on a biweekly basis rather than daily or every other day has been reported previously in humans with chronic lymphocytic leukemia and lymphocytic lymphoma with similar results compared to continuous single-agent or combination chemotherapy regimens.10,11 The ability to administer chlorambucil to cats on a biweekly basis rather than every 2 to 3 days while maintaining a prolonged duration of clinical remission is a logistic advantage.

One of the strengths of the current study is the high percentage of full-thickness surgical biopsies over endoscopic-obtained biopsies. Acquiring full-thickness surgical biopsies allows for a more thorough histopathological characterization of lesions due to the increased amount of tissue avail-
able for review. This results in a more complete understanding of the disease pathology, as evidenced by this study’s ability to additionally classify lesions as epitheliotrophic in 12 (67%) of the 18 samples available for slide review. A previous report suggests that endoscopic pinch biopsies are inferior to full-thickness biopsies obtained via exploratory laparotomy for differentiating between inflammatory bowel disease and small-cell GI lymphoma. Potential drawbacks of full-thickness biopsies include increased expense, time, and invasiveness/morbidity associated with obtaining them. None of the cats in this study had intra- or postoperative complications that resulted in a delay of treatment initiation.

The majority of cases for which adequate tissue remained for slide review and immunohistochemical analysis had a histological diagnosis of epitheliotrophic T-cell lymphoma. The combination of histological description, immunphenotype, and response to therapy has not been reported for cats with small-cell GI lymphoma. The majority of cases of GI lymphocytic lymphomas in a report by Fondacaro et al were described as having epitheliotropism, though no immunohistochemical analysis was performed. Carreras et al reported on 10 cases of feline epitheliotrophic intestinal lymphoma, all of which were described as consisting of small to intermediate-sized lymphocytes, and all stained positive for presence of the CD3 T-cell marker.

A median survival time of approximately 330 days was reported for nine of the cats that received prednisone with or without other chemotherapeutics. Multiple chemotherapy drugs were used in the treatment of these cats; however, no cat received chlorambucil, no standardized protocol was reported, and no toxicity information was available. That the majority of feline GI lymphomas are of T-cell origin with epitheliotropism is similar to a previous report characterizing canine GI lymphoma. Unfortunately, the response to therapy and clinical outcomes in those cases of canine GI lymphoma were not reported. Additional studies are needed to determine if a clinical significance is associated with epitheliotropism versus nonepitheliotropism in feline small-cell GI lymphoma. What is clear from this and previous studies on feline GI lymphoma is the more prolonged remission times associated with the small-cell form of the disease compared to the more typical large B-lymphocyte form of the disease.

Although not a primary objective of this study, seven cats with relapsed disease were treated with cyclophosphamide, with a 100% response rate and a median clinical remission time of 241 days. To our knowledge, this is the first study to report on the response to rescue chemotherapy for cats with relapsed small-cell GI lymphoma.

The strengths of this study include the high percentage of cats diagnosed with full-thickness surgical biopsy of the intestine, the histopathological and immunohistochemical characterizations of the majority of cases included in the study, and the reporting on the responses of cats with relapsed disease to rescue therapy. Additionally, one of the advantages of the currently reported dosing scheme is the relative infrequency of medicating the cat while maintaining a favorable clinical outcome.

Shortcomings of this study include the lack of thorough staging in all cats and the reliance upon resolution of clinical signs to signify disease remission. Also, the lack of standardized recheck examinations hinders the determination of adverse side effects associated with treatment. Finally, the large number of cats lost to follow-up, even with attempts being made to contact owners, is disappointing. All three of these limitations are inherent in a retrospective study design. Even with these study limitations, we are able to conclude that the treatment of feline small-cell GI lymphoma with the combination of chlorambucil and glucocorticoids is well tolerated and associated with a long initial remission time, similar to previous reports.

Future studies may focus on the optimal dosing schedule for chlorambucil in the treatment of small-cell lymphoma. All three studies on GI small-cell lymphoma utilized different treatment schedules, yet all were associated with a prolonged, first clinical response duration. The dose intensity of protocols used may be increased, as the hematological toxicities associated with the reported treatment protocols seemed to be low, with few treatment delays being necessary. Altering the dose intensity would allow us to determine if more frequent treatments or higher dosages would lengthen median remission times. However, as cats with GI small-cell lymphoma treated with chlorambucil already have prolonged clinical remission durations, the ability to detect significant differences in remission durations may be difficult.

Alternatively, it would be interesting to determine if continuous dosing with chlorambucil is required for the prolonged duration of remission times. Cats respond well to the currently published treatment protocols and in many cases receive chlorambucil for many months. The use of continuous chemotherapy in human studies has been associated with an increased risk of developing a second malignancy or hematological abnormality. Alkylator-purine analog combinations may increase the risk of therapy-related myeloid malignancy. An increased frequency of secondary malignancies, including epithelial neoplasms, was noted in cats receiving long-term chlorambucil for indolent chronic lymphocytic leukemia compared to a nontreated case population. Because chlorambucil is a slow alkylator, chronic use results in constant insults to normal DNA, and carcinogenesis is a risk. We did not compare the incidence of developing a second malignancy in cats with other forms of cancers associated with prolonged survival times; therefore, no statements may be made regarding an increased risk of secondary malignancy development associated with continuous chlorambucil treatment. However, it is striking that four (14%) of the 28 cats evaluated developed a second malignancy during their course of treatment.

Another potential explanation for the development of a secondary malignancy in this population of cats is that prolonged survival increases the chances of a mutation occurring in another cell type that could contribute to cancer formation. Finally, chronic continuous chemotherapy may
alter the immune system’s ability to identify and destroy abnormal cells. Second malignancies in this study included a poorly differentiated oral squamous cell carcinoma with metastasis to the regional lymph nodes, intraabdominal carcinomatosis, mammary carcinoma, and a gastric mast cell tumor. The second malignancies reported here are similar to the aforementioned study reporting an increased frequency of developing epithelial-based malignancies while being treated continuously with chlorambucil.16

Conclusion

Our finding of long initial remission times, particularly a median first remission time of 786 days, for cats with GI small-cell lymphoma treated with chlorambucil is consistent with those previously published. Dosing delays due to hematological toxicities were minimal in this study and previous reports; however, the finding of second malignancies in the current study is concerning and warrants additional examination.

References