Lymphoma is the most common haematopoietic malignancy in dogs, and is the most responsive to chemotherapy. Affected dogs are typically middle-aged. Neither gender nor neutering is a predisposing factor for developing lymphoma. In studies of canine lymphoma epidemiology, boxers, Scottish terriers, German shepherds and poodles were more often affected, and recent evidence suggests a high incidence in golden retrievers. The most common physical finding in dogs with lymphoma is peripheral lymphadenopathy, which is usually generalized but may be localized to a single lymph node or a region of the body. Involvement of other organs, such as spleen, liver, or bone marrow is an indication of advanced disease. Some dogs may be presented with bilateral ocular disease which can include uveitis, conjunctivitis or orbital involvement; involvement of other (extranodal) sites is rare in dogs.

Untreated lymphoma progresses rapidly (1–2 months) from presentation to terminal stages. With chemotherapy, however, considerable improvement in the duration and quality of the patient’s life can be expected. Advances during the past decade in not only chemotherapy, but also histologic subclassification and supportive care have dramatically improved the prognosis for a dog with lymphoma.

**Staging and Diagnosis**

Lymphoma is a systemic disease; therefore, it is important to determine the extent of organ involvement with lymphoma and to identify unrelated or secondary conditions that need to be treated or controlled before instituting appropriate therapy. Staging carries prognostic significance and enables the veterinarian and client to make informed and rational decisions as to the type of therapy best suited for the patient. Each dog is clinically staged based on the results of physical examination, clinical laboratory testing (i.e., CBC, biochemical profile, urinalysis, and bone marrow cytology), and imaging procedures (i.e., radiography and ultrasonography); see Table 1.

**Table 1: Clinical stages of canine lymphoma**

<table>
<thead>
<tr>
<th>Clinical Stage*</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>Involvement limited to single node or lymphoid tissue in single organ (excluding the bone marrow)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>Regional involvement of many lymph nodes, with or without involvement of the tonsils</td>
</tr>
<tr>
<td>Stage 3</td>
<td>Generalized lymph node involvement</td>
</tr>
</tbody>
</table>
### Stage 4
Involvement of liver and/or spleen, with or without generalized lymph node involvement

### Stage 5
Involvement of blood, bone marrow, and/or other organs

*Stages are further classified to clinical substage a (no clinical signs) or b (with clinical signs / hypercalcaemia). For example, Stage IIIa describes a dog with generalized lymphadenopathy and no clinical signs.

Cytologic examination of lymph nodes may be compatible with a diagnosis of lymphoma but does not always provide a definitive diagnosis. A definitive diagnosis is based on histologic examination of a lymph node biopsy. Examination of nodal architecture enables the pathologist to assign a grade, and immunohistochemistry for T and B lymphocyte markers; both of which are important for prognosis (see below) and selection of an appropriate treatment protocol. The most accessible lymph node is the popliteal lymph node. More recently the ability to provide immunophenotyping on well-prepared cytology samples means that a biopsy is not always necessary for diagnosis and T- and B-cell differentiation. While immunocytochemistry for T-cell and B-cell markers is ideal for high grade lymphoma; if the cytology suggests low grade lymphoma (sometimes termed indolent lymphoma), we recommend that a complete node be removed for accurate grading.

### Prognostic Factors
Recent studies have identified multiple factors that can help provide a prognosis for the individual patient. Currently lymphoma is one of the best characterized of canine tumours with regard to prognostic factors. It is clear that good clinical staging and ancillary laboratory testing provide important prognostic information. In the future, histopathologic “subtyping” of lymphoma using specific antibodies and cell markers may provide further prognostic information for individual patients. At present, however, the features known to be important in predicting outcome for dogs with lymphoma are described below:

#### Immunophenotype
Differentiation of phenotype is now widely available by immunostaining for CD-3 (T-cell) and CD-79a (B-cell) that can be performed on histopathology specimens. The majority (75-80%) of canine lymphomas reported have been B-cell. Some lymphoma cells fail to stain with either antibody; these so called “null-cell” tumours account for less than 5% of cases in most studies, and no prognostic significance has so far been ascribed to them. In some cases it may be a failure of the staining rather than a true immunophenotype. Most studies found that dogs with B-cell lymphoma were more likely to achieve a complete remission (81-84%) than dogs with T-cell lymphoma (50-67%).\(^1\)\(^-\)\(^3\) In addition, dogs with B-cell lymphoma have a much longer remission duration and survival than dogs with T-cell lymphoma. Often these studies found that immunophenotype was a stronger predictor of remission duration than any other potential factor studied.

It is important, however, not to delay starting chemotherapy while waiting for the immunophenotyping result to become available.

#### Substage
While the stage of disease is not always of prognostic significance, the presence or absence of clinical illness (substage b or a, respectively) appears to reliably predict remission duration and survival; dogs in substage b have shorter remission and survival times. Although the term “clinical illness” is a subjective one, further studies have specifically identified fever and dyspnoea, as being associated with a lower chance of a dog achieving a complete remission to induction chemotherapy. In other studies, 88% of dogs without anorexia, but only 58% of dogs with anorexia achieved complete remission. Of dogs that achieved a complete remission, those that were anorexic at entry had shorter overall disease control and survival.\(^4\)\(^,\)\(^5\)
**Stage of Disease**

Various studies have shown the prognostic importance of clinical staging; however, the details remain controversial. Some studies have suggested that dogs with less extensive diseases (i.e., stage 1 to 2 lymphoma) are more likely to achieve complete remission (CR) than are dogs in clinical stages 3, 4 and 5. In other studies, however, the percentage of dogs achieving remission was not correlated with stage of disease, but the duration of remission and survival were significantly higher in dogs with stage 1 to 2 lymphoma. It may be that the differentiation between stage 3, 4 and 5 lymphoma may be somewhat dependent on the aggressiveness of staging testing. Studies that report fine needle cytology for all sites (e.g. spleen and liver) and perform multiple bone marrow aspirates may tend to stage patients “higher”. In reality it seems that dogs with stage 1 or 2 disease, while rarely seen, may have the best prognosis.\(^6\)

**Hypercalcaemia**

Hypercalcaemia is often associated with anterior mediastinal lymphoma; therefore, thoracic radiographs should always be considered in dogs with confirmed hypercalcaemia. A dog with lymphoma and hypercalcaemia is, by convention, considered to be in clinical substage b. Dogs that are hypercalcemic (particularly those with an anterior mediastinal mass, “AMM”) are more likely to have a lymphoma derived from T-lymphocytes. It may be this factor rather than hypercalcaemia or an AMM per se that is prognostic. Although the finding of hypercalcaemia increases the chance that the dog could have a T-cell lymphoma, confirmation by immunohistochemistry is required (see also Immunophenotype).

**Histologic Grade**

Canine lymphoma is morphologically heterogeneous, almost always diffuse (versus nodular), and usually high grade. Counter-intuitively, dogs with high-grade lymphomas are more likely to achieve complete clinical remission than dogs with intermediate-grade lymphoma, possibly due to a higher proportion of “cycling” cells in the tumour.\(^7\) In addition, remission and survival times are usually longer in dogs with high-grade lymphomas. There is no correlation between grade or morphological classification and immunophenotype.\(^8\) - \(^10\)

**Low-grade** lymphoma is uncommon in dogs, accounting for approximately 5 to 10% of cases.\(^8;11\) In the few reported cases of low-grade lymphoma, response rates have been lower than for high- or intermediate-grade lymphoma, but survival times are often long, owing to a more indolent clinical course. In one study, dogs with low-grade lymphoma had a median survival time of 5 months when untreated, compared to 1.3 months if not low grade.\(^12\)

**Indolent lymphoma** is uncommon in dogs, accounting for approximately 5% of cases, but in some studies, up to 29%.\(^13\) This disease is also sometimes called low grade lymphoma, but this terminology has become confusing with recent changes in pathology nomenclature such that not all “low grade” lymphoma is actually indolent in behaviour.\(^14\) One study further characterized indolent lymphomas based on histopathologic criteria. They described follicular lymphoma (all appeared to be B-cell derived), marginal zone lymphoma and T-zone (T-cell derived) lymphomas, mostly in dogs with generalized lymphadenopathy.\(^15\) In the few cases that had clinical histories available, those that were treated with chemotherapy had long survivals (2 to 4 years), and few died due to lymphoma. There was a group of dogs with T-zone lymphoma that all had long survival times without therapy. We usually consider high-grade, T-lymphocyte derived lymphoma to have a worse prognosis; in contrast, from recent evidence it appears when it is indolent lymphoma, dogs with T-cell lymphoma may actually have a better prognosis. A very recent study found that dogs with T-zone lymphoma had a median survival time of >33 months, while dogs with marginal zone lymphoma had a median survival time of 21 month; there was not strong effect of chemotherapy on these figures, although individual dogs appeared to benefit.\(^13\)
Pretreatment Steroid Therapy

It has been postulated that administration of glucocorticoids prior to other chemotherapeutic agents may change the staging of dogs by causing incomplete remission, thereby masking more advanced disease, which is known to be a poor prognostic sign. It is also possible that glucocorticoid therapy may induce multiple drug resistance in the malignant cells, as has been observed in human patients.

In two studies, dogs with lymphoma that received glucocorticoids before initiation of combination chemotherapy had significantly shorter remissions and survival times than dogs that did not receive glucocorticoids before treatment. How long the glucocorticoid treatment was given (more than 2 weeks compared to less than 2 weeks) did not influence this finding.

Treatment

Once a definitive diagnosis has been made and after the patient has been staged accurately, the veterinarian should schedule a discussion with the owner regarding prognosis and treatment. One of the most important distinctions to make for the client is between remission and cure. When toxicities are discussed, the owner should be given criteria by which to distinguish mild side effects from those that can be life threatening. A copy of the protocol to be administered, with scheduled treatments, rechecks, and blood counts, will assist owners in remembering much of this information.

First-Line Protocols

One of the most common questions we are asked is “what is the best protocol to use for a dog with lymphoma?”. Of course this depends to a large degree on results of staging and the immunophenotype of the lymphoma, as well as the owner’s preferences with regard to cost, time commitment and risk of side effects; but it would be helpful to know whether an individual protocol has any distinct advantage over others. Over the last 20 years the Oncology group at Tufts University (now the Harrington Oncology Program) has reported the results of chemotherapy for more than 500 dogs with lymphoma. We have reviewed those protocols to compare the toxicity and efficacy (when possible) and to try to identify general trends.

COP

This protocol has formed the basis for all subsequent treatment protocols for canine lymphoma. While the staging system used in this study was different to that proposed by the W.H.O. system, it offers baseline information for dogs treated with a relatively inexpensive and safe protocol. It is still widely used by private veterinary practitioners and very acceptable to the majority of pet owners.

COPA

Remission duration was increased in dogs receiving doxorubicin and COP compared to those treated with COP alone. A further study performed 15 years later, but using the same 4 drugs in a sequential protocol (rather than using combinations) showed very different results. In that study, 36 dogs were treated with a 15 week discontinuous protocol. The complete and partial remission rates were 42% and 28% respectively, and the median remission duration was less than 50 days. The difference in outcomes probably relates to scheduling.

VELCAP-L

Ninety-eight dogs with lymphoma were treated with a 5-drug combination chemotherapy regimen (vincristine, L-asparaginase, cyclophosphamide, doxorubicin, prednisone (VELCAP-L)). The complete remission rate was 69%, with median remission duration of 55 weeks. Dogs with advanced stage of disease, constitutional signs, dogs that were older, and dogs that were dyspneic were less likely to achieve remission. Once in remission, dogs received maintenance chemotherapy for 75 weeks, and small dogs were likely to have longer
remission duration. Toxicity was frequent, but rarely fatal, and no predictive factors were found for a dog developing toxicity. It was concluded that VELCAP-L was an effective treatment for dogs in stage I-III lymphoma, particularly in young, small animals. These results are also very similar to those reported for other long 5-drug protocols (AMC and Madison-Wisconsin). The remission rate is slightly lower, but the remission duration is longer for VELCAP-L compared to other similar protocols.

VELCAP-S
Because palliation, rather than cure, is a major goal of chemotherapy in veterinary oncology, there has been recent interest in developing protocols that reduce the number of patient visits as well as cost and toxicity of treatment. Very long chemotherapy protocols (VELCAP-L: 75 weeks; Madison-Wisconsin: 135 weeks) had become the “standard of care” for dogs with lymphoma on the assumption that it was necessary to continue chemotherapy to maintain remission; however that assumption had never been tested. An intermittent protocol (VELCAP-S) that used an identical 15-week induction period to that in VELCAP-L but was followed by a period where treatment was ceased was studied to determine whether a long maintenance phase was needed. If dogs relapsed less than 16 weeks after stopping chemotherapy, the induction protocol was repeated and maintenance therapy was then instituted; whereas if dogs relapsed more than 16 weeks after stopping chemotherapy they received another pulse of induction therapy alone.

Eighty-two dogs were treated using this VELCAP-S protocol. Fifty-six dogs (68%) achieved complete remission for a median first remission duration of 20 weeks. Forty-eight dogs (86%) relapsed, of which 30 repeated the induction cycle. In 22 of these dogs, first remission was short, and they received maintenance chemotherapy; the other 8 dogs received 2 or 3 cycles of induction chemotherapy. Second remission rate for these 30 dogs was 87% (26 dogs). Overall disease control for the 38 dogs that remained on the protocol was 44 weeks; this was shorter but not statistically different than for dogs treated with the VELCAP-L protocol, in which maintenance chemotherapy was instituted in all dogs after an identical first induction. Dogs that were febrile and dogs that were dyspnoeic were less likely to achieve a complete remission during induction chemotherapy. Of the dogs that achieved a complete remission, those that were anorexic at entry had shorter overall disease control. The conclusion was that because of long-term disease control, discontinuous chemotherapy may reduce patient visits for some dogs. Delaying maintenance chemotherapy until after second remission is achieved did not markedly affect overall disease control.

VELCAP-SC
This more intense discontinuous protocol was designed to treat dogs with “high risk” characteristics such as substage b and T-cell immunophenotype. A 21-week, discontinuous, chemotherapy protocol using vincristine, L-asparaginase, cyclophosphamide, doxorubicin and prednisone followed by a consolidation phase of alkylating agents (CCNU, mechlorethamine and procarbazine) was used to treat 95 dogs with advanced stage lymphoma. Fifty-seven percent of the dogs had stage 5 disease, 63% had substage b, and 31% had a T-cell immunophenotype; thus the population in this study was one that would be expected to be particularly difficult to treat. The complete response (CR) rate was 70%, which compares favourably with other protocols being used to treat more typical populations. Only anorexia at presentation predicted whether a dog achieved complete remission. Of 40 dogs with normal appetite at presentation, 35 (88%) achieved a CR, while of 52 dogs that were inappetant, 30 (58%) achieved a CR. The median duration of the first CR was 24 weeks and 1 and 2-year CR rates were 17.4% and 15.5%, respectively. The median overall survival for all 95 dogs was 43 weeks. The 1 and 2 year survival rates were 44% and 13%, respectively.

VELCAP-HDC
Dose intensification of chemotherapy in dogs has usually been limited by a higher risk for toxicity. High dose chemotherapy with hematopoietic stem cell support, or bone marrow
transplantation (BMT), has become an important component of therapy for lymphoma and other malignancies in humans. We hypothesized that autologous BMT would allow dogs to receive intensified doses of myelosuppressive chemotherapy without increased toxicity, and that this intensification would improve remission duration and overall survival. Twenty-eight dogs were treated using a 12-week protocol based on the VELCAP-S protocol, concluding with high dose cyclophosphamide (HDC) supported by autologous bone marrow transplant. The standard dose of cyclophosphamide is 200mg/m² IV; HDC was given at 300 (3 dogs), 400 (12 dogs), and 500 mg/m² IV (13 dogs). Toxicity was common but mild, and the dose-limiting toxicity was neutropenia. There were no treatment-related fatalities, and only one dog, treated at the highest dose, required hospitalization for toxicity that was presumed sepsis which responded promptly to routine management. Lower clinical stage and higher cyclophosphamide dose both predicted longer remission duration and longer overall survival. Median remission duration for dogs treated at the highest dose was 54 weeks, compared to 21 weeks for dogs treated at the lower doses. Median survival time for dogs treated at the highest dose was 139 weeks, compared to 43 weeks and 68 weeks for dogs for dogs treated at the two lower doses, respectively. Thus, it was concluded that using autologous bone marrow to support chemotherapy dose intensification allows dogs to receive 2.5 times the standard dose of cyclophosphamide without any increase in clinical toxicity, and that this dose intensification results in significant prolongation of remission. This treatment modality should be available at the Animal Referral Hospital by the beginning of next year.

Half-Body Radiotherapy
In contrast to early reports, where high dose-per-fraction radiotherapy given to half the body at one treatment and to the other half 4 weeks later resulted in considerable patient morbidity; when half body radiation therapy was given in 2 consecutive daily 4Gy fractions (instead of a single dose) toxicities were mainly mild and self-limiting. Dogs in remission from lymphoma following chemotherapy appeared to benefit from the addition of radiation as a consolidation phase. In that study dogs received a short (11 week) protocol followed by radiation therapy fractionated as described above if they were in CR. There were 52 dogs that received radiation therapy and median first remission was 10 months; 31 relapsed and 20 went on to receive further chemotherapy. The second remission rate was 85%. The overall median remission (which included remission after first relapse) was 16 months. These results appear to be substantially better than those achieved with chemotherapy alone, and clearly this combination should be investigated further.

Efficacy was less, and toxicity (including treatment related death) was greater in another study where 7 or 8Gy fractions were used.

Discussion
It is difficult to accurately compare protocols for the treatment of canine lymphoma, particularly as the numbers reported are usually small, and the prognostic factors are often defined differently and analyzed with different statistical analyses. Also, and obviously, assignment to different treatments is done sequentially and not randomly. In addition the accumulation of new prognostic factors (such as CD-3 (T-cell) immunostaining) that were not available for earlier studies makes the effect on outcomes difficult to assess. However, we can comfortably infer that as protocols include more chemotherapy drugs in combination, there is an improvement in remission duration and survival time which is probably related to increased dose intensity.

In summary, the best protocol for lymphoma currently available for routine clinical use is a “5-drug protocol” consisting of cyclophosphamide, vincristine, prednisone, doxorubicin and L-asparaginase. As long as these drugs are being used, the exact protocol may not have much of an overall influence on canine patients. However, it does seem that using combinations of drugs wherever possible (rather than single drugs given sequentially) may be more effective. The use of “dose intensification” such as autologous bone marrow
transplant, or radiation therapy further improves on these data, but those techniques are limited to specialty practices. In the absence of referral to a veterinary oncologist, the practitioner is encouraged to use a protocol that they feel comfortable with, and make use of expert advice if problems are encountered during treatment.

The treatment options below are tiered according to risk of toxicity, cost, and efficacy. First-level protocols provide a low risk of toxicity at low cost but have low efficacy; as the level rises, so do efficacy, cost, and risk of toxicity.

**First Level:** For clients who cannot afford or will not accept a combination chemotherapy protocol due to the risks of toxicity, a protocol using prednisolone alone or in combination with chlorambucil may provide palliation with few risks of side effects. A CBC should be collected every 2 to 3 weeks to make sure that myelosuppression is not occurring.

**Second Level:** The COP protocol is a relatively inexpensive chemotherapy protocol with a low risk of toxicity. Dogs tolerate the treatments, and veterinarians find the protocol very manageable. CBCs should be taken 1 week after each dose of cyclophosphamide to ensure that myelosuppression (if it occurs) is not severe and that doses do not need to be adjusted. Doxorubicin administered every 3 weeks for five treatments is the most effective single chemotherapeutic agent. This treatment regimen results in a relatively high remission rate with relatively few serious life-threatening toxicities (<5%). Because the drug is given every 3 weeks, this treatment approach is less time intensive than most chemotherapeutic protocols. A second remission seems more likely if doxorubicin is used as first-line therapy and COP is used after relapse than if COP is used first. Overall remission time for the two-protocol treatment approach is similar to that of the COPA protocol.

**Third Level:** The most effective chemotherapy protocols use a five-drug combination of L-asparaginase, vincristine, cyclophosphamide, doxorubicin, and prednisolone. Similar remission rates and survival times have been obtained for the protocols that include these drugs. Although these protocols require more intense client–veterinarian communication and monitoring for toxicity, the overall level of satisfaction for owners, pets, and veterinarians is high. Most oncologists now recommend discontinuous protocols such as VELCAP-S; however, some clients will not restart chemotherapy when first remission is over. For such clients less intensive maintenance schedule may be preferred over restarting induction treatment at relapse. For dogs with T-cell lymphoma, protocols that rely heavily on alkylating agents, such as VELCAP-SC, should be used.

**Fourth Level:** The addition of radiation therapy or, if available, autologous bone marrow support to allow chemotherapy dose intensification represents the most aggressive treatment option for a dog with lymphoma. The potential for long-term remission and possibly cure is much higher than with other protocols. Dogs with T-cell lymphoma may not benefit to the same extent as those dogs with B-cell lymphoma. Although risks of toxicity are higher, the addition of radiation or chemotherapy dose intensification has not negatively affected the quality of life for treated dogs.

**Reference List**


