**Lymphoma Overview**

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Lymphoma treatment is characterized by early spectacular response, often followed by equally spectacular treatment failure. For the most part, lymphocytes have a limited life span. Each lymphocyte is committed to accomplish specific tasks, such as the production of antibodies, the recognition and destruction of viral infected or malignantly transformed cells, and the maintenance of memory so that antigens once recognized are less able to gain a foothold in the future. The immune effector cells of the body perform these tasks until the offending antigen is dealt with, then the simple economy of biology requires that what is no longer needed be down-regulated to conserve resources for other vital functions. In the case of lymphocytes, they accomplish this ecologic balancing act for the body by dying when they are no longer needed. Certain subsets of lymphocytes, such as stem cells and memory cells, have extremely long life spans in the body, and may in fact be immortalized. However, the vast majority of the lymphocyte population is in a state of flux, constant cycling through birth and death. The most critical key to the successful eradication of cancer cells by modern medicine is the triggering of programmed cell death, or apoptosis. All normal cells have the ability to detect genotoxic insults and to undergo apoptosis as a biologically appropriate response to potential mutagens. Part of the reason for lymphocyte labiality to therapeutic agents such as prednisone is that lymphocytes respond to these types of signals by rapid cell death. In the circumstance of treatment of lymphoma, we are simply exploiting the lymphocyte's own highly refined cell death machinery to take out a susceptible population of cells. Unfortunately, cancers may arise clonally, but they are genetically unstable; after successive rounds of replication, more and more mutations accrue in cancer cells. This leads to the phenomenon of drug resistance, among other consequences.

As yet, no consensus exists as to the most appropriate chemotherapy protocol for treatment of lymphomas in dogs and cats. The purpose of this discussion is to bring up the pros and cons of the currently available chemotherapy protocols in the context of known prognostic factors to better enable the practitioner to judge what might be the best approach for a given patient.

**Epidemiology**

Incidence rates for dogs with lymphoma have been reported at 24 cases/1 00,000 dogs at risk/year. The disease affects a wide range of animals, from the very young to the geriatric. The disease is generally seen in middle aged to older dogs, with no sex predisposition reported. Breeds at increased risk have been reported, including boxers, German shepherds, and golden retrievers. Familial distribution has been reported in bullmastiffs. In addition to breed predisposition, exposure to the herbicide 2,4D is associated with an increased risk in humans and dogs. While
retroviral etiology is known in cats, birds, rodents, and humans, no retrovirus has been identified in the dog as yet.

The Diseases We Call "Lymphoma"

One of the reasons that our response rates and remission rates vary when lymphoma is treated is that lymphoma is highly variable in its biology. While all lymphocytes start with a common progenitor stem cell, many different molecular genetic changes occur to arrive at the mature functional B, T, or NK lymphocyte phenotype. Cells at different points in their maturation and development can become malignant as a result of a variety of insults, and this results in a variety of different biologic behaviors when cancers arise. In a sense, each malignant transformation is unique, but common themes of behavior can be seen, In veterinary medicine, we are at the point that a cytologic diagnosis of "lymphoma" may not be enough information to choose appropriate therapy; histologic grading is proving to be valuable as well, The Kiel classification system describes lymphomas on the basis of their corresponding stages of normal lymphopoiesis, while the Working Formulation incorporates natural history and clinical course (low-, intermediate-, and high-grades), Most veterinary pathology studies have found a Working Formulation based approach to be most helpful (Carter et al, 1986; Greenlee et al, 1990; Teske, 1993), Most canine lymphomas are diffuse (vs., nodular) and are usually high or intermediate grade, Most are classified morphologically as diffuse large cell, immunoblastic, and small lymphocyte.

Prognostic Factors

Given the above discussion that lymphomas vary in biologic behavior, several prognostic factors can be determined from retrospective and prospective studies of canine lymphoma, Survival time of completely untreated patients is short (median 6-12 weeks from diagnosis), The importance of some prognostic factors have been debated in different studies, however it is clear that stage of disease and substage (a = asymptomatic, b=symptomatic for disease), serum calcium status, immunophenotype, and response to therapy are important predictors of long-term survival. Newer prognostic markers include assays to detect apoptotic competence of lymphoblasts, such as p53 and caspase-3 immunostaining.

Chemotherapy Protocols

In human medicine, as many as a dozen different chemo-therapy protocols utilizing as many as 8 agents in combination are currently in use for the treatment of non-Hodgkin's lymphoma, This underscores the fact that no protocol can be clearly advanced as "best" in the treatment of the various diseases that fall under the heading of lymphoma. A variety of chemotherapy protocols are available for the treatment of canine lymphoma. In establishing an optimal protocol, one must take into account the efficacy (as determined by remission and survival times), toxicity, and cost of therapy. Successful therapy with single agents can be achieved for
lymphoma patients. However, single agent remissions tend to be short due to the rapid evolution of drug resistance mechanisms. Drugs that have been shown to have single agent efficacy in canine lymphoma include: corticosteroids, cyclophosphamide, L-asparaginase, doxorubicin (Adriamycin), epirubicin, mitoxantrone, actinomycin D, and CCNU (Lomustine).

**Combination Chemotherapy**
COP: Most chemotherapy protocols currently in use for lymphoma are based on the combination cyclophosphamide, vincristine (oncovin) and prednisone (COP therapy). These drugs are given either concurrently or in sequential weeks. Median remission times of around 6 months can be expected for most lymphoma dogs using a COP protocol alone. Side effects may include leukopenia, alopecia, gastrointestinal upset, sterile hemorrhagic cystitis (cyclophosphamide) and the typical adverse effects of prednisone therapy. This combination can be economically reasonable, as vincristine is available in generic form.

**Rotating Sequential protocols:** Variations on the COP protocol contain additions of drugs such as L-asparaginase, cytosine arabinoside, methotrexate, and/or doxorubicin. In one popular protocol advanced by the Animal Medical Center, and modified at the University of Wisconsin, Madison, a rotating sequential combination of vincristine, L-asparaginase, prednisone, cyclophosphamide, doxorubicin, and methotrexate was used. Some of the best chemotherapy responses to date have been reported using this protocol, with 80% of patients attaining a remission (this is comparable to that achieved by COP therapy alone), and with median survival times extending past 12 months. 25% of dogs treated with this protocol have been reported to live more than 2 years. These findings were confirmed in a study of 55 dogs at the University of Wisconsin, in which 84% achieved a complete response, with median remission duration of 36 weeks and 25% of dogs alive at 2 years. Overall median survival time for this group was 51 weeks. This same protocol was used at UC Davis and 82 dogs were treated. 64% attained complete responses, with median relapse-free survival for those with complete remission being 217 days. Median overall survival durations for dogs with complete response was 366 days, with 26% 2 year survival reported for the UC Davis dogs.

Disadvantages of this protocol include the fact that it is complicated in terms of scheduling and has the potential for the side effects described above for the combination drugs and Adriamycin alone. Side effects of varying severity can be seen in over 60% of patients treated with this protocol. The cost of this therapy is considerable, because of the expense of the drugs and monitoring for toxicity over the 2-year potential treatment period.

**ACOPA protocols:** Other combination chemotherapy protocols include those used at Tufts University (ACOPA I and II protocols) and a short-term (12 weeks)
fractionated combination. The Tufts protocols involved combinations of COP plus L-asparaginase (Elspar) and doxorubicin using different schedules. In the earlier ACOPA I protocol reported by Stone et al in 1991, 76% of 41 dogs attained a complete remission, median remission duration 11 months and with 48% being in remission at one year. ACOPA I used vincristine, L-asparaginase and prednisone for induction and cyclophosphamide and doxorubicin for maintenance. ACOPA II was reported in 1997 and consisted of doxorubicin and prednisone induction with doxorubicin, vincristine, cyclophosphamide, prednisone and pulsed L-asparaginase maintenance therapy. ACOPA II was studied in 68 dogs, of which 65% attained complete remission with a median remission duration in this subset of dogs of 9 months and 40% in remission at one year and 21 % at two years. Interestingly, 37% of patients on the ACOPA I trial required modifications in dose or scheduling of chemotherapy because of toxicity, as compared with 62% of patients on ACOPA II. Thus on the surface it would appear that the ACOPA I protocol was superior (higher remission rates, longer remission duration, and lower dose-limiting toxicities). However more patients with advanced (Stage V) disease were entered into the ACOP A II arm, and a substantial number were lost to follow up while in complete remission. These factors may have biased results against this protocol.

**COPLA protocol:** At Michigan State University, we have used an independently developed but similar version of combination chemotherapy for canine lymphoma. (See attached protocol). Currently, median survival times with this protocol, plus rescue therapy, are around 50 weeks. However, we note only an 8-15% adverse effect rate for dogs treated with this protocol. Thus we feel that while the response durations are not as good with some of the other protocols, the adverse effect rates are compelling particularly in the treatment of geriatric patients. Seventy-five dogs with cytologically or histologically confirmed lymphoma received COPLA/LVP between January 1994 and June 1997. Toxicity was evaluated using the National Cancer Institute (NCI) to toxicity criteria. Age, weight, sex and response were evaluated for prognostic significance against first remission duration. A complete response was obtained in 61 dogs (80%), a partial response in 9 dogs (12%) and no response in 5 dogs (8%). The median first remission duration was 25 weeks with 17% and 5% of the dogs in first remission at 1 and 2 years respectively. Observed toxicity was low with 84% of dogs given and NCI score of 1 or 2. Median survival for dogs achieving complete response was 36 weeks versus 4 weeks for those achieving partial response or no response.

**Alternative Therapies**

Other forms of therapy that have been reported to be useful in the treatment of canine lymphoma include bone marrow transplantation and various immune therapies. Bone marrow transplantation has shown up to 25% long term survivals (cures), but 50% of patients so treated experienced a remission such as would be
expected from chemotherapy and 25% of patients died as a consequence of toxicity.

**Tumor vaccines:** Autologous tumor cell vaccines have produced prolonged remission and survival times when administered to patients who were in complete remission at the time of their administration. Mean survival times of 341 days for vaccinated dogs as compared to 138 days for chemotherapy treatment alone have been reported. Unfortunately, such vaccine products are not routinely available. Recently, some activity in the production of B cell anti-idiotypic vaccines has been discussed. At this point, controlled trials have not been carried out to assess the efficacy of this approach. Also, vaccines targeting the CD20 differentiation epitope of canine B cell lymphomas are being investigated currently, which means that therapy akin to the monoclonal antibody therapy used in human B cell lymphoma (Rituximab).

**Monoclonal antibodies:** A monoclonal canine lymphoma antibody was produced against an unknown but apparently highly conserved surface epitope in canine lymphoma. Median remission and survival times were reported in dogs receiving this treatment after induction of remission by standard chemotherapy as compared to chemotherapy alone. Jeglum et al reported on the results of a trial for 214 dogs treated with MA b 231 after induction therapy with 2 cycles of VCAA (L-asparaginase, vincristine, cyclophosphamid, and doxorubicin). 80% of the dogs achieved a complete response after induction with VCAA. The dogs that achieved a complete remission then received 5 days of slow IV infusion of the monoclonal antibody. Median first remission duration was 125 days, with median survival of 448 days. 41% of dogs were alive at one year. This monoclonal antibody product is no longer commercially available, however.

Relationship of classification of lymphomas and response to therapy - One reason for a lack of clear consensus as to the best treatment for lymphoma in dogs and cats is that this malignancy represents a number of different clinical manifestations with different biologic behaviors. Stage at diagnosis, histologic grade of malignancy, immunophenotype of lymphoma, and involvement of extranodal locations are all factors that must be considered when evaluating chemotherapy protocols for comparable remission and survival times. See the above discussion of prognostic factors for details.

**Radiation Therapy Considerations**

Lymphoma is generally exquisitely sensitive to radiation therapy. Thus, radiation therapy has a definite role in the treatment of lymphomas in veterinary medicine. Local lymphomas may be effectively treated in an emergency setting to restore vital organ function in the short term. Examples of this indication include life threatening anterior mediastinal lymphoma and CNS lymphoma. Lymphoma may also be
treated with curative intent by radiation therapy. Examples of this indication include treatment of lymphoma of the nasal sinus cavity. Dogs and cats treated for nasal lymphoma with full course radiation therapy are likely to be cured of local disease. Unfortunately, systemic relapse is expected so sequential or concurrent treatment with chemotherapy is necessary for these patients. Lymphoma may be treated with radiation therapy as a salvage procedure, as in the case of treating single refractory sites such as resistant peripheral nodal disease. These remissions may be short lived for patients with end stage lymphoma, but patient benefit may be achieved in the short term. Finally, radiation can be used adjuvantly for treatment of systemic lymphoma. Half-body radiation therapy has been evaluated for dogs with generalized lymphoma (Abrams-Ogg, et al). In this randomized prospective Phase III trial, 81 dogs with Stages III-IV lymphoma were induced into remission using a combination chemotherapy protocol that included asparaginase, cyclophosphamide, vincristine, epirubicin, and prednisone. Dogs were induced into a remission with this protocol for 6 weeks, then randomized into two treatment groups: maintenance chemotherapy (vincristine, cyclophosphamide, methotrexate) or half-body radiation therapy. The half-body radiation therapy arm consisted of patients treated with 800 cGy to the cranial half of the body on week 8, followed by 800 cGy to caudal half of the body on week 12. To prevent relapse in the caudal half of the body before the radiation fraction, vincristine was administered on weeks 8 and 10. Remission was achieved by 74% of dogs after the induction protocol. Dogs in the maintenance chemotherapy group achieved a median remission duration of 30 weeks, with 27% of dogs in remission at 1 year. Dogs in the radiation therapy arm had a median remission duration of 33 weeks, with 36% still in remission at one year. Interestingly, this remission was durable for 6 patients, with 27% of the half-body radiation group still in remission at 3 years. Although the results are not statistically significant there is a trend toward longer tumor control for the group treated with half-body radiation. Whole body radiation with bone marrow transplantation could also be considered in an investigational setting for dogs with lymphoma.

Summary
A great deal of effort and energy has been expended in the past few years to try to better establish prognostic factors for the treatment of lymphoma in dogs and cats. Several protocols are available, and factors related to the individual patient to be treated and the malignant behavior of that patient's tumor should be considered when setting up chemotherapy protocols. Doxorubicin containing protocols would appear to be best used for high-grade lymphoma and in patients with a poor prognosis, such as for histiocytic lymphomas, T cell disease in dogs, or for young animals. In older animals with less aggressive tumors, a COP protocol may achieve significant duration of remission without exposing the animal to the potential toxicities of doxorubicin until relapse, at which time doxorubicin can be used as an
Common protocols in use for canine lymphoma

The University of Wisconsin-Madison 25-week protocol is a rotating sequential protocol and is very dose intense, with arguably the longest overall survival durations for the largest portion of patients (30% live beyond 2 years).

Week 1 - Vincristine (0.5-0.7 mg/m2 IV), Asparaginase (400 IU/kg SC), Prednisone (2 mg/kg PO SID);
Week 2 - Cyclophosphamide (250 mg/m2 I), Prednisone 1.5 mg/kg PO SID;
Week 3 - Vincristine, prednisone (1.0 mg/kg PO SID);
Week 4 - Doxorubicin (30 mg/m2 IV), Prednisone (0.5 mg/kg PO SID);
Week 6 - Vincristine
Week 7 - Cyclophosphamide
Week 8 - Vincristine,
Week 9 - Doxorubicin.
If in complete remission at Week 9, this cycle is continued at 2-week intervals until
week 25, at which time therapy is discontinued.

The COPLA-LVP Protocol is a concurrent combination that is notable for low
side effect rate and high induction of remission, although overall survival times are
not as long as with the University of Wisconsin approach.

COPLA Protocol:
Cyclophosphamide 50 mg/m², PO, eod for 56 days (8 weeks)
Vincristine (Oncovin) 0.5 -0.7 mg/m, IV, starting day 1, q 7 days for 77 days (11
weeks),
Prednisone 20 mg/m, PO, sid for 7 days; then 20 mg/m, PO, eod until relapse or
adverse steroid effects, in which case taper dose and discontinue
L-asparaginase 10,000 IU/m², SC on days 1 and 8
Doxorubicin (Adriamycin) 30 mg/m², IV, on weeks 6, 9 and 12

CBC's and lymph node/mass measurements should be obtained weekly, in order to
modify treatment if deemed necessary.

MAINTENANCE LVP protocol
Chlorambucil (Leukeran) 4 mg/m², PO, eod
Vincristine 0.5 -0.7 mg/m², IV, q 14 days for 2 doses, q 21 days for 3 doses, q 28
days thereafter.
Prednisone 20 mg/m, PO, sid for 7 days; then 20 mg/m², PO, eod until relapse or
adverse steroid effects in which case taper dose and discontinue.

Cost-conscious protocols:

CCNU protocol
(Cycle is repeated every 21 days/or up to 4 cycles)
• CCNU (Lomustine) 50-90 mg/m², PO, q 21 days (administer in hospital)

Monitor CBC on Day 8 - if <3,000 total WBC or <1000 PMN institute antibiotic
therapy then reduce the subsequent dose of Lomustine by 25%, or if in stable
remission after 4 cycles continue every 6-8 weeks until relapse. Chronic
administration of CCNU results in profound and potentially unrecoverable
myelosuppression. If the dog is still in remission at the end of 4 cycles of CCNU
the interval between treatments can be extended to 6-8 weeks or low dose CCNU
(50 mg/m\(^2\)) PO, q21 days long term until relapse or adverse effects develop. Evaluation of serum chemistry panels are now recommended for patients treated with CCNU, as potentially fatal idiosyncratic hepatotoxicity has been reported. If patients have persistently elevated or increasing liver enzymes, it is recommended that lomustine therapy be discontinued.

LDP protocol-This is a mild chemotherapy protocol
Chlorambucil (Leukeran) 4 mg/m\(^2\), PO, eod
Actinomycin (Dactinomycin) D 0.5 to 0.75 mg/m\(^2\), IV every 2-3 weeks (for 6 cycles)
Prednisone 20 mg/m, PO, eod

*Have owner give prednisone and leukeran on alternating days. Attempt maintenance with LVP*