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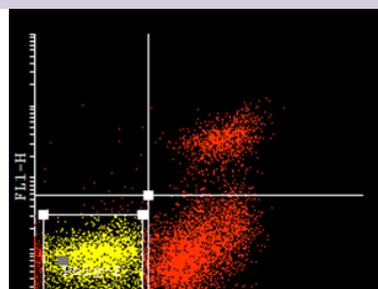
*1<sup>st</sup> Meeting of the European Canine Lymphoma Group*  
**State of the Art and Comparative  
Aspects in Canine Lymphoma**

**Workshop Proceedings**

Palazzo dei Congressi,  
Piazza Indipendenza 4  
CH-Lugano, June 22<sup>nd</sup> 2013

Edited by  
Franco Guscetti, Laura Marconato and Stefano Comazzi

A satellite workshop to the 12-ICML



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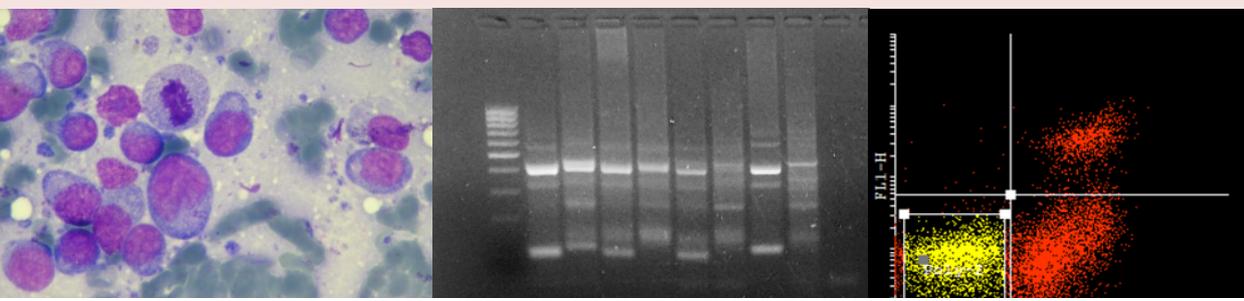
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# **1<sup>st</sup> Meeting of the European Canine Lymphoma Group, CH-Lugano, June 22<sup>nd</sup> 2013**

## **State of the Art and Comparative Aspects in Canine Lymphoma**

### **Workshop Proceedings**

Edited by

**Franco Guscetti, Laura Marconato and Stefano Comazzi**

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## Welcome from the Organizing Committee

Lugano, June 22<sup>nd</sup>, 2013

Dear colleagues,

humans and dogs share many heritable and environmental risk factors and are prone to the same types of cancers, with comparable clinical-pathological aspects. Ethical issues, shorter survival and remission times in dogs compared to humans, a broad genetic background and their size, elect dogs with spontaneous cancers as an appropriate tool for pathogenetic and therapeutic studies including anticancer drug development. This particularly applies to lymphoma, which is the most diffuse hematopoietic tumor in dogs and whose prevalence, risk factors, subtypes and clinical presentation share many similarities with human non-Hodgkin lymphoma.

We wish to acknowledge the kind invitation of 12th ICML congress organisers to join with the present satellite workshop. This can be interpreted as a sign of the potential interest towards canine lymphoma as a comparative model. To take advantage of, and to further develop this model common efforts and strategies are warranted. Also, there is the urgent need to standardize staging work-up and treatment protocols among veterinary oncologists to enable more insight into the biological mechanisms in specific disease entities, ultimately aiding in the development of treatment strategies, which are specifically tailored to the characteristics of the disease. The creation of the European Canine Lymphoma Network ([www.eu-can-lymph.net](http://www.eu-can-lymph.net)), born in 2009 and now linking more than 70 researchers from 25 European institutions involved in research and cure of canine lymphoma, has been a first significant step. The present workshop shall be the next.

The workshop is divided in three major sessions (pathogenesis, diagnosis and therapy) with a series of invited lectures focused on the state of the art on different aspects of canine lymphoma, with comparison with the human counterparts. We expect that the ensuing discussion will unveil hot issues, foster the development of common strategies, establish diagnostic and therapeutic guidelines, and reinforce collaboration within the network. In addition, a poster session with studies on various other topics will provide further material for discussions.

Enjoy the workshop!

The organizing committee,

Stefano Comazzi, Laura Marconato and Franco Guscetti

## Programme

12:45	<b>WELCOME AND INTRODUCTION</b> Stefano Comazzi, Franco Guscetti, Laura Marconato
13:00-14:50	<b>SESSION ON PATHOGENESIS</b> Chairs: Hugo Murua Escobar, Franco Guscetti
13:00-13:15	Comparative transcriptional analysis of canine and human Diffuse Large B Cell Lymphoma (DLBCL). Molecular signatures of NF- $\kappa$ B activation and sub-classification of DLBCL, Ted Hupp, UK-Edinburgh
13:15-13:30	Apoptosis in human and canine lymphoma: the Bcl-2 family of proteins, Franco Guscetti, CH-Zurich
13:30-13:45	Proliferation index (Ki67 and s-phase) in human and canine lymphoma, Fulvio Riondato, I-Turin
13:45-14:00	Role of canine lymphoma cell lines in research: comparison with human lines, Barbara Ruetgen, A-Vienna
14:00-14:15	The role of MMP, VEGF and PDGF in canine lymphomas, Luca Aresu, I-Padua
14:15-14:55	General Discussion
14:55 – 15:40	<b>COFFEE-BREAK AND POSTER VIEWING</b>
15:40-16:50	<b>SESSION ON DIAGNOSIS AND CLASSIFICATION</b> Chairs: Frédérique Ponce, Stefano Comazzi
15:40-15:55	Cytology in classification and therapy of canine lymphomas, Frédérique Ponce, F-Lyon
15:55-16:10	Flow cytometric immunophenotyping: how could it assist in lymphoma diagnosis and management? Stefano Comazzi, I-Milan
16:10-16:25	Classification and prognosis algorithms in canine lymphoma: Are we getting there? Joaquim Henriques, P-Lisboa
16:25-16:50	General Discussion
16:50-18:00	<b>SESSION ON THERAPY</b> Chairs: T. Hupp, L. Marconato
16:50-17:05	Role of immunotherapy in canine lymphoma: shaping the future? Laura Marconato, I-Bologna
17:05-17:20	Role of radiation therapy in canine non-Hodgkin lymphoma, Julia Buchholz, CH-Hünenberg
17:20-17:35	Role of surgery in the management of canine lymphoma: the example of splenic lymphoma, Damiano Stefanello, I-Milan
17:35-18:00	General Discussion
18:00	<b>END OF WORKS AND TAKE-HOME MESSAGE</b>

## Abstracts of invited lectures

### SESSION ON PATHOGENESIS

Chairs: Hugo Murua Escobar, Franco Guscetti

#### **01 Comparative Transcriptional Analysis of Canine and Human Diffuse Large B Cell Lymphoma (DLBCL). Molecular Signatures of NF- $\kappa$ B Activation and Sub-Classification of DLBCL**

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We present the first comparison of global transcriptional changes in canine and human diffuse large B-cell lymphoma (DLBCL), with particular reference to the nuclear factor-kappa B (NF- $\kappa$ B) pathway. Microarray data generated from canine DLBCL and normal lymph nodes were used for differential expression, co-expression and pathway analyses, and compared with analysis of microarray data from human healthy and DLBCL lymph nodes. The comparisons at gene level were performed by mapping the probesets in canine microarrays to orthologous genes in humans and *vice versa*. A considerable number of differentially expressed genes between canine lymphoma and healthy lymph node samples were also found differentially expressed between human DLBCL and healthy lymph node samples. Principal component analysis using a literature derived NF- $\kappa$ B target gene set mapped to orthologous canine array probesets and human array probesets clearly separated the healthy and cancer samples in both datasets. The analysis demonstrated that for both human and canine DLBCL there is activation of the NF- $\kappa$ B/p65 canonical pathway, indicating that canine lymphoma could be used as a model to study NF- $\kappa$ B-targeted therapeutics for human lymphoma. The model was further validated by identification of molecular signatures sub-classifying canine DLBCL into activated B-cell-like (ABC) or germinal centre B-cell-like (GCB) types.

## **02 Apoptosis in human and canine lymphoma: the Bcl-2 family of proteins**

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Deregulated apoptosis contributes to malignant transformation and progression and to the development of resistance to therapy. Intrinsic apoptosis has a main protective role against the establishment of neoplastic cell clones. The Bcl-2 family exerts a central function in this pathway by regulating the integrity of the outer mitochondrial membrane and the efflux of pro-apoptotic factors such as cytochrome c from the mitochondria into the cytosol. This results from the mainly stoichiometric interplay of three subgroups, i.e. the anti-apoptotic members (prototype: Bcl-2), the multidomain pro-apoptotic members (Bax, Bak) and the BH3-only proteins, which can act either as sensitizers or as activators.

Since the initial discovery of the oncogenic properties of Bcl-2 in human follicular lymphoma an ample body of basic research studies involving lymphoid cell lines and murine tumor models has implicated the involvement of members of the Bcl-2 family of proteins in lymphomagenesis. Three potential blocks have been postulated, including absence or loss of function of activator BH3-only proteins, or of Bax/Bak, and overexpression of anti-apoptotic members. Further, the status of mitochondrial priming, which can be functionally assessed using BH3-peptides, has been shown to predict response to therapy as a result of the relative abundance of Bcl-2 family members in the cell. Recently, the potential of this protein family as a therapeutic target has emerged with the development and current testing in clinical phase I/II trials of small molecule inhibitors of anti-apoptotic Bcl-2 proteins. They partly showed efficacy as single agents, e.g. for the treatment of CLL. At the sequence level, canine Bcl-2 family proteins display high levels of homology with their human counterparts especially at the BH-domains where they show almost 100% identity. Rare preliminary reports of use of such inhibitors in dogs with tumors are available, albeit this has not been reported yet with lymphoma.

In canine lymphoma, the Bcl-2 family of proteins has received little attention so far. Our approach is to develop immunohistochemical methods for Bcl-2 proteins in biopsies collected for histopathology with the intent of establishing, in the mid-term, predictive markers. Previous immunohistochemical studies of human lymphoma were based on the combined assessment of Bcl-2 family members variably including Bcl-2, Bcl-x, Mcl-1, Bax and Bak; while it was early shown that overexpression of Bcl-2 was associated with worse prognosis, data on prognostic significance were inconsistent for the remaining proteins. This may be due to the heterogeneity of the cases studied. A recent work has found a significantly higher apoptotic activity and expression of pro-apoptotic Bcl-2 members in human tumors with a germinal center vs. non-germinal center B-like profile. We have carried out immunohistochemistry using tissue arrays containing over 80 classified, archival canine lymphoma samples. We put much emphasis on antibody validation using recombinant proteins, normal canine tissues and western blots. So far, we have analysed the expression of the Bcl-2 family members Mcl-1 and Bcl-x, Bak and Bax, and the BH3-only protein Bad in this material. These proteins were found to be frequently and variably expressed across lymphoma immunophenotypes and subtypes. B-cell neoplasms significantly expressed more Mcl-1 than T-cell tumors. Bak and Bax were frequently expressed. Interestingly, the expression of Bax was significantly positively correlated with the percentage of cleaved caspase-3 positive apoptotic cells, indicating an association with the apoptotic activity of the tumors.

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### 03 Proliferation index (Ki67 and s-phase) in human and canine lymphoma

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Both s-phase fraction (SPF) and Ki67 index (Ki67) correlate with tumor grading of human non-Hodgkin lymphoma (NHL) and cut-off values to discriminate between aggressive and indolent forms have been suggested. Most importantly, both indexes present independent prognostic significance. However, contradictory results have been reported especially concerning Ki67. This is due in part to the heterogeneity of Ki67 and SPF values between and among different NHL histotypes. Other factors accounting for the contrasting results are the differences in the cut-off values adopted and in the method of Ki67 evaluation. More recently both indexes have been studied in specific lymphoma entities demonstrating the need for a different interpretation in relation to the histotype of NHL. These studies showed that a low Ki67 was associated to a worse prognosis in DLBCL and to a better prognosis in indolent NHL. Similarly, a better prognosis has been linked to a lower SPF in indolent lymphomas and to a higher SPF in aggressive lymphomas using different specific threshold values. Moreover, there is evidence of improved efficacy of the IPI (International Prognostic Index) score when combined with Ki67 evaluation. In patients affected by DLBCL and presenting with low or intermediate IPI score, a higher overall survival has been highlighted for Ki67 <70% and <75%, respectively; in mantle cell lymphoma a combined biological index (MIPIb) adding Ki67 to the MIPI (Mantle Cell Lymphoma IPI) score was useful to stratify patients into different risk groups.

Very few studies have been carried out on the assessment of SPF and Ki67 in canine lymphoma, and similarities with the human counterpart have been found. In a study by Teske, SPF varied among the different canine lymphoma entities but didn't show diagnostic or prognostic significance. In dogs, like in humans, Ki67 resulted as a very good indicator of grade of malignancy: in a study by Fournel-Fleury, 21% was identified as the cut-off value being able to discriminate between low- and high-grade NHL (the cut-off was reduced to 10% if mycosis fungoides cases were excluded). In this study Ki67 varied significantly among subtypes with marginal zone lymphoma (MZL) presenting the lowest index (3%). However, a certain degree of heterogeneity within each type was reported. In a recent study on canine indolent lymphomas, Flood-Knapik described significantly different Ki67

indexes in follicular lymphoma compared to marginal zone lymphoma, and hypothesized that Ki67 might be useful in detecting more aggressive MZL.

Our preliminary flow cytometric data confirm that both SPF and Ki67 are very good markers to differentiate between low-grade and high-grade lymphomas (cut-off values of 3,9% and 12%, respectively); furthermore, Ki67 may have prognostic significance in DLBCL, with a better outcome associated to intermediate values (20-40%). However, a much larger series is necessary to get reliable results, considering the very low incidence of some NHL subtypes in dogs. Moreover, combining flow cytometric determination of cell size (FSC), immunophenotype, proliferative activity (Ki67) and apoptotic activity (Annexin-V), we developed a scheme to help in classifying different entities. Thus, flow cytometric analysis of SPF and Ki67 are useful in stratifying canine lymphoma cases according to biological behaviour. Further studies are warranted to confirm their utility as prognostic indicators in canine NHLs and to define their role in characterizing specific entities for comparative studies.

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*Flood-Knapik et al. Vet Comp Oncol 2012 (epub ahead of print)*

#### **04 Role of canine lymphoma cell lines in research - comparison with human lines**

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Since the 50ies of the last century cell lines serve as worldwide used tools for *in vitro* and *in vivo* studies. Stable well characterised cell lines ensure stable and reproducible experimental conditions throughout a research project and are used for preclinical drug testing, antigen production, development of animal models, basic research on tumorigenesis and for quality control in diagnostic testing.

Establishment of leukemia/lymphoma cell lines is known to be difficult. Given the small amounts of sample material available and the lack of specific growth factors for optimal cell culture conditions the establishment of cell lines in veterinary medicine is particularly challenging. Nevertheless, also in humans success rates for the establishment of leukemia/lymphoma cell lines are poor. In humans, liquid sample material such as peripheral blood and pleural effusions show the highest success rate with 48% and 13%, respectively, whereas out of solid lymph node materials the success rate is only 3%. Approximately 1500 hematopoietic neoplastic cell lines have been described. Many of them were developed before the 'genomics revolution'; thus their characterisation is often based solely on their clinical origin in combination with immunophenotyping analysis.

In veterinary medicine only a few canine lymphoma/leukaemia cell lines are described and even a smaller number is well characterized. The first canine cell lines were established in the late 1980ies and early 1990ies and more recently in 2007, 2010 and 2013. Most of the well described cell lines, as in

humans, were established from effusion material or whole blood. One lymphoma cell line has been established from lymph node material and only two, OSW and CLBL-1, have been characterized in detail at the genomic level. The OSW cell line is characterized as T-cell line out of a peripheral T-cell lymphoma, whereas the CLBL-1 cell line is a B-cell line derived from a Diffuse Large Cell Lymphoma. As tools enabling genomic characterization are now available, previously established canine hematopoietic cell lines are revisited. Generation of a genotypic and phenotypic profile for each cell line is important to ensure its usefulness as *in vitro* and *in vivo* models of lymphoid neoplasia and for the development of adequate animal models for equivalent human disease entities.

Canine haematopoietic malignancies are highly comparable with their human counterparts with regard to clinical presentation, tumour biology and response to therapy. Dogs share evolutionarily conserved chromosome aberrations and mutations within key oncogenes, indicative of common pathogenesis mechanisms. Moreover dogs have also been sharing their environment with humans for thousands of years and are outbred individuals, which makes canine lymphoma a suitable large animal model in comparative medicine. These facts make also canine lymphoma cell lines valuable for canine and furthermore for comparative medicine.

In humans, as well as in dogs, these cell lines represent important tools providing repeatable and reproducible results and, after sufficient characterization, these cell lines have the potential to be representative for several aspects of the respective diseases.

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## 05 The role of MMPs, VEGF and PDGF in canine lymphomas

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Vascular Endothelial Growth Factor (VEGF) and enzymes involved in extracellular matrix (ECM) remodelling, including Matrix Metalloproteinases (MMPs), are recognized useful molecules for the early prognosis assessment of different human haematologic malignancies. Tumour cells in lymphoma and leukaemia can self-produce and secrete MMPs to promote invasion and increase their metastatic potential. These processes are controlled by tissue inhibitor metalloproteinases (TIMPs) that are co-secreted by MMP-producing cells. Among MMPs, MMP-2 and MMP-9 play a critical role in the modification of ECM and tumour invasion.

In canine lymphoma VEGF and active-MMP-9 plasmatic levels are significantly higher when compared with healthy dogs and in T-cell lymphomas compared with B-cell lymphomas. Increased levels of VEGF in plasma are also correlated to the biological behaviour in T-cell lymphoma. Decreased VEGF and act-MMP-9 levels are observed in dogs with B-cell lymphomas during the chemotherapy protocol. This data is in agreement with human studies indicating that VEGF and MMP-9 might predict treatment response. Conversely, in T-cell lymphomas, VEGF and MMP-9 levels are unaffected.

In lymph nodes affected by lymphoma, the most significant results are higher MMP-9 protein and mRNA expression levels in T-cell lymphomas compared with B-cell lymphomas and healthy control lymph nodes, indicating that MMP-9 may be associated with tumour phenotype. MMP-9 is also associated with prognosis since it is higher in HG T-cell lymphomas that are characterized by organ invasion and frequent bone marrow infiltration, and are considered highly aggressive. Biologically, T-cells are able to migrate across ECM barriers during the inflammatory process towards target tissues and the activation of MMP-9 can cause alteration of adjacent connective tissues and degradation of collagen. Interestingly, significantly higher levels of TIMP-1 mRNA are observed in T-cell lymphomas compared with B-cell lymphomas and controls. The *in vitro* model of T-cell lymphoma cell line (OSW) confirms this data. The most realistic hypothesis is that MMP-9 and TIMP-1 may act in concert in canine T-cell lymphoma: MMP-9 causes ECM degradation, whereas TIMP-1 shows an anti-apoptotic action. VEGF-A mRNA and protein expression are also correlated with prognosis and, moreover, the mRNA VEGF-A results are correlated with MMP-9 results in T-cell lymphoma. Future efforts should be directed to the functional VEGF polymorphisms, which have an effect on the regulation of gene expression.

Platelet Derived Growth Factors (PDGFs) and receptors are known to induce tumour growth by directly stimulating growth, to stimulate angiogenesis and to recruit pericytes. Peripheral T-cell lymphomas and cutaneous T-cell lymphomas shows the most significant results with a high level of protein and mRNA expression of PDGF-B and PDGF-R $\beta$ . Conversely, PDGF family seems not to be involved in the pathogenesis of Diffuse Large B-cell lymphomas and Marginal Lymphomas. The PDGF-B gene has been identified as the human homologue of the *v-sis* oncogene, and the strong expression of PDGF-B and PDGF-R $\beta$  suggests that this autocrine signalling may be important in the malignant transformation of T-cell lymphomas. Conversely, this mechanism seems not to be associated with B-cell lymphomas.

## SESSION ON DIAGNOSIS AND CLASSIFICATION

Chairs: Frédérique Ponce, Stefano Comazzi

### 06 Cytology in classification and therapy of canine lymphomas

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Following the Human Classifications of Non-Hodgkin's Lymphomas (NHLs), the goal is to define canine disease entities, with their particular morphology, immunophenotype and clinical data. They must be recognized by pathologists and have clinical relevance.

**The first rationale for a precise morphological classification** of NHLs appears obvious as the examination at the cellular level is now an essential step to make the diagnosis of the various subtypes of NHLs. In our experience, there appears to be a good correlation between morphology and phenotype allowing the pathologists to formulate a tentative diagnosis of B or T-phenotype by cytologic analysis in most cases. However, the immunophenotype always has to be precisely determined by immunolabelling.

As in humans, the cytological diagnosis is based on: i. the architectural pattern (e.g. recognition of a follicular organization, aspect of the background) ; ii. the cell type ; iii. the differentiation block in the lymphomatous process (concept of transformation during the course of the disease) ; iv. the proliferating power (mitotic index, Ki67 index).

**The second rationale for a precise morphological classification** is to allow oncologists to predict outcome and responses to chemotherapy. Hence, there are many distinct diseases each with their particular clinical presentation at the time of diagnosis, their own prognostic relevance and associated with distinctive responses to chemotherapy. This suggests that recognition and classification of the different subtypes of lymphomas is clinically justified in dogs, as in humans.

**The third rationale for a precise cytological classification** is to allow, in the context of the daily diagnostic work, a precise diagnosis of the majority of subtypes and a regular cytological follow-up of the clinical course of indolent lymphomas until transformation. Cytological monitoring allows an earlier detection of transformation from a low to a high-grade lymphoma. In addition, fine needle aspirations are easily and quickly performed in case of infiltration of deeply located organs.

In humans, there is still significant skepticism towards the role of cytology, and excisional biopsy is frequently regarded as essential for a precise classification of NHLs. However, cytology is an accurate method of diagnosing and typing common forms of human's NHL. Awareness of the diagnostic limitations and pitfalls is of key importance in promoting the acceptance for a cytologic diagnosis. Excisional biopsy should be considered where the cytologic diagnosis is equivocal or lymphadenopathy persists without identified cause.

**Out of the new World Health Organization classification**, two **B-cell entities** will be presented as examples and will be compared to their humans counterparts: i. the Diffuse Large B cell lymphoma ;

ii. The Marginal Zone lymphoma and the concept of transformation towards higher grade as in humans (indolent and aggressive). The definition of canine **T-cell and NK cell** neoplasms, based on morphological immunophenotypic features and postulated cell of origin is less well known than for B-cell tumors and still imprecise. However, two particularly striking clinico-morphological entities will be pointed out: i. the small clear cell/T-zone lymphoma with indolent clinical course, as a specific canine entity ; ii. the aggressive T-cell Lymphoma with Large Granular cells, compared to humans aggressive N/K cell lymphoma/leukemia, hepatosplenic T-cell lymphoma and enteropathy type.

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### **07 Flow cytometric immunophenotyping: how could it assist in lymphoma diagnosis and management?**

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Immunophenotyping plays a central role in refining the cyto-morphological diagnosis of canine lymphoma (cNHL). Flow cytometry (FC) offers some advantages over other immunophenotyping techniques: 1) the large panel of antibodies allows to easily detect aberrancies (thus confirming clonality) and to more precisely determine lymphoma subtypes; 2) the possibility to perform multi-colour labeling helps to pinpoint also poorly represented cell populations, which is useful for staging and evaluation of minimal residual disease; 3) the accurate quantification of both the number of positive cells and the amount of positivity allows to quantitate antigenic expression and improves the evaluation of maturative and activation status; 4) FC is rapid and relatively cheap and this facilitate its introduction in a clinical setup as an important step for diagnosis of c-NHL.

However some disadvantages remain: 1) FC needs a fresh sample (within 24 hours from sampling), however some preservative solutions are under evaluation to prolong storage and facilitate shipping

of samples to reference labs; 2) the lack of information about the architecture of tissues limits the use of FC in some nodular (follicular L), and most splenic (marginal zone and mantle cell L) cNHL and a contemporary evaluation of cyto-morphological aspects is mandatory; 3) the lack of lineage specific markers for some populations (plasma cells, immature B cells, NK cells) and of antibodies labeled with different fluorochromes limits the diagnostic power of FC in veterinary in comparison to human medicine.

In veterinary medical clinical diagnostics the use of FC is relatively recent and lacks standardization among different institutions and labs. The establishment of a consensus among research groups working on FC in canine onco-haematology is mandatory. It should define common strategies, similar protocols and panels, and result in standardized reports and contribute to increase case numbers on specific lymphoma subtypes and pathological conditions. Moreover, a closer cooperation between clinical pathologists working on FC, molecular biologists working on genetic aspects and histopathologists could bring to a better definition of a common chartflow to properly define lymphoma subtype, staging the disease and predict prognosis in order to help the clinicians to tailor the appropriate therapy.

Some hot topics that could require discussion are: 1) is bone marrow evaluation mandatory for clinical staging and which technique should be used? 2) are there specific immunophenotypic signatures that could be considered of prognostic impact? 3) may FC help in monitoring minimal residual disease and predict recurrence? 4) which data from immunophenotyping of cNHL (either by FC or IHC) are clinically useful and how should they be reported?

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## **08 Classification and prognosis algorithms in canine lymphoma: Are we getting there?**

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Non-Hodgkin lymphoma is one of the most common neoplasms in dogs. Despite the advances in the veterinary oncology field, when considering lymphoma as a general disease entity, the available chemotherapy with CHOP based protocols induces response in about 80% of patients obtaining an overall remission of about 6-11 months [1]. In more recent years, the WHO classification of canine hematopoietic diseases, which groups lymphomas by morphology, phenotype, genetic and molecular aspects, allowed its identification as specific entities of B and T cell neoplasms [2]. When applying the WHO classification on canine B cell neoplasms, a high incidence of Diffuse Large B-Cell Lymphoma (DLBCL) subtype is found; this is the most common entity found in dogs [2]. Very few clinical studies exist in the veterinary field regarding pathogenesis and clinical outcome in dogs with DLBCL. Preliminary results suggest that DLBCL is associated with shorter remission time than the historical reports (personal data). In human medicine it has been nearly 10 years since two distinct molecular and prognostic subtypes of DLBCL were differentiated by gene expression profile (GEP) analysis into Germinal Center (CG) and non-Germinal Center B-cells (nGC) with different clinical prognosis and response to therapy [3]. These different subtypes could be differentiated easily using an immunohistochemistry panel, a widely available technique. The use of a classification algorithm that replicates the GEP results could stratify patients according to their survival and consequent response to therapy [3]. Recently, molecular profiling has been performed in a cohort of canine lymphomas, but DLBCL subtypes were not identified [5]. Recent studies (personal data) failed to apply Hans' algorithm to canine DLBCL (cDLBCL), following inconsistency of BCL6, MUM1 and CD10 immunostaining. These results can be due to different genetic causes in cDLBCL or suggest a non-Germinal Center (nGC) origin of cDLBCL, which may behave in an aggressive way as in nGC human counterpart. As in humans, at the present time, there is no consensus on which biological prognostic markers should be routinely assessed in canine DLBCL or what is the actual benefit of the IHC algorithms. Further studies focusing on the comparison between various algorithms reported in human DLBCL, and association with GEP results of the canine lymphoma samples are required. The author will present a review of the DLBCL algorithms used in people and its applicability to canine lymphomas.

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## SESSION ON THERAPY

Chairs: Ted Hupp, Laura Marconato

### **09 Role of immunotherapy in canine lymphoma - shaping the future?**

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Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of canine lymphoma, and it shares many features with the human counterpart, including clinical presentation, biological behavior, genetics and treatment response. The present standard of care for canine DLBCL includes dose-intense multidrug chemotherapy. Although chemotherapy elicits remission and extends overall survival, the disease is essentially incurable, therefore new approaches are needed.

In the last decades, much attention has been paid to immunotherapy, which attempts to foster elimination of malignant cells through the immune system. Active immunotherapy offers two main advantages: i. it elicits a tumor-specific immune response, ii. it potentially establishes a long-lasting tumor immunity via the capacity to exhibit memory, thereby limiting the likelihood of relapse.

The clinical experience in dogs with lymphoma is still at an embryonic stage. In 14 dogs with B-cell lymphoma, a genetic cancer vaccine targeting telomerase in combination with chemotherapy significantly increased survival time when compared with 8 historic controls treated with chemotherapy only. Since diagnosis was obtained by cytology different disease entities may have been included. Also, chemotherapy and type of vaccination differed among dogs. Sorenmo et al. demonstrated the immunogenicity of a cell-based vaccine in dogs with lymphoma using CD-40 activated B-cells. Time to progression (TTP) and lymphoma-specific survival (LSS) were not significantly different between vaccinated and non-vaccinated dogs; however, vaccination potentiated the effects of salvage therapy and improved the rate of durable second remissions and LSS following rescue therapy. Overall, the tolerability and efficacy of the vaccines in these studies were compelling enough to justify evaluation of alternative vaccines considering the criteria of easier/faster production, better cost-effectiveness, or stronger immune response.

Typically, active immunotherapy in people with lymphoma consists of vaccines that use the immunoglobulin idiotype as a tumor-specific antigen. However, a possible disadvantage of active immunotherapy is its reliance on the patient's immune system, which may be compromised or deregulated by the tumor itself or by previous chemotherapy. Consequently, efforts to enhance the efficacy of active immunotherapy are ongoing with an emphasis on optimization of antigen delivery and presentation and modulation of the immune system toward counteracting immune-suppression. A new promising approach in people is the delivery of an autologous vaccine consisting of hydroxyapatite ceramic powder and proteins purified from the patients' tumors, such as Heat Shock Proteins (HSPs). A randomized controlled double-blinded trial was conducted at the Centro Oncologico Veterinario, with the primary aim of showing that an autologous vaccine with hydroxyapatite and tumor derived HSP-peptide complexes (HSPPCs) is safe and therapeutically effective in dogs with DLBCL. Nineteen dogs with DLBCL were prospectively enrolled; among these, 12 received chemo-immunotherapy (Group 1) and 7 received chemotherapy only (Group 2). Median duration of first remission was significantly longer in Group 1 compared with Group 2 (282 vs 41 days, respectively;  $p=0.0004$ ). Dogs in Group 1 were significantly less likely to experience a relapse

compared to dogs in Group 2 (risk ratio: 0.583333; 95%CI: 0.36-0.94). In Group 1, 5 dogs were still alive at the time of analysis; in Group 2, no dogs survived. Median survival was significantly longer in Group 1 than in Group 2 (473 days versus 159 days, respectively;  $p=0.0018$ ). Overall, treatment was well tolerated in both groups. In conclusion, our results suggest that HSPPCs is well tolerated and synergizes with chemotherapy to improve TTP and LSS in dogs with DLBCL.

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## 10 Role of radiation therapy in canine non-Hodgkin lymphoma

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In veterinary medicine radiotherapy (RT) is commonly and successfully used for localized lymphoma, such as nasal, spinal, cutaneous, rectal, osseous and mediastinal forms. The strong radiation sensitivity of lymphocytes justifies inclusion of RT also into the treatment regimen of multicentric lymphoma. Dogs are mainly suffering from non-Hodgkin lymphoma (NHL) and RT is not used routinely. If radiotherapy is used for not localized NHL, mainly half or total body radiation is/has been performed, which consequently carries a higher risk of toxicity.

Already in 1968 Johnson et al. stated that canine lymphomas closely resemble their human counterparts. They observed objective remissions to total-body irradiation (TBI) in dogs with lymphoma. In 1989, Laing et al. evaluated the technique of half-body radiation therapy to treat canine lymphoma patients administering 7 Gray (Gy) in a single exposure (Phase I-II study). During these first studies, the overall tumor response was poor compared to combination chemotherapy protocols and the toxicity was unacceptable, including deaths due to tumor lysis syndrome, sepsis, thrombocytopenia, and with up to 80 % of dogs showing signs of radiation sickness. Since then more commonly 4 Gy fractions on 2 consecutive days have been administered to each half of the body 3-4 weeks apart to reduce toxicity. To increase efficacy, RT was incorporated in protocols to be given in combination with chemotherapy rather than using the two treatments separately, in an attempt to take advantage of complementary effects of the two modalities. Another approach has been to treat relapsed canine lymphoma using low-dose total body irradiation (LDTBI) with the additional rationale to induce an immune response. Recently, also low-dose rate irradiation was administered to half of the body in order to shorten the inter-radiation interval. This means that half of the body gets treated first with this low dose rate allowing for sublethal damage repair to occur, and allowing for safe treatment of the other half of the body after 2 weeks already, thereby minimizing tumor cell

repopulation between the two RT-fractions. Results of this study were very promising with median survival times of about 3 years; chemotherapy was continued for a long time though (about 2 years). Potential side effects after half-body/TBI are myelosuppression (mainly neutropenia and thrombocytopenia), alopecia, vomiting/diarrhea and anorexia. These side effects are minimal if patients in clinical complete remission are irradiated. If macroscopic lymphoma is irradiated, tumor lysis syndrome is a possible severe, potentially life-threatening complication. Main indications for RT of canine lymphoma are 1) chemotherapy-resistance, 2) consolidation for dogs already in complete remission after systemic multi-drug chemotherapy, and 3) local and/or locoregional lymphoma.

Our group tried to follow the idea of involved-field RT for canine NHL to spare as much tissue as possible, but so far results are very preliminary. The use of functional imaging could be helpful to identify eligible patients.

Bone marrow transplantation (BMT) has not been used much clinically in dogs so far, although 95% of all human BMT protocols were first perfected in dogs. The dog was considered a suitable animal model also for BMT following myeloablative therapy. As a consequence of the recent improvements of lymphoma subtype diagnostics, dogs might become a more important model again for new approaches.

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## **11 Role of surgery in the management of canine lymphoma: the example of splenic lymphoma**

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Splenic masses are frequent in dogs with half of cases being non-neoplastic lesions and the other half consisting of hematopoietic and non-hematopoietic splenic tumors. Two thirds of these malignancies are hemangiosarcomas, whereas hematopoietic tumors of the spleen make up 5-24% of cases. While primary lymphoma of the spleen is often an incidental finding in canine oncology, an invasion of the spleen by nodal lymphoma (e.g. in canine multicentric lymphoma), is frequently detected during standard staging work-up. Little information regarding clinical, pathologic, therapeutic and prognostic findings is available on primary splenic lymphoma in dogs. The most frequently reported canine splenic lymphoma histotype is marginal zone lymphoma (MZL). However, according to recent literature aimed at recognizing specific disease entities, MZL has a low incidence in dogs.

The detection of splenic masses is often incidental during abdominal palpation or ultrasonography, and patients can be completely asymptomatic. In some cases, symptoms are due to hemoabdomen caused by spontaneous rupture of the spleen. Splenectomy is routinely performed few days after the detection of a splenic mass in order to prevent a rupture, or within 24h in the presence of hemoabdomen. The diagnosis of canine splenic MZL is then obtained by histology performed after splenectomy. Upon confirmation of canine splenic MZL, clinical staging must follow. Nonetheless, amongst the cases observed at our institution, the involvement of peripheral lymph nodes, liver, blood and bone marrow was rare. As per our experience, in absence of systemic involvement, surgery without adjuvant therapy is sufficient to ensure prolonged survival with good quality of life, without further progression of the disease. Nevertheless, these results must be interpreted with caution. No prospective studies on treatment efficacy have been carried out by stratifying canine splenic MZL cases on the base of clinical, hematological, pathological and molecular variables.

In humans, splenic MZL is a rare condition, generally with good prognosis, which may be classified as an indolent lymphoma. Hemoglobin concentration, platelet count, high lactate dehydrogenase level, and extrahilar lymphadenopathy affect lymphoma-specific survival. Treatment options include surgery with or without chemotherapy, chemotherapy alone, and in some cases a “wait and see” approach. Splenectomy alone results in a prolonged survival time with good quality of life; however there is still no consensus on the best treatment strategy. The recent identification of risk group categories on a large sample population could be the basis for selecting future risk-tailored treatment approaches for splenic MZL in humans. In conclusion canine splenic MZL seems to share the favorable prognosis of human splenic MZL; however, in people, nodal MZL has a more aggressive biologic behavior. Whether the same holds true for dogs is currently unknown.

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## Poster abstracts

### P01 Minimal Residual Disease assessment based on IgH rearrangements and flow cytometry in canine Diffuse Large B-cell Lymphoma

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**Introduction.** Canine diffuse large B-cell lymphoma (cDLBCL) accounts for 30-40% of lymphoma cases. The persistence of cells unidentifiable through cytology is termed Minimal Residual Disease (MRD). Currently, MRD is measured by flow-cytometry (FC). The aim of the study was to determine the diagnostic and prognostic role of rearranged IgH (PARR analysis) in the lymph node (LN), peripheral blood (PB) and bone marrow (BM) of cDLBCL compared with FC.

**Materials and methods.** PB, BM aspirate and LN tissue from 17 cDLBCL were available for PARR analysis at diagnosis (T0) and at the end of treatment (T1). The concordance between PARR and FC was analyzed for LN, PB and BM.

**Results.** At T0, the B-cell origin of the lymphomas was confirmed by IgH Major rearrangements in the LN of all dogs. Eleven dogs (64,7%) showed simultaneous IgH rearrangements in BM and PB, whereas 6 dogs (35,3%) had no rearrangements in none of the samples. At T1, IgH monoclonal rearrangement was found in 13 (81%), 8 (50%) and 6 dogs (38%) in LN, PB and BM, respectively.

At T0, the concordance rate between FC and PARR was 100%, 82%, and 47% for LN, PB and BM, respectively. The highest concordance rate was obtained in LNs (PARR-positive/FC-positive), whereas the highest discordance was obtained in BM (PARR-positive/FC-negative).

At T1, the concordance rate was overall reduced, accounting for 47%, 50%, and 63% in LN, PB and BM, respectively. The highest discordance was found in LNs (PARR-positive/FC-negative), whereas the highest concordance was found in BM (PARR-negative/FC-negative).

**Discussion.** We propose the use of both, PARR and FC, in tandem to offset the possibility of false-negative MRD assessment; in case of discrepant results, dogs should be closely monitored to detect a possible early recurrence. The concordance rate was largely dependent on the time point, being higher in T0. The discordance between PARR and FC results in dogs in clinical remission may be due to the limited number of available cells, which reduces the sensitivity of FC.

### P02 Is it really hopeless to target CD20 in canine B-cell lymphoma?

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**Aim.** Radiolabelled anti-CD20 (Rituximab) has a proven effect in treating human non-Hodgkin lymphoma but there are only a few and ambiguous data about its use against canine B-cell

lymphoma. We aimed to perform a pilot study with nude mice xenografts to compare the effectivity of  $^{177}\text{Lu}$ -anti CD20 against human and canine cell-lines.

**Materials and Methods.** Altogether 20, 5-6 weeks old (18-22 gram weight) nude mice (BALB-C nu/nu) were xenografted. Ten animals were grafted with human cells (Burkitt and Jurkat), the other ten with canine (own cell lines) by injecting B-cells into the left and T-cells into the right thigh muscle. Five mice from both groups were treated with a single intravenous application of 10 (low-dose) and 20 MBq (high-dose)  $^{177}\text{Lu}$ -antiCD20 MoAbs, respectively. Then there was a 6 weeks follow-up period when mice body weights and tumor diameters (volumes) were checked twice weekly and whole body hybrid imaging (nanoSPECT/CT) was carried-out in representative mice from each group every week to estimate standard uptake values (SUV) by B- and T-cell lymphomas.

**Results.** In serial SPECT/CT examinations both the human and the canine B-cell lymphoma xenografts showed significantly higher (1.4-2.1x) SUV-values compared to T-cell lymphomas. Low-dose treatments resulted in smaller B-cell lymphomas than T-cell lymphomas both in human and in canine xenografts but these differences were not significant. High-dose treatments resulted in significantly reduced B-cell lymphoma growth both in human and in canine models 2 weeks post-application and later on. Only 6 out of 10 mice treated with high dose radiopharmaceutical lived 6 weeks postapplication.

**Conclusion.** In our double xenograft models  $^{177}\text{Lu}$ -antiCD20 seems to have an equal efficacy potential against human and canine B-cell lymphomas. Our preliminary results however need further confirmations before considering the possibility of using CD20 targeting treatments in canine B-cell lymphomas. The radiotoxicological properties of the pharmaceutical need to be taken into account to establish the doses.

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### **P03 Comparative expression profiling of four canine lymphoma cell lines**

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**Introduction.** Since many of the lymphomas and leukaemias of the dog resemble morphologically those of humans it is logical to expect that they also have an underlying and recurring basis in genetic injury detectable by current technology. Prior to this study, several marker genes have been evaluated as potential prognostic or diagnostic markers for canine B- and T-cell lymphomas. In the course of this project, we aim at assigning unique expression signatures to canine B- and T-cell Non-Hodgkin's Lymphoma (NHL) subtypes in consequence of gene expression alterations induced by tumorigenic processes.

**Methods.** Comparative gene expression profiling of two canine lymphoma B-cell lines (CLBL-1, GI-1) and two T-cell lines (OSW, CL-1) were conducted by quantitative real-time PCR (qPCR) assays for important tumorigenic genes playing key roles in lymphopoiesis, differentiation, proliferation, cell

survival and apoptosis. Comparative Cq method-based data evaluation was done by normalizing mean Cq values against three reference genes and using non-malignant lymph node samples for calibration.

**Results.** Preliminary relative RNA expression data showed for the CLBL-1 cell line a down-regulation of Bcl-XL, c-kit, the analysed MAP kinase signalling related genes as well as the tumour suppressor genes PTEN, Rb and p16. In contrast, the T-cell line OSW is mainly driven by the elevated expression of c-kit but also exhibits downregulation of p16 and PTEN.

**Conclusions.** Expression analysis has great potential in the field of veterinary oncology to advance our knowledge of tumorigenic processes. Applying gene expression profiling as a quantitative molecular framework for the study of lymphomas represents a novel innovative approach in veterinary cancer research.

#### **P04 Safety and efficacy of a genetic vaccine targeting telomerase plus chemotherapy for B-cell canine lymphoma therapy**

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**Background.** We have recently shown that a genetic vaccine targeting dog telomerase (dTERT) and based on Ad/DNA-EP technology can induce strong immune response and increased overall survival of dogs affected by B-cell malignant lymphoma (ML) in comparison with historical controls when combined with COP chemotherapy regimen.

**Objectives.** Here, we have conducted a double arm clinical trial including 21 dogs for each arm, (vaccine vs. control), measured the antigen-specific immune response and evaluated potential toxic effects of the immunotherapy along with following patients survival for three years and half.

**Methods:** Changes in hematological parameters, local/systemic toxicity or organic dysfunction and fever were monitored over time during the treatment. The immune response was measured by ELISPOT.

**Results.** No adverse effects attributable to treatment were observed in any dog patient. dTERT-specific immune response was induced in 19 of 21 treated animals. The overall survival time of vaccine/COP treated dogs was significantly increased over contemporaneous COP-treated animals (>76.1 vs 29.3 weeks, respectively,  $p < 0.0001$ ), while no differences were found between the groups regarding the relapse-free interval.

**Conclusions.** Ad/DNA-EP-based cancer vaccine against dTERT in combination with COP chemotherapy is safe and significantly prolongs the survival of ML canine patients while having no remarkable effect on the relapse-free interval. These data suggest that the dTERT has therapeutic efficacy and support the evaluation of this approach for other cancer types.

**P05 NK-cell Large Granular Lymphocyte leukemic lymphoma in a dog**

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Large granular lymphocytes (LGL) are defined as a lymphoid subset that constitutes 1–10% of the circulating pool of lymphocytes in dogs and are characterized either by CD3 positivity (in > 90% of the cases) or negativity for both T- and B-cell markers (in < 10% of cases, natural killer origin). A 11-year-old, male, mongrel dog was submitted to the referring veterinarian following a 2-month history of progressive generalized weakness, vomiting, weight loss and anorexia. Physical examination revealed severe jaundice and hepatomegaly. Results of a complete blood count (CBC) were within reference intervals (RI). Serum biochemical analysis revealed increases in ALT (175 U/L; RI, 20-150 U/L), AST (295 U/L; RI, <80 U/L) and total bilirubin (6,3 mg/dL; RI, 0,1-0,6 mg/dL). Ultrasound-guided fine-needle aspiration of the liver was also performed. Cytological examination of hepatic and blood smears revealed the presence of a large number of lymphoid cells characterized by an abundant amount of pale bluish cytoplasm containing numerous distinct magenta granules variably in size and shape (LGL), with densely stained reniform nuclei and without apparent nucleoli. On immunocytochemistry, neoplastic cells were negative for both T- and B-cell markers. A final cytological diagnosis of LGL leukemic lymphoma of NK origin was made. Due to the poor clinical condition of the animal the owner did not allow any chemotherapeutic approach and the dog died spontaneously few days later. Large granular lymphocyte leukemias of NK-type are surface CD3-negative disorders with variable clinical behaviour in dogs, as reported in humans.

**P06 A CLBL-1 based canine B-Cell lymphoma model - comparative evaluation of *in vitro* and *in vivo* characteristics**

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**Introduction.** In cancer research, cell lines are a major tool for studies of the generation of neoplasias in animal models mimicking the original neoplasias *in vivo*. Canine lymphoma is the major hematopoietic malignancy in dogs and considered as a valuable spontaneous large animal model for human Non-Hodgkin's Lymphoma (NHL). Herein we used a canine B-cell lymphoma cell line (CLBL-1) and Rag2<sup>-/-</sup>γ<sub>c</sub><sup>-/-</sup> mice to establish an *in vivo* model and analysed the key features specially focusing on the comparability of the induced tumours to the original cell line and primary material.

**Methods.** The canine CLBL-1 cell line was injected into Rag2<sup>-/-</sup>γ<sub>c</sub><sup>-/-</sup> mice. The generated tumour material was used to establish the cell line CLBL-1M. Both cell lines were compared for their expression of leukocyte differentiation antigens and their doubling time features. Further, the cell lines were stimulated with IL-2 and DSP30 to examine the stimulatory effect on cell proliferation.

**Results.** FCM studies for the expression of leukocyte differentiation antigens revealed comparative pattern of marker antigen expression. Doubling time as well as the reaction on DSP30 and IL-2 stimulation showed that both cell lines respond in the same way as primary, xenograft-derived lymphoma material.

**Conclusions.** The herein described CLBL-1 *in vivo* model provides a highly stable tool for B-cell lymphoma research allowing various further *in vivo* studies.

### **P07 Prognostic value of Hans' algorithm and Bcl-2/C-MYC double hit score in canine Diffuse Large B-cell Lymphoma (DLBCL)**

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**Introduction.** Relevant animal models of human cancers are highly warranted to accelerate transfer of innovative therapies into human clinical practice. Non-Hodgkin lymphoma is frequent in dog, with diffuse large B-cell lymphoma (DLBCL) being the most common subtype. In order to validate spontaneous DLBCL in dog as a model for preclinical studies in humans, we applied Hans' algorithm and a canine-adapted immunohistochemical double-hit score to canine DLBCLs and evaluated their prognostic value.

**Methods.** 49 DLBCLs diagnosed between 2005 and 2011 in the College of Veterinary Medicine of Nantes (France), with available 2-year follow-up, were evaluated for CD10, BCL-6, MUM-1, BCL-2 and c-MYC (clone Y69) by automated immunohistochemistry. Thresholds for positivity were 30% for CD10, BCL-6, MUM-1; 10% for BCL-2 and 80% for c-MYC.

**Results.** Thirty-three dogs (67%) presented with stage III disease according to the World Health Organization scheme. Six (12%) samples were classified as immunoblastic, while 43 (88%) were centroblastic. Seven cases (25%) were germinal center (GC)-DLBCLs according to Hans' algorithm and 42 cases (75%) were non-GC DLBCLs. 26 cases (53%) carried overexpression of c-MYC. A high immunohistochemical double-hit score (DHS) (c-MYC and BCL-2 positivity) was observed in 6 cases (12%). Twelve dogs (24%) received no treatment, 26 (53%) received prednisolone alone, and 11 (22%) received CHOP-like chemotherapy regimen. Median overall survival (OS) according to treatment options was 19, 45 and 220 days, respectively. In multivariate analysis, 4 parameters (absence of treatment, high WHO clinical stage, non-GC phenotype and high DHS) were associated with shorter OS.

**Conclusion.** Hans' algorithm applied in the dog showed that non-GC DLBCL is more frequent than GC DLBCL. As observed in human DLBCL, a high DHS is associated with worse outcome. Our results suggest that spontaneous canine DLBCL may be used as a model for non-germinal-center DLBCL for preclinical investigations.

**P08 The use of canine lymphoma and leukemia cell lines in the study of cytotoxic activity of common antineoplastic agents used in dogs.**

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**Introduction.** Hematopoietic neoplasms in dogs represent about 25% of all cancers and, among them, lymphoma is the most commonly diagnosed. Traditional chemotherapy protocols using classical cytostatics (doxorubicin, vincristine, prednisone, cyclophosphamide) administered alone or in combination are used for lymphoma treatment. However, there is a lack of data describing the effects of different antiproliferative agents on induction of apoptosis in established canine lymphoma/leukemia cell lines. The aim of our study was to evaluate the concentration-dependent cytotoxicity and the ability of common antineoplastic drugs, used as single agents, to induce apoptosis in selected canine lymphoma and leukemia cell lines.

**Materials and Methods.** The canine cell lines CL-1 (primitive ab T-cell leukemia), GL-1 (B-cell leukemia), CLBL-1 (B-cell lymphoma) and the human Jurkat line (T-cell leukemia) were used. The cells were treated for 24 or 72 h with either etoposide in the concentration range of 0.005-20 µg/ml, lomustine (0.05-50 µg/ml), prednisolone (0.01-10 µg/ml), 4-HO-cyclophosphamide (0.01-100 µg/ml), chlorambucil (0.01-20 µg/ml), dexamethasone (0.005-24 µg/ml), vincristine (0.000001-1 µg/ml), cytosine arabinoside (0.001-1 µg/ml) or doxorubicin (0.05-10 µg/ml). Early apoptotic events and DNA fragmentation were detected by flow cytometry using the Annexin V-FITC Apoptosis Detection Kit and ethanol-fixed propidium iodide stained cells, respectively. Cell viability was determined using the MTT test. The cytotoxic activity was expressed as IC<sub>50</sub>.

**Results.** Vincristine and doxorubicin strongly decreased the viability of canine cells; CL-1 with IC<sub>50</sub> of 0.00001 and 0.08 mg/ml respectively, GL-1 with IC<sub>50</sub> of 0.01 and 0.08 mg/ml respectively and CLBL-1 with IC<sub>50</sub> of 0.0005 and 0.05 mg/ml respectively, whereas 10 mg/ml cyclophosphamide induced the highest level of apoptosis in all tested cell lines (70-80 % dead cells). The most sensitive to all drugs was CLBL-1 cell line, which in contrast to the CL-1 and GL-1 was also sensitive to the effects of steroidal anti-inflammatory drugs.

**Conclusions.** Our study provides a useful frame to test the efficacy of anti-cancer drugs.

**P09 The diagnostic and prognostic value of the serum C-reactive protein and haptoglobin levels in canine lymphoma**

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**Introduction.** The aim of this study was to determine the prognostic significance of serum C-Reactive Protein (CRP) and haptoglobin (HAPT) levels in dogs diagnosed with malignant lymphoma.

**Materials and methods.** From thirty dogs diagnosed with malignant lymphoma by (immuno)cytology, serum was collected at day 0 and at control visits. CRP and HAPT levels were determined and used for the Canine Lymphoma Blood Test (CLBT)<sup>1</sup>. The CLBT software utilises kNN (k-nearest neighbours) algorithms and decision trees taking into consideration the CRP and haptoglobin levels of the dog along with risk factors, as age and sex, and the presence of lymphadenopathy.

**Results.** The CLBT proved to be sensitive (92,19%) and specific (79,49%) for determining the clinical status (detectable disease vs. remission) of canine lymphoma patients. A high CLBT % (>80%) on day 0 was associated with a significantly less favorable prognosis achieving a median Time to Progression (TTP) of 14 weeks (MST 19 weeks) in comparison with a value of <80% (median TTP 37 weeks, MST 59 weeks).

The lowest achieved CLBT value during treatment proved also to be important, with median TTP of 50 weeks for values <20% being significantly longer (Mann Whitney p=0.0087) than for patients reaching a lowest CLBT% of 20-40% (30 weeks), 40-60%, (18 weeks), 60-80% (20 weeks), and >80% (7 weeks) respectively.

**Conclusion.** Ratcliffe et al.<sup>2</sup> have shown that serum CRP and HAPT levels are sensitive and specific for diagnosing canine malignant lymphoma. Now we showed a good correlation between clinical status (detectable disease vs. remission) and CLBT results of canine lymphoma patients during treatment. Even more importantly the CLBT% at presentation and during treatment both appear to be of prognostic value. As CRP is a valuable prognostic factor in human NHL, this test might also be of use for human NHL patients.

<sup>1</sup>*Pet-screen Advanced Lymphoma Blood Test, see [www.pet-screen.com](http://www.pet-screen.com) for more details*

<sup>2</sup>*Ratcliffe, L., Mian, S., Slater, K., King, H., Napolitano, M., Aucoin, D., and Mobasher, A., Proteomic identification and profiling of canine lymphoma patients. *Veterinary and Comparative Oncology*, 2009. 7: p. 92-105.*

## **P10 Lymphomas in dogs: spontaneous models to decipher the genetics of lymphomagenesis in dogs and humans**

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**Background and Aim.** There are over 400 genetically distinct breeds of dogs, each of them corresponding to a genetic isolate. The consequence of breeding practices is that most breeds naturally develop specific cancer types, reflecting the presence of predisposing alleles. Moreover, dogs benefit of an excellent medical follow-up, allowing access to different samples, and dogs also share our own environment, allowing to decipher the genetic and environmental features of

cancers. This is especially interesting for lymphoma, for which dogs represent powerful models. Indeed, most human lymphoma subtypes are encountered in dogs, with some subtypes being associated to a higher frequency in specific breeds (Pastor et al., 2009; Villamil et al., 2009; Rowell et al., 2011; Marconato et al., 2013). We focused on lymphoma occurring in large families of Dogue de Bordeaux (DDB) and Bernese Mountain Dog (BMD) with the objective to identify predisposing genetic regions and somatic alterations involved in lymphomagenesis and tumoral progression.

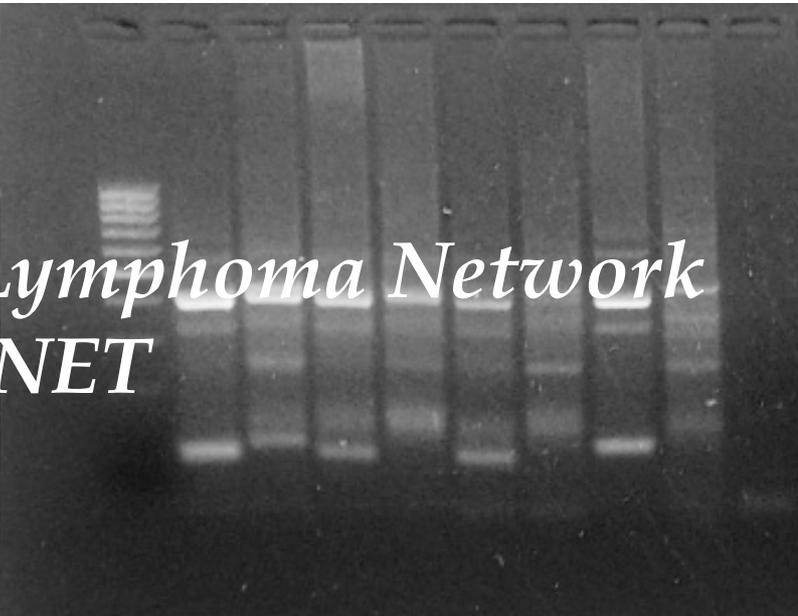
**Materials and Methods.** We thus collected blood and tumour samples, clinical and genealogical information of affected dogs in BMD and DDB families, using the French CaniDNA archive developed in the lab (<http://dog-genetics.genouest.org>). DNA and RNA were extracted using Macherey-Nagel kits. Dogs were genotyped on Illumina 170 000 SNP-arrays. Histopathological diagnosis was performed from FFPE tumour samples by veterinary histopathologists FN and JA.

**Results.** We collected blood and lymphoma tissue samples from 150 dogs from affected breeds, of which 55 affected BMDs and 6 DDBs, belonging to large pedigrees, have been analysed. First, we demonstrated a familial segregation of lymphoma; second, a genome-wide association study (GWAS) has been performed on 46 BMD cases and 156 controls. Preliminary data showed a significant region, associated with lymphoma, on canine chromosome CFA 23.

**Conclusions.** We collect different types of lymphoma in different predisposed breeds, expecting that the discovery of genes and pathways involved in given breeds will shed light on the allelic combinations predisposing to lymphomas in humans. We anticipate that these results will open the field of targeted clinical trials for the benefit of both dogs and humans.

## Notes





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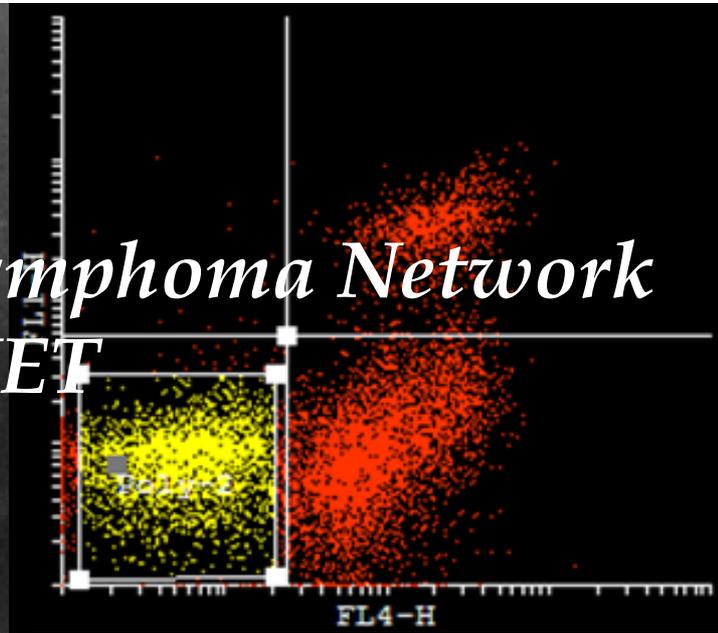
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## 1<sup>st</sup> Meeting of the European Canine Lymphoma Group

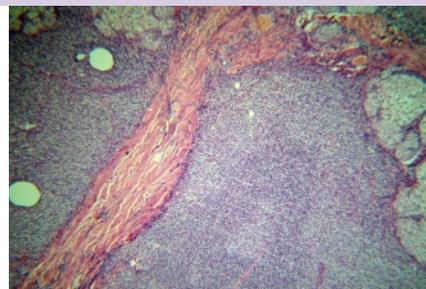
### Workshop Proceedings

Palazzo dei Congressi,  
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