Lymphoma – Revisited
All LSA’s Are Not Created Equal

- Rodney D Ayl BSc BVSc MRCVS
- Dip ACVIM (Med Onc) Dip ACVR (Rad Onc)
- Based on: “Canine Lymphomas: Association of Classification Type, Disease Stage, Tumor Subtype, Mitotic Rate, and Treatment With Survival” V. E. Valli, et al. Vet Path 2013 50(5) 738-748

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**Lymphomas**

*Figure 14.* Lymph node. **Lymphoblastic T-cell** lymphoma. Homogeneous proliferation of medium-sized cells, with round nuclei, fine chromatin, and small but visible nucleoli. HE.

*Figure 15.* Lymph node. **Pleomorphic large T-cell** lymphoma. Large cells with marked irregular nuclear outlines. HE.

*Figure 16.* Lymph node. **Immunoblastic T-cell** lymphoma. Large cells with abundant, acidophilic, unipolar cytoplasm and a high nucleolar volume. HE.

*Figure 17.* Lymph node. **Plasmacytoid T-cell** lymphoma. Medium- and large-sized cells, with extended, deeply acidophilic cytoplasm and pale juxtanuclear area in some cells. HE.

*Figure 18.* Intestinal mass. **Aggressive large granular T-cell** lymphoma. Variable cell size, with round or indented nuclei, slightly clumped chromatin, and distinct nucleoli. HE.

*Figure 19.* Lymph node. Fine-needle aspirate. **Aggressive large granular T-cell** lymphoma. Note the presence of coarse azurophilic granules in the cytoplasm. May-Grünwald-Giemsa technique.

*Figure 20.* Lymph node. **Prolymphocytic T-cell** lymphoma. Majority of small cells with regular nucleless commonly with slightly irregular nuclei, a visible nucleolus, irregularly clumped chromatin, and small rim of pale cytoplasm. HE.
There has been an ongoing debate in human medicine about whether an aggressive chemotherapy combination is needed for indolent lymphomas.

- The updated results from the German Study Group for Indolent Lymphoma (StiL) "clearly show" that the simpler 2-drug combination of bendamustine plus rituximab is more effective and less toxic, Dr. Rummel said.

- Speaking before his presentation at the plenary session here at the 2012 Annual Meeting of the American Society of Clinical Oncology, Dr. Rummel told journalists that he expects these new results to change clinical practice, especially in the United States, where the R-CHOP regimen is still widely used.
The standard treatment for these more challenging types of lymphoma — which include indolent follicular, Waldenström's, marginal zone, and mantle cell lymphoma in elderly patients — has been the R-CHOP chemotherapy regimen (rituximab [Rituxan, Genentech] plus cyclophosphamide [Cytoxan], hydroxydaunorubicin [Adriamycin], vincristine [Oncovin], and prednisone).
New long-term results from the German trial of human patients with indolent and slow-growing lymphoma confirm that a simpler 2-drug combination can be used in these patients instead of a more aggressive chemotherapy approach.
Typical therapy for some specific subtypes of human lymphomas

- **Follicular lymphoma** - Watchful waiting, for asymptomatic patients with normal blood cell counts and no critical visceral involvement; medical treatments include alkylating agents (eg, chlorambucil or cyclophosphamide; cornerstone of treatment) and rituximab

- **Splenic marginal zone lymphoma** – Splenectomy

- **Extranodal B-cell lymphoma of MALT** – Antibiotic treatment of Helicobacter pylori infection; surgical excision; systemic chemotherapy for widespread disease
Mantle cell lymphoma – Combination chemotherapy; bortezomib for relapsed disease

Diffuse large B-cell lymphoma – Current standard therapy is rituximab with cyclophosphamide, hydroxydaunorubicin (Adria), vincristine (Oncovin), prednisone, and bleomycin (R-CHOP)

Mediastinal diffuse large B-cell lymphoma – CHOP with adjuvant field radiation therapy

Burkitt lymphoma – Most adult treatment regimens include brief high-dose combination chemotherapy with CNS prophylaxis with and without cranial irradiation
There is marked variation in the biology of untreated canine lymphomas that requires the management of each type of lymphoma to be carefully weighed.

Diagnosis of lymphomas in dogs has followed systems of classifications developed for humans and has recently been accurately characterized by an adaptation of the latest World Health Organization (WHO) system.
This classification was developed from recommendations of an international group of pathologists based on unique aspects of each neoplastic disease to provide an updated system of classification.

This classification provided a system of categorizing lymphoid neoplasms according to their level of cellular maturation that also provided a level of prognostic information.
All cases routinely had immunohistochemistry (IHC) performed with CD3 (T-cell), CD79a (B-cell), and additional IHC, including CD18 (pan-leukocyte marker), when needed to evaluate histiocytic proliferation.

Cases considered non-diagnostic (benign vs. neoplastic or hyperplasia vs. neoplasia) on initial evaluation were examined by PCR methods (PARR Assay – clonality).
Histological examination included:

- Assessment of tissue architecture
- The mitotic rate
- The general cell type
- For diffuse large B-cell lymphomas, the grade according to mitotic rate was included
- For lymphoblastic lymphomas, the nuclear shape was included (B- versus T-cell)
- These additional data were collected to assist in interpreting differences in survival times.
The lymphoblastic lymphomas of B- and T-cell types were tallied separately for nuclei of round or convoluted types to determine whether this morphologic detail was associated with a difference response to therapy and survival.

Lymphomas of mantle cell and marginal zone types appeared to arise as primary neoplasms in the spleen. These lymphomas were identified in peripheral areas by their relationship to a germinal center, cytological features, and low mitotic type.
Hemosiderin In Node
B- and T-cell lymphomas were grouped according to histological grade based on mitotic rate.

By this classification, B- and T-cell lymphomas of lymphoblastic type were analyzed together because both by definition have a high mitotic rate and are similar clinically in response to therapy, with difficulty in obtaining a remission.

In contrast, the lymphomas with histological grades of low mitotic rate of B- and T-cell types were not combined to allow comparison of phenotype impact on survival of indolent lymphomas.
For stage of disease (as distinguished from grade), the more detailed WHO system of Ia or Ib to Va or Vb was compressed (as is customary in the human staging system) to a 1–3 system.

- **WHO Stages:**
  - I and II were considered local disease in 1 node or region (Stage 1)
  - III and IV representing cases of generalized lymphadenopathy usually including some involvement of spleen and liver (Stage 2)
  - V was considered advanced disease (Stage 3) that included systemic involvement.
Combinations of lymphoma diagnostic categories were then divided into 3 major groups, determined by:

- Mitotic rate
  - Low
  - Intermediate
  - High grade.
- These 3 groups were further sub-divided into 4 distinct subgroups based on treatment protocol.
Immunoblastic B-cell LSA
A. High-grade lymphomas (LBL and PTCL)

Group 1: Protocols containing doxorubicin (Doxorubicin), including;

- a.) L-asparaginase, vincristine, doxorubicin, and prednisone (CVTX)
- b) L-asparaginase, vincristine, doxorubicin, and prednisone (Wisconsin)
- c) Cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) Note that first 2 treatment protocols above (CTVX, Wisconsin) each contain doxorubicin and are based on the CHOP protocol. Variations in the order of dosage and timing occurred with the administration of the same drugs.
Group 2: Other protocols not containing doxorubicin, including:
- a) Mechlorethamine, vincristine, procarbazine, and prednisone (MOPP)
- b) Cyclophosphamide, vincristine, prednisone (COP)
- c) Prednisone and 1 other treatment, either chlorambucil or lomustine (CCNU)

Group 3: Only prednisone

Group 4: No treatment
B. Intermediate-grade lymphomas (DLBCL CB, DLBCL IB)
- Group 1: Any doxorubicin-containing protocol
- Group 2: Any non–doxorubicin-containing protocol
- Group 3: Only prednisone
- Group 4: No treatment

C. Low-grade lymphomas (LGBC and TZL)
- Group 1: Any doxorubicin-containing protocol
- Group 2: Any non–doxorubicin-containing protocol
- Group 3: Only prednisone
- Group 4: No treatment
Large Granular NK-cell Lymphoma
# Lymphoma Diagnoses

## Benign lymphoid hyperplasia
- Atypical follicular hyperplasia: 3.1%
- Benign lymphoid hyperplasia: 1.2%
- Fading follicular hyperplasia: 0.4%

## Benign tumor
- Thymoma: 0.1%

## Indolent B-cell lymphoma
- Marginal zone lymphoma: 6.5%
- Mantle cell lymphoma: 1.6%
- Follicular lymphoma: 0.3%

## Low-grade B-cell lymphoma
- Diffuse large B-cell lymphoma LO IB: 3.8%
- Diffuse large B-cell lymphoma LO CB: 3.7%
- T-cell-rich large B-cell lymphoma: 0.9%
- B-cell small lymphocytic lymphoma: 0.8%
- B-cell chronic lymphocytic leukemia: 0.2%
- Diffuse intermediate B-cell lymphoma: 0.8%

## Intermediate-grade B-cell lymphoma
- Diffuse large B-cell lymphoma mid CB: 8.3%
- Diffuse large B-cell mid IB: 2.1%
- Plasmacytoma: 1.5%
- Lymphoplasmacytoid lymphoma: 0.1%

## High-grade B-cell lymphomas
- Diffuse large B-cell lymphoma HI CB: 26.4%
- Diffuse large B-cell lymphoma HI IB: 3.2%
- Burkitt-like lymphoma: 2.2%
- B-anaplastic large cell lymphoma: 0.5%
- B-cell lymphoblastic lymphoma: 1.5%
- B-cell lymphoblastic lymphoma cleft: 0.3%
- Plasmablastic lymphoma: 0.7%

## Indolent T-cell lymphoma
- T-zone lymphoma: 11.2%

## Low-grade T-cell lymphomas
- T-cell anaplastic large cell lymphoma: 0.5%
- Enteric T-cell lymphoma: 0.1%
- Cutaneous T-cell lymphoma: 1.2%

## High-grade T-cell lymphomas
- Peripheral T-cell lymphoma: 13.6%
- T-cell lymphoblastic lymphoma: 1.6%
- T-cell lymphoblastic lymphoma cleft: 1.4%
Lymphoblastic T-cell LSA
Subtypes of lymphomas in dogs with survival data and response to varying types of treatment protocols

1) Each case in this study had been phenotyped, which has been shown to be essential for the diagnosis of all human lymphomas (except follicular types that can be recognized as B-cell type on the basis of tumor architecture).

2) Because follicular lymphomas are rare in animals, virtually all cases require determination of B- or T-cell type for a firm diagnosis of lymphoma subtype.

3) The long survival of the low-grade lymphomas, most of which require conservative or no therapy, also makes a specific diagnosis imperative.
4) One of the more surprising findings of this study is the relatively short survival of dogs with benign lymphoid hyperplasia, many of which had follicular hyperplasia that must be differentiated from true follicular lymphoma. Thus, for a dog to have a follicular hyperplasia, there must have been a strong immune stimulation.

5) It is possible to diagnose lymphomas by cytological examination, but it is not yet possible to give a specific diagnosis of lymphoma subtype by interpretation of a fine needle aspirate.
6) In contrast, it appears that a cytological preparation (if made like a blood smear) of a uniform population of smaller cells that have a “hand mirror” type of cytoplasmic extension is almost certainly derived from a node with T-zone lymphomas. NOTE: An imprint of a T-zone lymphoma does not have the hand mirror cytoplasmic features.

7) Although flow cytometry (single cell suspension) can be rapid and informative and may provide a very high likelihood of lymphoma and even phenotype, it is unable to provide a diagnosis of specific lymphoma subtype.
Normal Node
Currently, most lymphoma therapy in animals is largely palliative, suggesting that collaboration is needed with oncologists to test specific protocols on single subtypes of lymphoma with a goal of therapy that is closer to curative.
1) The inclusion of doxorubicin to other intensive protocols showed high probability for prolonging survival of dogs with intermediate-grade lymphomas.

2) The survival of dogs with low-grade T-zone lymphomas deserves further study because many of the dogs with that diagnosis were given treatments of various kinds, however use of chemotherapy was associated with reduced survival for dogs with that diagnosis. It may be though, that the dogs treated for TZL presented in very late stage with prominent lymphadenopathy, whereas those that were untreated were apparently asymptomatic with minor lymphadenopathy.
3) Dogs with T-zone lymphomas may have a dual presentation because some lived for years with no therapy and others reached a stage at which therapy was indicated. It may be that the cases with the larger cells termed “intermediate” type are those that tend to progress, whereas those of small cell type may be a cohort that does well with no therapy. There is very little difference in the mitotic rates of these types of T-zone lymphomas, with usually none in a 400 field of the small cell type and 0–1 in the tumors with cells of intermediate size.
4) The management of diffuse large B-cell lymphomas (DLBCL) of immunoblastic nucleolar type (IB) may not be sufficiently different from management of the more common type with centroblastic nucleolar configuration (CB) to warrant treating these as separate diagnoses. In the older classification of the NIH Working Formulation, the DLBCL of IB type was classed as high grade and the DLBCL of CB type was considered to be of intermediate grade; that has since been questioned in human studies.
Plasma Cell Hyperplasia
5) There appears to be considerable heterogeneity within the morphologic types of human large B-cell lymphomas that relates to significant differences in survival. Some of the subtypes associated with shorter survival are distinguished morphologically, as in this study, whereas others are identified by specific immunohistochemical markers.

6) There is marked variation in the survival of dogs with DLBCL of varying mitotic rates. The median survival in dogs with fewer than 20 mitoses per 400 field was found to be 188 days; in contrast, the median survival of dogs with 21 or more mitoses per 400 field was 31 days. Because most chemotherapeutic drugs act on cells at some stage of mitosis, it is important to recognize the level of cell proliferation and how those lymphomas can most effectively receive targeted treatment. The assessment of mitotic rate is essential for obtaining a specific diagnosis, and the most efficient manner of determining this parameter is from a histological section.
7) A remaining problem in diagnosis of canine lymphomas is that with disease progression, marginal zone lymphomas have cells with a single large nucleolus that may appear as a diffuse proliferation with a low mitotic rate and can be confused with low-grade lymphomas of DLBCL-IB type, which also have a single large nucleolus. Usually, there is sufficient architectural evidence of nodular late-stage MZL that this entity can be identified on that basis if sufficient tissue is available for examination. In addition, MZL has a nucleus of intermediate cell size (1.5 red cell diameters) with abundant cytoplasm, but that of the IB type of DLBCL is truly of large cell type (2–2.5 red cell diameters), with this difference in nuclear size sufficient to suggest one diagnosis over the other.
Pyogranulomatous Lymphadenitis
8) A further area of diagnostic differentiation identified by this study is whether it is advantageous to differentiate between lymphoblastic lymphomas (LBL) of convoluted (LBC) or smooth round (LBL) nuclear types in terms of treatment or survival. There were only 30 cases of T-cell LBL total and 18 cases of B-cell LBL in this study, too few to determine meaningful survival differences.

9) Like the exfoliation of cells of T-zone lymphomas in cytological preparations, the appearance of the cells of lymphoblastic lymphomas is also distinctive to the experienced observer. In both cytological and histological examination, the dispersed chromatin of LBL of B- or T-cell type is characteristic and appears more densely stained with obscured nucleoli.
10) A report on the diagnostic criteria of LBL by both cytological and histological examination is needed together with a collaborative trial of intensive therapy, which now appears to increase survival times in humans.

11) At present, these appear to be the most difficult subtype of canine lymphoma to bring into remission and to attain long-term survival with results similar to human cases of lymphoblastic leukemia/lymphomas.

12) Burkitt-like lymphomas (BKL) appear to have longer survival, with some living for 2 to 3 years following diagnosis.

13) In humans, there is similar concern that some cases may be difficult to distinguish from DLBCL with a high mitotic rate.
Small Cell Lymphoma
The cases of benign hyperplasia and of lymphomas were therefore placed by diagnosis into the following 7 categories:

1. Benign (BLH): fading follicular hyperplasia, atypical follicular hyperplasia, and benign lymphoid hyperplasia
   - Treatment: Underlying disease

2. Low-grade B-cell (LGBC): mantle cell lymphomas of node or spleen, marginal zone lymphomas (MZL) of node or spleen, follicular lymphomas, centrocytic lymphomas, lymphoplasmacytoid, and plasmacytic lymphomas
   - Treatment: May or may not benefit from chemo – human CHOP + monoclonal ab vs. chlorambucil
3. High-grade (LBL): T- and B-cell lymphoblastic lymphomas, including types with both round and convoluted nuclei and Burkitt-like lymphomas
   - Treatment: Pred alone – no benefit; chemo of any sort - benefit

4. Low-grade T-cell (TZL): T-zone lymphomas and T-cell anaplastic lymphomas
   - Treatment: No survival benefit from chemo but chemo better than pred alone

5. Centroblastic B-cell (DLBCL CB): large B-cell lymphomas of low, intermediate, and high mitotic rates
   - Treatment: Significant survival benefit from any chemo; pred no better than no treatment

6. Immunoblastic B-cell (DLBCL IB): large B-cell lymphomas of low, intermediate, and high mitotic rates
   - Treatment: Survival benefit from dox>other chemo; pred no benefit

7. T-cell high-grade (PTCL): peripheral T-cell lymphomas
   - Treatment: No apparent chemo benefit
Most of the current lymphoma treatment protocols are based only on chemotherapy and seem to have reached their limitations when it comes to improving overall survival times with a reasonable quality of life.

Treatment of lymphoma in humans faced a similar limitation in the late 1990’s.
After their introduction, monoclonal-based therapies became the standard in treating humans either in conjunction with chemotherapy during the induction phase, following relapse, or as monotherapy during maintenance.

Using monoclonal antibody therapy in combination with chemotherapy may be less stressful on the dog’s system than only the chemotherapeutic drugs used today. And, it may lead to new beginnings for treatment and quality of life for canine lymphoma patients in the future.
A conditional license has been granted by the U.S. Department of Agriculture (USDA), for B-cell and T-cell canine-specific monoclonal antibody therapies as aids in the treatment of lymphoma in dogs.

- AT-005, the first T-cell biological therapeutic, is a caninized monoclonal antibody engineered using the Aratana technology platform.
- AT-005 provides a targeted immunotherapy that specifically recognizes, with high affinity, the target cell-surface antigen CD-52, expressed on the cancer (lymphoma) T-cells in dogs.
Plasma Cells With Russell Bodies
Upon binding to the target, the dog’s immune system is better able to identify and help to eliminate the lymphoma cells.

ACI is currently enrolling cases in a nationwide clinical trial to evaluate the monoclonal antibody (AT-005) that is conditionally licensed by the USDA to aid in the treatment of dogs with lymphoma. The study is designed to assess the benefit of adding AT-005 to a single-agent CCNU chemotherapy protocol for dogs with intermediate to high grade T-cell lymphoma.

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Many types of lymphoma have this receptor on their surface at high levels, a property that makes them stand out from normal immune cells in the body.

An antibody targeting this receptor (known as IMMU-114) has recently been developed and is being investigated as a possible new treatment for both humans and dogs with B cell lymphoma.
Doctors at North Carolina State University’s (NCSU) Veterinary Teaching Hospital are now using bone marrow transplants (BMT) to treat canine patients with lymphoma.

- A new application of an existing technology responsible for saving the lives of thousands of humans each year.
- Since bone marrow transplants are so new to veterinary medicine, long term clinical results are not yet available, which can make it difficult to speak authoritatively about cure rates and average survival times.
Macrophages In A Lymph Node
However, for humans, the procedure results in a 50-60% cure rate in those with B-cell lymphoma, and if history is any indicator, dogs could potentially enjoy a similar outcome as they so often mirror humans when it comes to cancer treatments.

In the meantime, veterinary professionals and pet guardians can consider the success we have seen so far in using this procedure with dogs. But, as with humans, cure is not guaranteed, and some dogs will relapse.

Expense, duration of treatment, complications
Rabacfosadine is a novel targeted anti-cancer therapy that has demonstrated significant efficacy in dogs with multicentric lymphoma.

- For the most part, it has been well tolerated, with potential side effects including bone marrow suppression, skin toxicity, and gastrointestinal upset.
GS-9219, a prodrug of the nucleotide analogue 9-(2-phosphonomethoxyethyl) guanine (PMEG), which delivers PMEG and its phosphorylated metabolites to lymphoid cells with preferential cytotoxicity in cells with a high proliferation index such as lymphoid malignancies.

- Discovered by Gilead Sciences, Inc., and licensed to VetDC for use in animal cancer, Tanovea™ (formerly VDC-1101) was designed to preferentially target and attack cancer cells implicated in lymphoma, one of the most common and deadly cancers impacting the pet community today.
In previous clinical studies, Tanovea™ has been shown to be highly effective in pet dogs with lymphoma, demonstrating a 77% overall response rate. Tanovea™ was generally well-tolerated and demonstrated high rates of response in both dogs naïve to previous treatments as well as in dogs that relapsed or failed previous chemotherapy. Furthermore, Tanovea™ is currently being evaluated for use in cats with lymphoma.
Survivin is a protein that is very important in cancer cell growth and survival

- Dog lymphomas express survivin, and that high survivin levels are associated with a worse outcome after chemotherapy.
- EZN-3042 is a form of gene therapy that targets survivin and reduces survivin protein in cancer cells. EZN-3042 reduces survivin levels in canine cancer cells, resulting in reduced growth and enhanced cell death.
VDC-597 is an oral agent that has been used both in normal dogs and in humans with cancer. It works by inhibiting the function of proteins in certain cells that are necessary for cell growth and prevention of cell death.

- These proteins seem to be more important in certain kinds of cancer than in normal cells. VDC-597 has been shown to inhibit the growth of canine lymphoma cells in the laboratory, and has been shown to be safe in normal dogs.
Metastatic Carcinoma
Verdinexor (KPT-335) works by preventing powerful tumor suppressing proteins from leaving the nucleus of cells, an exodus that allows cancer to grow unchecked.

- It's the first new therapeutic option for dog lymphoma in more than two decades; potentially offering vets another alternative for treating the disease, which is the most common form of canine cancer.
- "Verdinexor is a really different from chemotherapy, the current standard of care for lymphoma."
Transitional Cell Carcinoma
Lymphomas in animals and humans require a specific diagnosis for selecting the most appropriate chemotherapy.

The accurate diagnosis of lymphomas can be difficult and may require multiple diagnostic modalities, including clonality testing.

Next-generation collaborative studies by pathologists and oncologists are required for each subtype of lymphoma to determine the optimal treatment for each entity.
Thank You!