Lymphoma is the most commonly-encountered systemic cancer of dogs. It’s critical for the small animal clinician to recognize this disease in its various forms, and once the diagnosis is made, to be able educate clients on the appropriate diagnostic tests, the available therapies, and the expected benefits, costs and risks of treatment. As a first step, referral for care by an ACVIM board-certified oncologist should always be offered, but canine lymphoma patients can be successfully and safely treated by general practitioners, provided that protocol guidelines are followed, and proper measures for minimizing personnel exposure to chemotherapy agents are in place.

The cause of lymphoma in dogs is unknown. Exposure to environmental factors such as herbicides and magnetic fields have been associated with higher risks in some studies. Chromosomal and genetic aberrations have been found in malignant lymphocytes. The differential probability of specific breeds developing lymphoma also supports the role of genetic factors in lymphomagenesis. For example, Basset Hounds, Boxers, Bulldogs, Bullmastiffs, Golden Retrievers, Rottweilers and Scottish Terriers are at increased risk. It’s less common to see Chihuahuas, Dachshunds, miniature and toy Poodles, Pekingese and Pomeranians with lymphoma, since these breeds have a decreased risk of developing this cancer.

Lymphoma is a malignancy of lymphocytes; their generally mobile nature means that lymphoma is often, but not always, a disseminated disease. Nonetheless, the distribution of lymphoma cells is non-random, and presumably recapitulates the normal trafficking/homing behavior of the non-malignant parent cells. Thus, lymphoma can be classified by anatomic site, which is one of the two most important factors in predicting the cancer’s response to chemotherapy and patient outcome. The second critical predictor is histologic grade, which can be conveniently divided into two classes: intermediate-high (diffuse large cell; immuno- or lympho- or centroblastic) or low (small cell; lympho- or centrocytic). With these two pieces of information in hand, one can normally formulate an appropriate treatment plan and reasonably anticipate the long-term outcome for an individual patient. Let’s now look at dogs with lymphoma in the clinical setting.

Pre-treatment considerations

*What are typical presentations for canine lymphoma?* The high-grade, multicentric form is most common, and should be familiar to most practitioners [when not otherwise stated, this is the form that’s being referred to in these guidelines]. The non-febrile dog that is feeling well and has moderately-to-markedly enlarged, firm peripheral lymph nodes, including those that are not normally palpable (axillary; inguinal; facial), has lymphoma until proven otherwise. Infectious causes are unlikely, and fine needle aspirate cytology (FNAC), not a trial of antibiotic therapy, is the next immediate diagnostic step. Sometimes such lymphadenopathy is discovered by owners, who will report progression over a week or two; in other cases, nodal enlargement is discovered incidentally on routine exam or during grooming.

Occasionally, patients present with mild, progressive, non-specific or predominantly gastrointestinal (GI) signs of illness without peripheral lymphadenopathy, and upon subsequent evaluation by abdominal radiography or sonography, are found to have substantially enlarged intra-abdominal nodes (with or without splenic & hepatic changes); FNAC often demonstrates high-grade lymphoma. Far less commonly, high-grade lymphoma presents as isolated hepatosplenomegaly or a GI tract mass.

In some dogs without lymphadenopathy, lymphocytosis (>5000/μL) is noted on a complete blood count (CBC). When the patient being screened is ill and the cells have a blast morphology, the diagnosis is likely acute lymphoblastic leukemia (ALL); when the patient is clinically normal and the cells are small-to-intermediate in appearance, the diagnosis is likely chronic lymphocytic leukemia (CLL). Both are lymphomas of the bone marrow (designated as anatomic site E by the World Health Organization...
[WHO]), with ALL being the high-grade form, and CLL, the low-grade form. Dogs with lymphoma may also present for polyuria and polydipsia, caused by hypercalcemia. Symptomatic patients with confirmed, moderate-to-high elevations in serum calcium (total >14 mg/dL) without an anal sac mass presumably have T-cell lymphoma, and an immediate hunt for the tumor should begin. If obvious lymphadenopathy is absent, then the mediastinal (thymic) form of lymphoma is possible (this site is incriminated in ~40% of dogs with lymphoma and hypercalcemia), and chest radiographs to rule this possibility in or out are the next step.

Not uncommonly, in dogs, lymphoma can also present exclusively as solitary-to-generalized skin or oral cavity lesions, whose appearance can vary from diffuse areas of erythema to crusted plaques to subcutaneous nodules. Other primary anatomic sites of lymphoma in dogs that are very infrequently encountered (classified as site F by the WHO) include the CNS, eye and kidneys.

How is the diagnosis of lymphoma confirmed? Typically, for high-grade nodal forms, FNAC is sufficient. In a recent study, the accuracy of cytology in canine B- and T-cell lymphomas was >92%; that is, when the diagnosis is made, it’s likely to be histologically correct. When the diagnosis cannot be confirmed (for example, when there’s only a preponderance of intermediate-sized lymphocytes), a biopsy is needed, which is often most expeditiously obtained by removal of an enlarged peripheral lymph node. Analogously, if the spleen is the sole site of disease, a splenectomy is indicated. In many of these cases, a low-grade or indolent lymphoma may be diagnosed, and the treatment plan will likely quite different (see below) than those typically chosen for high-grade forms. For dermal or oral lymphomas, a punch biopsy is almost always needed to provide the diagnosis and prescribe appropriate treatment.

What staging is necessary? The WHO clinical staging scheme for canine lymphoma, familiar to most clinicians, consists of five stages ranging from Stage I (single lymphoid node/organ involvement) to Stage V (involvement of blood, marrow or other non-lymphoid/hepatic organs). While it may be gratifying to define the extent of a patient’s disease, this estimate is likely to only reflect the sensitivity of the technology used to make the measurements. For example, in one study, 80% of dogs diagnosed with Stage III (multicentric nodal) lymphoma had circulating tumor cells (Stage V) when a sensitive molecular test (PARR – see below) was used to screen for blood involvement. Moreover, currently, it’s primarily the anatomic site and histologic grade (and the patient’s performance status) that informs treatment decisions, not stage. Thus, regardless of stage, dogs with high-grade, non-cutaneous lymphoma are always treated with successive multi-agent chemotherapy protocols until complete chemoresistance is encountered, at which time no further therapy is possible. Of course, more information is always helpful when managing patients, and staging in itself poses minimal risk, but the financial burden of such evaluation can often later limit the ability of owners to pay for chemotherapy or for managing adverse effects (the treatment-induced GI or septic event that requires hospitalization), so should only be embarked on after careful consideration of true benefit. In simple terms, the biggest gain of staging would be determining whether there is extranodal involvement that indicates a much poorer-than-normal prognosis, such as intestinal or renal infiltration, which is rare. Should staging be undertaken, there are few questions that are frequently asked, such as: Do I need to acquire FNAC specimens from multiple enlarged nodes? No, a single node is representative. Should I pool samples from multiple nodes for FNAC to save costs? No, if nodes are heterogeneously affected, “dilution” from a less-affected node may preclude a diagnosis being made, ultimately costing more. If peripheral and intra-abdominal nodes are involved, should I perform FNAC from each site? No, the information is likely to be redundant. The same applies to the spleen; an obviously-infiltrated (“Swiss cheese”-appearing) spleen does not require separate FNAC if more-accessible peripheral nodes are involved. Should I routinely perform a bone marrow aspirate cytology? Such an analysis is helpful when unexplained cytopenias are encountered, or potentially, when a leukemia is being profiled, but the results are unlikely to alter the treatment plan.

In some rare cases, staging is indispensable. For example, such evaluation is needed if the lymphoma is to be treated by non-systemic means, such as surgery or radiation (e.g., for a single oral lymphoma lesion), or when there’s risk of a serious adverse effect, such as acute tumor lysis syndrome (ATLS), when half-body radiation is being given to a bulky lymphoma burden.
How are flow cytometry and PCR for Antigen Receptor Rearrangement (PARR) testing useful? Flow cytometry (“flow”) is an antibody-based test that looks at single living cells in liquid suspension (acquired by FNA) for specific defining proteins, often designated by a CD (Cluster of Differentiation) number. Flow is analogous to immunohistochemistry on fixed/frozen biopsy specimens, but is much quicker. Flow is the most expedient means of assigning a phenotype (B-, T- or null [undetermined] cell lineage) to the malignant lymphocytes. Identifying an abnormal preponderance of one type of lymphocyte in a node or organ is consistent with lymphoma, but in itself this finding should not be taken as definitive (with the possible exception of an FNA sample from the thymus). Only in a minority of cases (~10%), where malignant lymphocytes aberrantly express CD markers of both B and T cells can flow results be considered strongly (but not conclusively) in making the diagnosis of lymphoma. It’s also important to remember that flow results interpreted in isolation can sometimes be misleading to the clinician. For example, flow of a peripheral blood sample with small-cell CD34⁺ lymphocytosis might be labeled “CLL” by the flow cytometrist, even when the patient has significant lymphadenopathy that is most consistent with multicentric lymphoma. That distinction is important to make, for a multicentric lymphoma patient will fare much better with standard lymphoma chemotherapy, than with the less-intense treatment that is used for managing CLL.

PARR is a genomic DNA-based test that amplifies a tiny portion of the B or T cell receptor that serves as the fingerprint of the lymphocyte. When a sample, such as blood, or node/spleen aspirate contains lymphoma, PARR demonstrates that too many cells have the same fingerprint, and are thus clonal – a signature of cancer. Because PARR is not perfectly specific (some non-neoplastic immune reactions can appear clonal), the assay’s probably most useful in making a strong circumstantial case for lymphoma that is cytologically suspicious, but not definitive, when a biopsy is not permitted or feasible (for example, with probable splenic lymphoma and marked thrombocytopenia). Like flow, PARR can also assign a B- or T-cell phenotype to malignant lymphocytes.

The most common perception of the utility of flow and PARR at diagnosis is that it’s critical to distinguish B- from T-cell lymphomas. While it’s true that dogs with high-grade T-cell lymphoma collectively fare worse than those with B-cell types, it’s unclear how useful phenotype is for predicting the fate of an individual patient, since unknown idiosyncratic factors seem to most strongly determine outcome. Moreover, since B- and T-cell lymphomas are often treated identically, phenotypic determination should be considered optional.

What evaluation/testing is essential at the time of diagnosis? Some dogs with lymphoma have paraneoplastic uveitis that requires topical treatment to prevent blindness, so an eye exam is always warranted. Recording the size of palpable peripheral lymph nodes is helpful at successive weeks of treatment to determine the response to individual agents in the protocol. Similarly, for the skin form, a topographic map of lesion location with accompanying digital photographs is incredibly helpful in directing therapy. A CBC, chemistry panel and urinalysis (acquired no longer than 1-2 days prior to initiating therapy) are mandatory. The hemogram may show particular abnormalities that require precautions or treatment (e.g., blood loss or immune-mediated hemolytic anemia; significant neutropenia or thrombocytopenia). The chemistry panel and urinalysis provide critical information on calcium status, the rational dosing of chemotherapy drugs, and potentially, the ability of the patient to handle large-scale tumor die-off (i.e., risk of ATLS) upon induction. Finally, it’s critical for a patient belonging to a breed with a high likelihood of carrying the “MDR1 mutation” (ABCB1-1Δ; breeds include Australian Shepherd, Collie, Longhaired Whippet, Silken Windhound and Shetland sheepdog) to be genotyped for the polymorphism, as mutants are at greatly increased risk for adverse reactions to vincristine and doxorubicin.

Treatment considerations

High-grade lymphomas are primarily treated with cytotoxic chemotherapy. The clinician’s goal with such treatment is complete remission (no evidence of cancer by standard diagnostic methods), and the restoration of pre-lymphoma performance status. Patient owners, of course, want the same thing: their dog back to normal, with minimal disruption of quality of life and lowest possible expense. Common
questions prior to starting treatment are, not surprisingly: How sick will my dog get? Will he/she lose his/her hair? How long will he/she live? How much will it cost? Obviously, the answers vary, but most patients don’t get very sick with standard chemotherapy; only dogs with continuously-growing hair lose significant amounts of their coat; survivals of one year are not uncommon; and expenses with chemotherapy usually run in the several thousands of dollars. Accurately and confidently communicating with owners about what to expect when treating lymphoma is key in many cases to allaying natural fears about cancer and chemotherapy, and permitting treatment to go forward.

Induction is administration of the first chemotherapy agent(s) to produce remission, and should commence as soon as the diagnosis is made, and any staging, if performed, is completed. Vincristine is a potent anti-lymphoma drug that is commonly used to initiate induction in dogs. As a general principle, the “best” drugs are offered first, at the highest dosages that have been shown to be tolerated by almost all dogs. Of course, when an individual patient’s drug doses are calculated, any idiosyncratic factors that could reduce drug tolerance should be considered, including liver dysfunction, obesity, MDR1 mutation or concurrent administration of drugs that inhibit drug excretion/metabolism pathways, such as ketoconazole. Drug doses are generally calculated based on body surface area (BSA), and most veterinary formularies have tables that permit conversion from weight in kilograms to BSA in M$^2$ for dogs. It’s important to double-check a patient’s weight before beginning chemotherapy, as the wrong dose can lead to serious adverse effects or death. Additionally, at least two trained individuals should verify each individual dose prior to administration.

In multiagent protocols, a patient’s probability of developing GI-related illness or myelosuppression is highest throughout the first cycle, since the individual’s drug tolerance is unknown, and each agent is being administered for the first time. Even if well-tolerated, doses of a particular agent are rarely escalated at succeeding treatments, but conversely, are always reduced if significant adverse effects occur (neutropenia <1000/μL; platelets < 50,000/μL; GI-related illness requiring hospitalized care; prolonged/profound anorexia & weight loss). Once lowered, it’s not usual practice to return to starting dosages. Finally, there’s no predictable correlation between susceptibility to one agent and another; thus, the dog that does not initially tolerate a standard dose of vincristine does not automatically require an extrapolated reduction of the doxorubicin dose at the first administration.

What is the best therapy for high-grade forms of lymphoma? Chemotherapy regimens incorporating multiple efficacious drugs produce superior outcomes when compared to treatment with single agents. At least a dozen multiagent protocols have been described for dogs, with most being variants of a combination of Cytoxan (C [cyclophosphamide]), hydroxydaunorubicin (H [doxorubicin]), Oncovin (O [vincristine]) and prednisone (P), referred to collectively as CHOP. CHOP is the standard recommendation of most veterinary oncologists for canine high-grade lymphoma. The three cytotoxic agents are administered on a rotating weekly basis, with prednisone administration typically concluding after the first cycle. Other drugs, such as L-asparaginase, CCNU or methotrexate are sometimes included in modifications of CHOP, but it remains unclear whether such additions meaningfully improve outcomes. There is evidence that T-cell lymphomas respond less frequently to doxorubicin than their B-cell counterparts, and one study reported improved outcomes in T-cell lymphomas treated with a combination of mechlorethamine, Oncovin, procarbazine and prednisone (MOPP) with L-asparaginase, but there is no clear-cut superiority to this more intense, expensive protocol, so CHOP remains the frontline recommendation of most oncologists, and MOPP is usually reserved for the rescue setting.

Most patients can be induced as outpatients. Dogs that are sick, or hypercalcemic, or have subjectively large cancer burdens or renal dysfunction that are thought to predispose to ATLS, are often hospitalized for monitoring and intravenous fluid therapy for the first few days after the administration of their initial chemotherapy.

L-asparaginase is sometimes very effective as a sole induction agent (with prednisone) when there is lymphoma-related hepatic dysfunction that precludes safe dosing of any of the three principal CHOP agents (doxorubicin and vincristine have impaired clearance, resulting in overdosage; cyclophosphamide is not converted to the active form, resulting in underdosage). In many cases, liver function returns to normal in 2-5 days, permitting follow-up administration of vincristine to continue the induction process.
Naturally, for some owners, the time commitment (~weekly treatments) and costs of CHOP are too onerous, and alternative chemotherapy regimens are sought. Table 1 lists the most commonly employed alternates, and their efficacy and approximate monthly cost (at NCSU-CVM) (COP is Cytoxan, Oncovin & prednisone; CCNU [lomustine] is given as a single agent).

**How is the lymphoma patient managed over the course of treatment (using CHOP as an example)?** Dogs with high-grade lymphoma can have one of four responses to chemotherapy: complete remission (CR), partial remission (PR), stable disease (SD), or progressive disease (PD). With CHOP, most dogs should attain a CR within the first cycle (~1 month) of chemotherapy; in fact, some patients will have dramatic softening of their nodes within 24 hours of initiating treatment, and will be in a CR one week after a single vincristine dose, but others may take longer. For dogs with peripheral lymphadenopathy, physically measuring lymph node size prior to administration of each chemotherapy dose is sufficient to assign remission status, and all other sites of disease (e.g., the spleen) are assumed to respond synchronously (and are thus not verified), as long as the patient is doing well. For patients with only internal disease (e.g., mediastinal lymphoma), repeated imaging is usually performed prior to successive CHOP cycles to assess remission; thus, the management of these patients is more costly than for the typical dog with peripheral disease.

One of the most noticeable adverse effects of CHOP and other chemotherapies in dogs is GI illness, seen 3-5 days post-treatment. In general, 75% of dogs will have no significant signs, 20% will have signs that are manageable with at-home symptomatic care, and 5% might require 1-3 days of hospitalized care. Significant myelosuppression (neutropenia, manifesting as lethargy, inappetance and fever) is uncommon (<10% of patients), but when it occurs, is usually observed 5-8 days post-treatment. With IV antibiotic therapy and supportive care, most patients recover uneventfully in 1-3 days.

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Response rate (%)</th>
<th>Median remission (months)</th>
<th>Median survival (months)</th>
<th>Cost ($ per month)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOP</td>
<td>85</td>
<td>8.5</td>
<td>12</td>
<td>850</td>
</tr>
<tr>
<td>COP</td>
<td>75</td>
<td>4.7</td>
<td>8</td>
<td>625</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>75</td>
<td>5.3</td>
<td>7.3</td>
<td>600</td>
</tr>
<tr>
<td>CCNU</td>
<td>53</td>
<td>1.4</td>
<td>4</td>
<td>475</td>
</tr>
</tbody>
</table>

There are a several scenarios that are commonly encountered in treating dogs with high grade multicentric LSA that are helpful in illustrating general principles of management. The most important fact to remember is that when a particular agent is perceived by the clinician to stop “working”, then it should no longer be used, and when possible, a substitute agent (ideally with a similar mechanism of action) can be administered. What “working” means is not a fixed definition, though; in later stages of treatment, purely stable disease might represent an acceptable goal, but in the early stages, reasonable efficacy generally means inducing/maintaining some form of remission, with a duration of effect of no less than two weeks (i.e., brief lymph node shrinkage followed by enlargement 5 days post-administration indicates ineffectiveness of the drug).

Many dogs treated with CHOP rapidly obtain a CR, which is maintained throughout the protocol. In those cases, one may know nothing about the efficacy of any individual agent besides the one that induced remission, and treatment is given automatically as long as the patient has a good quality of life, CBCs show drug tolerance, and the cancer remains undetectable, until the protocol is completed (see below). At the other end of the spectrum are those patients that prove to be refractory (resistant) to all CHOP agents, and then successive trials of other second-line protocols/agents (“rescue” or “salvage”; Table 2) are indicated, although the prognosis is poor, presumably due to inherent chemoresistance. There are two other common scenarios. In the first, the dog attains a PR with the first CHOP cycle, which
remains stable thereafter. While technically refractory, such patients may have a good quality and quantity of life, as long as chemotherapy is continued indefinitely. Finally, there is a subset of patients that attain a CR or PR with CHOP that subsequently progress while still receiving therapy. It is sometimes possible to reverse or arrest that progression by substituting a new agent for the drug administered immediately prior to overt loss of remission. Thus, as an example, if a dog formerly in CR presents with lymphadenopathy one week following cyclophosphamide administration, one might continue with the that week’s planned administration of doxorubicin or vincristine, and then substitute another alkylating drug, chlorambucil, when the suspect drug (cyclophosphamide) is due. This strategy is sometimes successful, although early failure usually presages a poorer outcome for that patient. And once doxorubicin is lost from CHOP, most clinicians opt to offer a rescue protocol rather than trying drug substitution. It’s important to note that remissions obtained in the rescue setting are much shorter (Table 2) than those obtained up-front with CHOP, COP or even single-agent doxorubicin (Table 1).

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Response rate (%)</th>
<th>Median remission (months)</th>
<th>Cost ($ per month)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOPP</td>
<td>65</td>
<td>2.2</td>
<td>1650</td>
</tr>
<tr>
<td>CCNU/L-asparaginase</td>
<td>82</td>
<td>2.4</td>
<td>550</td>
</tr>
<tr>
<td>CCNU</td>
<td>27</td>
<td>3.1</td>
<td>475</td>
</tr>
<tr>
<td>Dacarbazine</td>
<td>35</td>
<td>1.5</td>
<td>900</td>
</tr>
</tbody>
</table>

Dogs with high-grade, multicentric lymphoma that are not treated have short survival times of one or two months; most are euthanized because of the development of signs of systemic illness, GI disease, airway-obstructive lymphadenopathy, and general loss of quality of life. It’s well known that prednisone at immunosuppressive doses (30 mg/M² once daily) can delay this outcome by a few weeks. As most practitioners know, corticosteroid administration prior to injectable chemotherapy reduces the duration of remission, so it should be made certain that chemotherapy is not desired prior to embarking on this palliative course. For owners that want treatment with minimal side effects and cost, a combination of prednisone and oral cyclophosphamide (50 mg/M² once daily for four consecutive days per week) is a reasonable approach that may prolong quality of life for two to three months. Of course, there are also some owners that are attracted by inclination to alternative or “natural” therapies for lymphoma; however, effective treatments in this category have not been convincingly described, nor can be recommended.

Not all canine lymphomas are best treated with CHOP; there are two noteworthy exceptions. Epitheliotropic lymphoma is a cutaneous T-cell variant that is usually CHOP-resistant and is commonly treated with CCNU (with prednisone). Eighty-percent of dogs have a response (only 17-30% CRs, however) averaging three months’ duration. Low-grade “indolent” lymphomas of the spleen and/or lymph node, or in the bone marrow (CLL), are sometimes vigilantly monitored but receive no treatment; with progression, chronic chlorambucil and prednisone are often prescribed, yielding long survival times.

Radiation therapy is infrequently used for treating canine lymphoma, but is sometimes employed for definitive treatment of solitary lesions, palliation of disease related to mass effect (e.g., massive mandibular lymphadenopathy; rectal, brain or mediastinal lymphoma); or half- or total-body consolidation therapy to treat minimal residual disease.

Post-treatment considerations

With successful induction, CHOP chemotherapy is usually discontinued after 5 or 6 cycles, since there is no demonstrable advantage to continued (maintenance) treatment. During the post-CHOP phase, one should monitor the dog monthly for relapse (loss of CR) by physical examination and any other test
that might be helpful for the particular patient, based on their initial presentation; for example: CBC (lymphoblasts); total calcium (hypercalcemia); thoracic radiographs (mediastinal mass).

A suspected relapse should be confirmed by FNAC. If the owner is willing, then the best approach is to re-start CHOP, substituting mitoxantrone for doxorubicin when the cumulative dose of the latter drug has reached the safety limit of 180 mg/M². The majority of patients whose lymphoma responded well to CHOP the first time will respond equally well again, although the duration of that response, in most cases, will be shorter. The guidelines for drug substitution or protocol abandonment described above once again apply.

Ultimately, virtually all canine lymphoma patients become increasingly, and eventually, completely chemoresistant, as successive rescues are attempted. At that point, it’s common for dogs to return to a state similar to their initial presentation, with the oncologist prescribing weekly trials of various single agents that, unfortunately, usually have minimal to modest, short-lived effects on the cancer. Treatment is typically discontinued once the dog’s quality of life is significantly impacted by the disease or chemotherapy, and survival then is often measured in weeks.

Prognosis

The survival of patients with high-grade lymphomas treated with aggressive chemotherapy is best predicted by the predominant anatomic site. On average, dogs with the multicentric form survive 12 months, with 25% alive at 24 months. Dogs with epitheliotropic lymphoma survive 6 months. Dogs with lymphoma of the GI tract, liver or bone marrow (ALL) live for 2 months; with CNS involvement, survival is usually half that – just 1 month. Dogs with low-grade, indolent lymphomas (e.g., T zone; CLL) can survive for years, sometimes without treatment, but unfortunately remain incurable.