Correlations between numerical chromosomal aberrations in the tumor and peripheral blood in canine lymphoma

J.J. Devitt^a D.G. Maranon^b E.J. Ehrhart^a A.M. Bachand^b S.E. Lana^a
S.M. LaRue^a

^aAnimal Cancer Center, Departments of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, and ^bDepartment of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO (USA)

Abstract

Lymphoma is the most frequently diagnosed hematopoietic malignancy in dogs. Untreated, the survival times are approximately one month. Chemotherapy is the current standard of care and can initiate and temporarily maintain remission, with average remission times of one year. Canine lymphoma is an established model of human non-Hodgkin's lymphoma, and studying this disease in dogs can provide insight to both human and canine disease. Cytogenetic abnormalities can aid in diagnosing tumors as well as in giving a more accurate prognosis for the specific mutations present. Evaluating peripheral lymphocytes instead of tumor cells is less invasive for the affected dog and technically easier. This study was designed to investigate a correspondence between numerical aberrations detected in the tumor and the peripheral blood in dogs with lymphoma. Twenty-five dogs with lymphoma had one lymph node excised, a peripheral blood sample drawn, and a bone marrow aspirate performed. Portions of the lymph node were submitted for immunophenotyping and cytogenetic analysis. The peripheral blood sample was cultured for cytogenetic analysis and the bone marrow aspirate was used for staging purposes. A significant correspondence between the numerical aberrations in the tumor and the peripheral blood was found. The findings in this study pave the way toward an alternative method for evaluating lymphoma. When tumor analysis is not pos-

sible, the peripheral blood offers a viable option for cytogenetic assessment. Additionally, this may provide a method to evaluate the efficacy of the treatment protocol during the course of treatment.

Copyright © 2009 S. Karger AG, Basel

Lymphoma is one of the most common neoplasms seen in the dog and can be located in any lymph node, organ, and/or bone marrow (Baskin et al., 2000; Vail and Young, 2007). It is a locally and systemically aggressive tumor, with a survival time of 30 days if untreated. Canine lymphoma is similar in clinical signs, histopathology, and treatment response to non-Hodgkin's lymphoma (NHL) in human beings and is therefore a widely recognized model for NHL (Weller et al., 1982; Hahn et al., 1994; Khanna et al., 2006). Chemotherapy can successfully initiate and temporarily maintain remission. However, despite intense efforts within the veterinary oncologic research community to improve treatment outcome, long term survival remains poor, with median survival between 12 and 18 months (Vail and Young, 2007). One

This study was supported by NIH/NCI training grant number T32 CA009236.

factor that may be contributing to the failure to gain a more durable remission is the lack of predicative assays that can classify affected dogs into clinically relevant subpopulations. In the treatment of human lymphoma patients, appropriate classification based on histological subclassification, immunophenotyping, and cytogenetic analysis has led to the identification of subtypes that respond well or poorly to traditional treatment (Rudiger et al., 2002; Kim et al., 2004; Lee et al., 2006). These predictors of response can then be used to select subgroups with poor prognoses for entry into clinical trials evaluation of more aggressive or novel protocols. In veterinary medicine, immunophenotyping is commonly used to distinguish between B- and T-cell lymphomas but there is considerable heterogeneity of treatment response within the two groups. Histological subclassifications have been developed, but their lack of clinical impact is evidenced by the fact that many treatment facilities do not require histology as part of routine staging (Carter et al., 1986). Numerical aberrations occur more frequently in canine tumors than structural changes like deletions, translocations, and rearrangements (Pathak and Wurster-Hill, 1977; Hahn et al., 1994; Reimann et al., 1994; Thomas et al., 2003). This allows for aneuploidy to be detected using fluorescent probes located at any cytological location along the chromosome of interest. Previous cytogenetic research on canine lymphomas has determined that dogs with tumors bearing trisomy 13 as their primary aberration have a better prognosis than dogs with tumors bearing other aberrations (Hahn et al., 1994; Thomas et al., 2003).

From a clinical standpoint, being able to classify subsets of canine lymphoma quickly, reliably, and non-invasively would allow clinicians to select the most appropriate treatment for the specific tumor type. The preferred tissue for aberrant chromosomal analysis is samples derived directly from the tumor; however, in instances when the tumor analysis is impractical or impossible, the peripheral blood presents a viable alternative. Our laboratory has determined that chromosomal aberrations are detectable in lymphocytes from the peripheral blood in previously untreated lymphoma dogs. Evaluating chromosomal changes from peripheral blood offers a huge advantage over evaluating cells obtained from the tumor as it provides a method of monitoring the dog once the disease has gone into remission. However, first a correspondence between the aberrations in the blood and tumor needed to be determined which was successfully achieved in this study. The most likely origin of the aberrant peripheral blood lymphocytes is due to extravasation of

tumor cells from the lymph nodes. The positive results garnered in this study indicate promise for use as a diagnostic tool using the peripheral blood of dogs with lymphoma.

Materials and methods

Collection of sample material

Twenty-five dogs with naïve lymphoma were identified by the Oncology Service of the Colorado State University Veterinary Medical Center. The age at diagnosis ranged from 4 to 11 years (mean 8 years) and included 8 females and 17 males. Ten different breeds were represented along with five mixed breed dogs. Clinical stages were determined according to the World Health Organization staging criteria for canine lymphoma (WHO, 1979) and all dogs were in stages III–V. Dogs underwent surgical lymph node excision, bone marrow aspiration, and had a peripheral blood sample collected. Ten clinically normal dogs and five dogs with solid tumors, both female and male of various breeds, had one peripheral blood sample collected to serve as controls.

Histology, staging, and immunophenotyping

A portion of the excised lymph node was submitted to the Colorado State University Diagnostic Laboratory for immunophenotyping via flow cytometry and histology. Tumors were classified according to the National Cancer Institute's Working Formulation. Bone marrow aspirates were cytologically evaluated for tumor spread.

Cell culture

The portion of lymph node allotted for cytogenetic studies was prepared for short-term culture (16 h) in RPMI-1640 medium supplemented with 20% fetal bovine serum, 4 mmol/l L-glutamine, 200 U/ml penicillin, and 0.2 mg/ml streptomycin. Cultures were set up with a final cell concentration of $0.75 \times 10^6/\text{ml}$ in 10 ml of RPMI-1640 medium and incubated at 37°C, 5% CO₂. For the final hour of incubation, 100 ng/ml colcemid was added. The cells were processed using a traditional hypotonic fixation method using 75 mM KCl and 3:1 methanol:acetic acid.

Each peripheral blood sample was cultured with 1 ml of blood in 10 ml of RPMI-1640 medium supplemented with 20% fetal bovine serum, 4 mmol/l L-glutamine, 200 U/ml penicillin, 0.2 mg/ml streptomycin, and 10 µg/ml pokeweed mitogen (Sigma-Aldrich). Cultures were incubated at 37°C with 5% CO₂ for 96 h with 100 ng/ml colcemid added during the last hour of incubation. The cells were processed using a traditional hypotonic fixation method.

FISH probe development

The four clones were selected from the CHORI-82 bacterial artificial chromosome (BAC) library (<http://bacpac.chori.org/library.php?id=253>) and represented sequences of canine chromosomes 11, 13, 14, and 31. These four chromosomes were chosen based on previous findings of their predominance in canine lymphoma (Thomas et al., 2003). The names of each BAC are as follows: CH82-110N04 (CFA11), CH82-139P07 (CFA13), CH82-408F05 (CFA14), and CH82-190A04 (CFA31). The midpoint megabase location of each BAC DNA insert is as follows: CFA11

Fig. 1. Trisomy 13 labeled in Spectrum Red in a tumor lymphocyte (top) and peripheral blood lymphocyte (bottom) from dog 11. The top figure demonstrates normal copy numbers of CFA11 (Spectrum Green). Note that the blood sample has both an interphase and metaphase cell displaying the aberration.

(37.55), CFA13 (62.56), CFA14 (91.41), and CFA31 (20.24). BAC DNA was isolated using the Qiagen Large-Construct Kit. One microgram of BAC DNA was fluorescently direct labeled using a nick translation kit (Roche). The labeling protocol was as follows: 15°C for 3 h and 65°C for 10 min.

Fluorescence in situ hybridization

The probes were hybridized to normal canine metaphase cells to verify cytogenetic location and copy number. Two slides were made for each blood and tumor sample, with each slide hybridized with two probes. Scoring two probes per slide was found to be more rapid than scoring a slide with all four probes primarily due to being able to score the slide directly rather than combining multiple pictures into one image. Chromosomes 11 and 13 were

labeled with Spectrum Red (Abbott Molecular) and chromosomes 14 and 31 were labeled using Spectrum Green (Abbott Molecular). To prepare slides for FISH analysis, 20 μ l hybridization mix (50% formamide), 4 μ l canine COT-1 DNA (400 ng/ml), fluorescently labeled probes, and sterile water were combined in an Eppendorf tube with a final volume of 30 μ l. The COT-1 DNA was prepared from whole blood samples garnered from at least five different dogs of both genders. The sample slides and probes were coverslipped and sealed using rubber cement. They were co-denatured at 80°C for 5 min and 37°C for at least 5 min. The slides were incubated at 37°C for 24 h. Afterwards, the slides were washed in the following series at 45.5°C: 50% formamide in 2 $\times$ SSC pH 5.8, 2 $\times$ SSC pH 7.4, and 2 $\times$ SSC pH 7.4 + 0.1% Igepal CA-630. Slides were counterstained with DAPI/AF.

Image analysis

Each FISH slide had 200 cells evaluated using a Nikon fluorescent microscope and corresponding MetaMorph cytogenetic software (Molecular Devices, Sunnyvale, CA) (Fig. 1). A cutoff of 1% was established to determine the presence of an aberration meaning at least two cells demonstrating the aberration were required for inclusion in the statistical data summarized in Table 1. Both metaphase and interphase cells were scored.

Statistical analysis

The frequencies and relative frequencies of the aberrations and measures of blood test validity were measured. The overall agreement (OA) of the aberrations being found in both the lymph node and peripheral blood were calculated as follows: $OA = (n_{Node = 0, Blood = 0} + n_{Node = 1, Blood = 1})/25$. The sensitivity (Se) corresponds to the ability of the peripheral blood to detect the presence of aberrations while the specificity (Sp) reflects the ability to detect the absence of aberrations. The predictive value (PPV) is the probability that the presence of an aberration found in the peripheral blood corresponds to the presence of the same aberration in the lymph node while the negative predictive value (NPV) reflects that the absence of the aberration corresponds in both the blood and lymph node.

Results

Histology, staging, and immunophenotyping

The tumors were classified according to the National Cancer Institute's Working Formulation for Non-Hodgkin's Lymphoma, which divides the tumors into low, intermediate, and high grade. Of the 22 dogs with histological grading, none of the dogs had low grade tumors, 8 dogs (36%) had tumors of intermediate grade, and 14 dogs (64%) had high grade tumors. Dogs with intermediate grade disease had a median survival time of 332 days whereas dogs with high grade disease had a shorter median survival (232 days). Six morphological subgroups were identified with between one and eight representatives in each group. The two dogs with diffuse small cleaved cell (intermediate grade) had a median survival

Table 1. Staging information and specific numerical aberrations for 25 dogs with lymphoma

Dog	Sex/age	Breed	Classification ^a / grade	Immuno- phenotype	Stage	Aberrations in lymph node	Aberrations in blood
1	♀/8	Mixed breed	DSC/intermediate	T	IIIa	+31	–
2	♂/6	Rhodesian Ridgeback	DL/intermediate	B	IIIa	–11, +11, +13, –14	–11, +11, +13, –14, +31
3	♂/10	Australian Shepherd	LB/high	T	IVb	–13	–
4	♂/5	Golden Retriever	IB/high	B	IIIa	–11, +11, –13, +13, –14, –31, +31	–11, +11, +13, –13, +13, –14, –31, +31
5	♀/6	Bernese Mountain Dog	DSC/intermediate	B	–	–11	–
6	♂/4	Doberman Pinscher	IB/high	B	Va	–11, +11, –13 +13, –14, –31, +31	–11, +11, –13, +13, –14, –31, +31
7	♂/8	Boxer	DL/intermediate	B	IIIa	+13, –31, +31	+13, –31
8	♂/8	Golden Retriever	–	B	IIIa	–	–11, +13
9	♂/11	Golden Retriever	–	–	Va	–11, +13	–11, +13
10	♂/11	Golden Retriever	DL/intermediate	–	IVa	–	–
11	♂/8	Golden Retriever	SNC/high	B	IVa	+13, +31	+13, +31
12	♀/4	Golden Retriever	IB/high	B	IIIa	–	–
13	♂/6	Scottish Terrier	IB/high	B	–	–11, +11, +13, –31	–11, +11, –13, +31
14	♂/7	Bouvier des Flandres	–	B	IVa	–11	–11
15	♂/6	Golden Retriever	DM/intermediate	T	–	+13, +31	–
16	♂/11	Mixed Breed	LB/high	T	IIIa	+13	+13
17	♀/6	Mixed Breed	LB/high	T	IIIa	+11, –31, +31	+11, –11
18	♀/10	Mixed Breed	IB/high	B	Va	–11, +13	–11, +13
19	♂/7	Mixed Breed	DL/intermediate	B	–	–11, –13, +13, +31	–11, +11, –13, +13
20	♀/7	Weimaraner	IB/high	B	–	+13	+13
21	♀/10	Golden Retriever	IB/high	–	–	–11, –13	–11
22	♂/10	Jack Russell Terrier	DSL/low	–	–	–11, +11, +13, +31	–11, +31
23	♂/8	Mixed Breed	IB/high	B	–	–	–
24	♀/9	Border Collie	DL/intermediate	B	IVb	–	–
25	♂/9	Miniature Schnauzer	SNC/high	–	Va	+11, +13, –14	+13

^a DL: diffuse large cell; DM: diffuse mixed small and large cell; DSC: diffuse small cleaved cell; DSL: diffuse small cell; IB: large cell immunoblastic; LB: lymphoblastic; SNC: small non-cleaved cell.

time of 322 days. Eight dogs had large cell immunoblastic lymphoma (high grade) and had a median survival of 322 days. Five dogs had diffuse large cell lymphomas (intermediate grade) and had a median survival of 226 days. Three dogs had lymphoblastic lymphoma (high grade) and had a median survival of 162 days. The log-rank test and Wilcoxon test *P* values were not significant. The staging information and aberration correlations are summarized in Table 1.

Of the 20 dogs with immunophenotype data, 15 (75%) had tumors of B-cell origin and 5 (25%) had T-cell lymphoma. The T-cell dogs had a median survival time of 332 days whereas the B-cell dogs had a median survival time of 322 days. The log-rank test and Wilcoxon test *P* values were not significant.

FISH analysis

Seven numerical aberrations were scored in both the lymph node and concurrent peripheral blood sample. The FISH reactions were performed using two color reactions of Spectrum Red and Spectrum Green meaning that each sample garnered two slides for scoring. The combination of probes on the slides was randomly chosen. For example, the probe for CFA11 (red) could be paired with either the probe for CFA14 or CFA31 (green). Two hundred cells, both metaphase and interphase, were scored for each slide sample.

Dogs with lymphoma

The quantitative values for the actual number of aberrant cells scored are summarized in Table 2. In the majority of cases, the specific aberration was detected in both

Table 2. Number of aberrations scored (n = 200 cells) in the tumor and blood in canine lymphoma patients. The table includes all scored aberrations; however, only samples demonstrating >1% abnormal cells (≥ 2 cells) were included in the statistical data summarized in Table 1.

Dog	Aberration	Tumor	Blood	Dog	Aberration	Tumor	Blood	Dog	Aberration	Tumor	Blood	Dog	Aberration	Tumor	Blood
1	-11	0	0	8	-11	0	0	15	-11	0	0	22	-11	7	3
	+11	0	0		+11	0	0		+11	0	0		+11	3	0
	-13	0	0		-13	0	0		-13	0	0		-13	0	0
	+13	0	0		+13	0	0		+13	4	0		+13	12	0
	-14	0	0		-14	0	0		-14	0	0		-14	0	0
	-31	0	0		-31	0	0		-31	0	0		-31	0	0
	+31	4	0		+31	0	0		+31	6	0		+31	20	15
2	-11	15	8	9	-11	15	3	16	-11	0	0	23	-11	0	0
	+11	12	4		+11	0	0		+11	0	0		+11	0	0
	-13	0	1		-13	0	0		-13	1	0		-13	0	0
	+13	12	10		+13	9	2		+13	171	7		+13	0	0
	-14	12	4		-14	0	0		-14	1	0		-14	0	0
	-31	0	1		-31	0	0		-31	0	0		-31	0	0
	+31	1	2		+31	0	0		+31	0	0		+31	0	0
3	-11	0	0	10	-11	0	0	17	-11	0	14	24	-11	0	0
	+11	0	0		+11	0	0		+11	10	14		+11	0	0
	-13	2	0		-13	0	0		-13	0	0		-13	0	0
	+13	0	0		+3	0	0		+13	0	0		+13	0	0
	-14	0	0		-14	0	0		-14	0	0		-14	0	0
	-31	0	0		-31	0	0		-31	3	0		-31	0	0
	+31	1	0		+31	0	0		+31	3	0		+31	0	0
4	-11	20	3	11	-11	0	0	18	-11	7	5	25	-11	0	0
	+11	6	3		+11	0	0		+11	0	0		+11	3	0
	-13	15	13		-13	0	1		-13	0	0		-13	0	0
	+13	3	3		+13	69	15		+13	172	37		+13	186	79
	-14	8	10		-14	0	0		-14	0	0		-14	6	0
	-31	6	9		-31	0	0		-31	0	0		-31	0	0
	+31	20	3		+31	10	6		+31	0	0		+31	0	0
5	-11	3	0	12	-11	1	0	19	-11	10	12				
	+11	0	0		+11	0	0		+11	0	4				
	-13	0	0		-13	0	0		-13	12	2				
	+13	0	0		+13	0	0		+13	10	16				
	-14	0	0		-14	0	0		-14	0	0				
	-31	0	0		-31	0	0		-31	0	0				
	+31	0	0		+31	0	0		+31	3	0				
6	-11	10	3	13	-11	10	3	20	-11	0	0				
	+11	3	3		+11	8	3		+11	0	0				
	-13	6	8		-13	0	6		-13	0	0				
	+13	8	15		+13	6	0		+13	153	17				
	-14	3	2		-14	0	0		-14	0	0				
	-31	2	4		-31	0	3		-31	0	0				
	+31	3	4		+31	6	0		+31	0	0				
7	-11	0	0	14	-11	20	11	21	-11	6	3				
	+11	0	0		+11	0	0		+11	0	0				
	-13	0	0		-13	0	0		-13	2	0				
	+13	162	18		+13	0	0		+13	0	0				
	-14	0	0		-14	0	0		-14	0	0				
	-31	3	3		-31	0	0		-31	0	0				
	+31	3	0		+31	0	0		+31	0	0				

Table 3. Frequencies and relative frequencies of aberrations and measures of blood test validity (total n = 25)

Aberration	Node (-), blood (-) n (%)	Node (-), blood (+) n (%)	Node (-) total	Node (+), blood (-) n (%)	Node (+), blood (+) n (%)	Node (+) total	OA	Se	Sp	PPV	NPV	% node (+)
Monosomy 11	13 (92.9)	1 (7.1)	14	1 (9.1)	10 (90.9)	11	0.92	0.91	0.93	0.91	0.93	44
Trisomy 11	17 (94.4)	1 (5.6)	18	2 (28.6)	5 (71.4)	7	0.88	0.71	0.94	0.83	0.89	28
Monosomy 13	20 (100)	0	20	2 (40)	3 (60)	5	0.92	0.60	1.00	1.00	0.91	20
Trisomy 13	11 (100)	0	11	2 (14.3)	12 (85.7)	14	0.92	0.86	1.00	1.00	0.85	56
Monosomy 14	20 (100)	0	20	2 (40)	3 (60)	5	0.92	0.60	1.00	1.00	0.91	20
Monosomy 31	19 (95)	1 (5)	20	2 (40)	3 (60)	5	0.88	0.60	0.95	0.75	0.90	20
Trisomy 31	13 (86.7)	2 (13.3)	15	6 (60)	4 (40)	10	0.68	0.40	0.87	0.67	0.68	40

OA: overall agreement, Se: sensitivity, Sp: specificity, PPV: predictive value, NPV: negative predictive value.

the lymph node and peripheral blood. The proportion of aberrant cells was consistently higher in the lymph node than in the blood. There were some examples of an aberration detected in the lymph node but not the corresponding blood sample. There were fewer examples of the aberration found in the blood but not in the lymph node. The overall agreements for the peripheral blood and lymph node samples in relation to each aberration were calculated (Table 3).

Control dogs

Each normal dog and solid tumor dog had at least one peripheral blood sample cytogenetically analyzed using the same technique of two-color-hybridization per slide. Only a single monosomy was detected at one time point from four of the normal dogs (CFA11) and at one time point from one solid tumor dog (CFA13).

Discussion

The four chromosomes that were cytogenetically analyzed using single locus probes were chosen based on previous studies implicating CFAs 11, 13, 14, and 31 as some of the most commonly aberrant chromosomes in canine lymphoma (Hahn et al., 1994; Thomas et al., 2003). The comparison between the tumor and peripheral blood aberrations revealed a significant correspondence. Occasionally, the aberration was found in either the tumor or blood but not both. In the cases in which the aberration was detected in the tumor but not the blood, it is possible that the sample count was not sufficient to detect the aberration. When the aberration was found in the blood but not the tumor, one explanation

could be tumor heterogeneity. Only a small portion of the excised lymph node is prepared for culture. It is possible that different clonal populations are spatially separated and not detected in the analyzed samples (LaRue et al., 1994). The overall agreement among each aberration was convincing in six out of the seven aberrations scored.

Trisomy of CFA13 has been reported as a predictor of longer remission and survival times (Hahn et al., 1994). It has also been described as the most common numerical aberration found in canine lymphoma (Hahn et al., 1994; Thomas et al., 2003). This study was in accordance with the predominance of trisomy 13. However, prognostic significance was not found with any of the aberrations, likely due to a small sample size. Correlations between histological classifications and immunophenotype and specific aberrations were not found, probably due to a small case number.

It is likely that subsets of lymphoma based on cytogenetic abnormalities exist in canine lymphoma, just as in human non-Hodgkin's lymphoma and other hematopoietic diseases. Once that is established, a peripheral blood sample from a dog diagnosed with lymphoma could be cytogenetically analyzed to better aid the clinician and owner toward an appropriate treatment protocol that is ideal for the particular subset of lymphoma. Significance for the number of aberrations in the peripheral blood would need to be determined to reduce the number of cells scored and time involved for analysis. Cytogenetic analysis of the tumor is still preferred but this method offers a viable alternative when tumor analysis is not possible. This could pave the way toward a method of monitoring dogs with lymphoma during their course of treatment.

References

- Baskin CR, Couto CG, Wittum TE: Factors influencing first remission and survival in 145 dogs with lymphoma: a retrospective study. *J Am Anim Hosp Assoc* 36:404–409 (2000).
- Carter RF, Valli VE, Lumsden JH: The cytology, histology and prevalence of cell types in canine lymphoma classified according to the National Cancer Institute Working Formulation. *Can J Vet Res* 50:154–164 (1986).
- Hahn KA, Richardson RC, Hahn EA, Chrisman CL: Diagnostic and prognostic importance of chromosomal aberrations identified in 61 dogs with lymphosarcoma. *Vet Pathol* 31: 528–540 (1994).
- Khanna C, Lindblad-Toh K, Vail D, London C, Bergman P, et al: The dog as a cancer model. *Nat Biotechnol* 24:1065–1066 (2006).
- Kim GE, Koom WS, Yang WI, Lee SW, Keum KC, et al: Clinical relevance of three subtypes of primary sinonasal lymphoma characterized by immunophenotypic analysis. *Head Neck* 26:584–593 (2004).
- LaRue SM, Fox MH, Withrow SJ, Powers BE, Straw RC, et al: Impact of heterogeneity in the predictive value of kinetic parameters in canine osteosarcoma. *Cancer Res* 54:3916–3921 (1994).
- Lee MY, Tan TD, Feng AC, Liu MC: Clinicopathological analysis of 598 malignant lymphomas in Taiwan: seven-year experience in a single institution. *Am J Hematol* 81:568–575 (2006).
- Pathak S, Wurster-Hill DH: Distribution of constitutive heterochromatin in carnivores. *Cytogenet Cell Genet* 18:245–254 (1977).
- Reimann N, Rogalla P, Kazmierczak B, Bonk U, Nolte I, et al: Evidence that metacentric and submetacentric chromosomes in canine tumors can result from telomeric fusions. *Cytogenet Cell Genet* 67:81–85 (1994).
- Rudiger T, Weisenburger DD, Anderson JR, Armitage JO, Diebold J, et al: Peripheral T-cell lymphoma (excluding anaplastic large-cell lymphoma): results from the Non-Hodgkin's Lymphoma Classification Project. *Ann Oncol* 13:140–149 (2002).
- Thomas R, Smith KC, Ostrander EA, Galibert F, Breen M: Chromosome aberrations in canine multicentric lymphomas detected with comparative genomic hybridisation and a panel of single locus probes. *Br J Cancer* 89: 1530–1537 (2003).
- Vail DM, Young KM: Hematopoietic tumors, in Withrow SJ, Vail DM (eds): *Withrow and MacEwen's Small Animal Clinical Oncology* 4th ed., pp 699–784 (Saunders, Philadelphia 2007).
- Weller RE, Theilen GH, Madewell BR: Chemotherapeutic responses in dogs with lymphosarcoma and hypercalcemia. *J Am Vet Med Assoc* 181:891–893 (1982).
- WHO: Report of the Second Consultation of the Biological Behavior and Therapy of Tumors of Domestic Animals: Bulletin of the World Health Organization, 1979.