Canine and Feline Lymphoma

Canine and Feline Lymphoma: Challenges and Opportunities for Creating a Paradigm Shift

David J. Argyle and Evi Peccau

The Royal (Dick) School of Veterinary Studies and the Roslin Institute, The University of Edinburgh, Easter Bush, Roslin, Midlothian EH25 9RG.

Abstract

With rare exceptions, Non-Hodgkin Lymphoma (NHL) remains a fatal disease in dogs and cats. Although our understanding of the disease continues to grow through research, the impact of this research has yet to be translated into significant increases in survival times for affected patients. To highlight the importance of this disease, the editorial team of Veterinary and Comparative Oncology have constructed this themed issue highlighting current research in this field. This short introductory review aims to provide a synopsis of some of the key areas of global research interest, identify the challenges to clinical progress and finally will offer an optimistic view of new developments which aim to enhance the lives of our patients.

Introduction

Non Hodgkin Lymphoma (NHL) remains one of the most important diseases in human and veterinary oncology. Despite incremental advances in our understanding of lymphoma biology and treatment, the disease remains one of high mortality in veterinary species. In dogs, NHL accounts for approximately 10% of all malignant tumours and 83% of all haematopoietic malignancies [1]. NHL is initially highly responsive to standard chemotherapy,

as with human patients, with first-remission rates of approximately 90%; however, drug resistance occurs in most cases, resulting in disease recurrence[1]. Our understanding of this disease in dogs and cats has significantly lagged behind human medicine in terms of pathophysiology of the disease, classification, diagnosis and novel treatment options. However, with advances in molecular biology, pathology and genetics, we are developing the toolbox necessary to explore this disease more fully. In addition, international collaborative groups are developing, such as the European Lymphoma Network[2], helping to drive research forward through increasing critical research mass. Importantly, collaborative research and the development of molecular and immunological toolboxes are allowing the dog and cat to be explored as significant and important models of human lymphoma biology and therapy.

In studying the literature around canine and feline NHL, and bringing together key publications in this issue of VCO, it becomes easier to crystallize some of the greatest clinical challenges to improving outcomes in this devastating disease. However, the exponential increase in our understanding of cancer biology and the overt collaborative approaches to advancing knowledge offers an optimistic view of Lymphoma research, ensuring that the following challenges can be transformed into significant opportunities.

Establishing an Appropriate Clinical and Pathological Classification System

One of the major hurdles to enhancing clinical development has been our inability to develop a good sub-classification system for NHL, similar to that for people. As far back as 1966, veterinarians have tried to apply the human classification systems to canine NHL[3-7]. In order to use all diagnostic criteria such as cellular morphology, cell lineage, topography and

biology, the Revised European-American Classification of Lymphoid Neoplasms (REAL) was created which led to the currently used World Health Organization (WHO) system of classification[8-10]. The application of standardised criteria to differentiate different types of Canine malignant lymphoma showed an accuracy of 83% amongst 17 different pathologists[11]. Feline lymphomas have also been classified according to the National Cancer Institute working formulation (NCI WF)[12].

In addition to marker classification systems, anatomic location remains an important consideration with regards to prognosis and treatment options for dogs and cats. Multicentric lymphoma is the most common form in dogs, followed by the alimentary form and other types. A poorer prognosis has been assigned to the gastrointestinal form, compared to the multicentric presentation[13,14]. The anatomic location of lymphoma in cats has changed significantly over the last 2 decades with an increasingly high rate of gastrointestinal lymphoma accompanied by a decreased FeLV/FIV prevalence. Despite the decrease in this latter negative prognostic factor, gastrointestinal lymphomas still have short overall responses even on multi-agent chemotherapy[15].

The importance of an internationally recognised classification system for lymphoma cannot be overstated, as the current systems are still far from perfect. It is essential that the oncology community develop such systems to further identify different disease entities in companion animals and to assign prognostic value to specific tumour types. For example, indolent lymphoma has been shown to behave in a different manner than high-grade lymphomas and therefore require a different treatment approach[16,17].

The use of immunological markers and gene and protein profiling is underpinning a revolution in developing classification systems for cancer that will inform treatment and

prognosis. The introduction of gene expression profiling in human medicine has identified novel molecular lymphoma subtypes that are histologically indistinguishable[18]. In diffuse large B-cell lymphoma (DLBCL), the distinction of the germinal center B-cell–like (GCB) DLBCL and activated B-cell–like (ABC) DLBCL subtypes is beginning to translate into the clinic, as these diagnostic categories have significantly different survival rates after standard treatment. Similarly, the molecular distinction using gene expression profiling of DLBCL and Burkitt's lymphoma (BL) is of major clinical importance, as BL requires more intensive treatment strategies. These examples evidence that the routine application of gene expression profiling in veterinary species may eventually lead to the establishment of the molecular classification of lymphoma. Gene expression profiling has been performed in canine lymphoma[1]. However, many of the current studies in veterinary medicine suffer from low patient numbers and insufficient power. Developing a robust classification system based upon molecular signatures combined with pathological and clinical criteria will require significant international collaboration. This collaboration needs to be extended to data scientists to develop appropriate models and algorithms that can be translated into clinical practice.

Understanding Molecular Pathophysiology

Cancer is a difficult disease to define as an entity but represents break down in cellular homeostasis leading to unrestricted growth and proliferation of tissues[19]. Much of this unrestricted growth is driven by changes in Oncogenes and/or Tumour Suppressor Genes that control cellular processes. The NHLs represent a heterogeneous group of malignancies that arise from the lymphoid system and, as with other cancers, have been explained

through the concept of multistage carcinogenesis. In recent years, the cancer stem cell hypothesis (where a cancer is driven by a small sub-populations of rare primitive cancer stem cells) has been used to explain the formation of many solid cancers[20]. However, no lymphoma stem cell pool has, as yet, been defined in lymphoma. The pathophysiology of lymphoma is more likely closely linked with B and T cell development and a revolution in molecular biology techniques is allowing this to be dissected in human and veterinary medicine.

The development of both T and B cells lends itself to the potential for DNA damage. As an example, B-cell development begins in the primary lymphoid organs with subsequent differentiation in secondary lymphoid tissues such as lymph nodes, spleen, or tonsils[18]. During development B cells are subjected to a range of DNA modifications that could predispose to the development of lymphoma. B cell development in the bone marrow is initiated during the development of the B cell receptor through V(D)J recombination. BCR expression is followed by the exit of B cells from the bone marrow to become naïve B cells. Activation of B cells through interaction with antigen leads to germinal centre activation involving somatic hypermutation (SHM) and class-switch recombination (CSR). Finally, there is the production of B cells and memory cells. V(D)J recombination, SHM, and CSR especially represent critical processes which could predispose to these malignancies and all have been linked to lymphomagenesis[18].

Diffuse Large B Cell Lymphoma (DLBCL) represents the most common type of malignant lymphoma in both human and veterinary medicine[1]. DLBCL is heterogeneous with respect to clinical presentation and pathology but molecularly can fall into distinct sub-classes. In a generation we have gone from single pathway analysis to a position when we can examine

thousands of genetic changes in a cancer sample using gene array “chips” or newer technologies such as high throughput sequencing and RNA sequencing (RNA-seq). RNA-seq uses Next Generation Sequencing (NGS) to rapidly analyze the changing transcriptome in a cancer cell. These technologies in cancer discovery have been used to Identify common cancer signatures across lymphoma phenotypes and Identify potential targets for drug development[21]. In human oncology, Array profiling has identified three molecular subtypes Activated B-cell–like diffuse (ABC), Germinal Center B-cell–like (GCB) and Primary Mediastinal B-cell lymphoma (PMBL). These subtypes use distinct oncogenic signaling pathways and respond differently to conventional treatment. In canine Lymphoma we have identified distinct molecular sub-types, which resemble ABC and GCB types[1], but more work is required to characterize these subtypes with much larger numbers of patients. By building a toolbox of reagents and genetic profiles, and with international and national collaborative research, it will be possible to generate the numbers of patients required to advance our understanding of the molecular pathology of this disease. This will help us define patients that require conventional treatments to be tailored, and potentially identify new therapeutic targets or biomarkers for diagnosis and early relapse.

Developing New Treatments and Overcoming Drug Resistance

In Veterinary and Human Lymphoma treatment, conventional cytotoxic chemotherapy is the mainstay of current therapies. These are largely based on the CHOP (Cyclophosphamide, Doxorubicin, Vincristine and Prednisolone) protocols and, in veterinary oncology, very little has changed in the last 20 years in terms of advancing treatment options or improving survival times. The major significant change has been the adoption of shorter protocols,

removing the maintenance phase of treatment[22] and studies to evaluate whether shorter protocols are possible without significant effects on mean survival time (MST)[23,24]. Veterinary chemotherapy protocols are rarely curative as significant changes in chemotherapy intensity often have the negative effect of reducing overall quality of life. In addition, the development of multi-drug resistance is often an inevitable consequence of conventional chemotherapy treatments, obviating the need for new approaches.

New approaches will require the development of new therapies and/or the development of technologies for overcoming drug resistance. Cytotoxic chemotherapy drugs have traditionally been administered based on the Maximally Tolerated Dose (MTD) principle[25,26] but the development of chemotherapy-resistant cells and tumour recurrence or relapse seem to be an inevitable consequence of the "Darwinian" tumour evolution. High dose chemotherapy, which targets the chemotherapy-sensitive cells, actually allows the population of resistant cells to expand, a process known as "competitive release". Overcoming competitive release using alternative treatment approaches is an active area of research in human medicine and is yet to be exploited in veterinary oncology. As an example, ATP-binding cassette (ABC) drug transporters consuming ATPs for drug efflux is a common mechanism by which cancer develops multidrug resistance (MDR). Non-chemotherapy drugs that are ABC substrates ("ersatzdroges") are being explored as a means to suppress MDR phenotypes[27-29].

The development of small molecule, targeted drugs has been seen hotly pursued in human oncology, especially drugs that target the kinome. To date, there are only two tyrosine kinase inhibitors, licensed for the use in dogs. These drugs have largely been used for treating mast cell tumours (with c-kit mutations), but they have also shown effects in other

cancers[30]. Alternatively drugs such as CDK-inhibitors are being explored in solid cancers. CDK is essential for the progression of the cell cycle, which means it could represent a treatment target within rapidly growing tumour cells. Currently trials are ongoing in human medicine incorporating CDK inhibitors as treatment for solid tumours and non-Hodgkin lymphoma[31,32]. The role that these drugs play in canine and feline lymphoma is yet to be realized, but, as with conventional drugs, the selection pressure created by using one single drug supports the development of drug resistance. With no veterinary second-generation drugs on the horizon, and with the development of resistance, the benefits from these drugs are potentially short-lived.

The development of small molecules to target specific pathways and driver mutations was considered to be a major breakthrough in cancer treatments. However, monoclonal antibodies have now far exceeded small molecules in terms of the market share of biologics being used in cancer treatment. In human medicine monoclonal antibodies have meant a breakthrough in lymphoma, with significantly improved outcomes targeting CD20 expression[33]. CD20 is a cluster of differentiation molecule consistency expressed on the human B lymphocyte cell membrane, with the anti-CD20 monoclonal antibody rituximab now approved in drug protocols. Rituximab does not bind canine CD20, driving a need to develop canine-specific antibodies. The use of "human" monoclonal antibodies in veterinary oncology is usually not feasible due to the development of an immune response to foreign protein but species-specific (e.g. caninized) monoclonal antibodies are being developed for canine lymphoma[34]. This is a truly exciting prospect, as it will deliver new and affordable reagents to the veterinary oncology community[35,36.]

Horizon Scanning and Precision Medicine

The tools that have or are being developed for dissecting the lymphoma genome offers an incredible opportunity to refine our understanding of this devastating disease. However, as an oncology community we must form strong collaborations across the disciplines in order to exploit the vast amount of data we now have the ability to generate. Currently, the amount of data we are generating far exceeds our ability to analyze and understand it, impeding progress in diagnosis, classification of the disease and treatment.

As discussed, the development of the appropriate reagents for mining veterinary genomes, proteomes and metabolomes is rapidly expanding, coupled with a reduction in costs. To fully exploit these technologies we must improve clinical data recording, consider large-scale multicentre clinical studies and embrace the importance of bioinformatics, statistics and mathematical modelling[21]. This will require a paradigm shift in how we traditionally approach veterinary medicine, removing research and discipline silos and mapping a landscape for a "comparative oncology" ecosystem. This will involve developing systems that will allow us to integrate clinical, biological and epidemiological data to provide the optimum clinical care for our patients, i.e. the development of true precision medicine[21].

Concluding remarks

As evidenced by the range of research papers in this edition of VCO, there is much cause for optimism in the field of lymphoma research. There are continuous improvements in conventional therapies, diagnosis, imaging and drug development in veterinary oncology. Equally, we have an unparalleled opportunity to study lymphoma without any species boundaries as evidenced by the clear pathological and molecular similarities of this disease

between dogs and humans[1]. Data derived from studies in spontaneous lymphoma in dogs and cats could serve to improve animal health and also serve as an important link between basic cancer research and human and veterinary clinical trials. Creating such a platform of interdisciplinarity supports progress in clinical cancer practice, offering an opportunity to increase the predictability of human clinical trails and reduce the cost and time of getting new drugs into patients.

Our exponential growth in understanding of the molecular events in lymphoma is being matched by the development of exciting therapeutic strategies such as species-specific monoclonal antibodies. In addition, we are living in a data-driven age, which can be exploited for the good of our patients. Data science will be vital to understanding the complexity of lymphoma at the cell and population level and the integration of clinical and biological data to improve treatment outcomes and design specific therapies. Precision medicine proposes the customization of healthcare, with medical decisions, practices, and/or products being tailored to the individual patient and this should be the direction of travel for veterinary oncology also.

References:

1. Mudaliar MA, Haggart RD, Miele G, Sellar G, Tan KA, Goodlad JR, Milne E, Vail DM, Kurzman I, Crowther D, Argyle DJ. Comparative gene expression profiling identifies common molecular signatures of NF-κB activation in canine and human diffuse large B cell lymphoma (DLBCL). PLoS One. 2013 Sep 4;8(9):e72591. doi: 10.1371/journal.pone.0072591.
2. Comazzi S, Marconato L, Argyle DJ, Aresu L, Stirn M, Grant IA, Guscetti F, Hendrickx T,

Ibisch C, Lawrence JA, Polton GA, Teske E. The European canine lymphoma network: a joining initiative to generate consensus guidelines for the diagnosis and therapy in canine lymphoma and research partnership. Vet Comp Oncol. 2015 Dec;13(4):494-97.

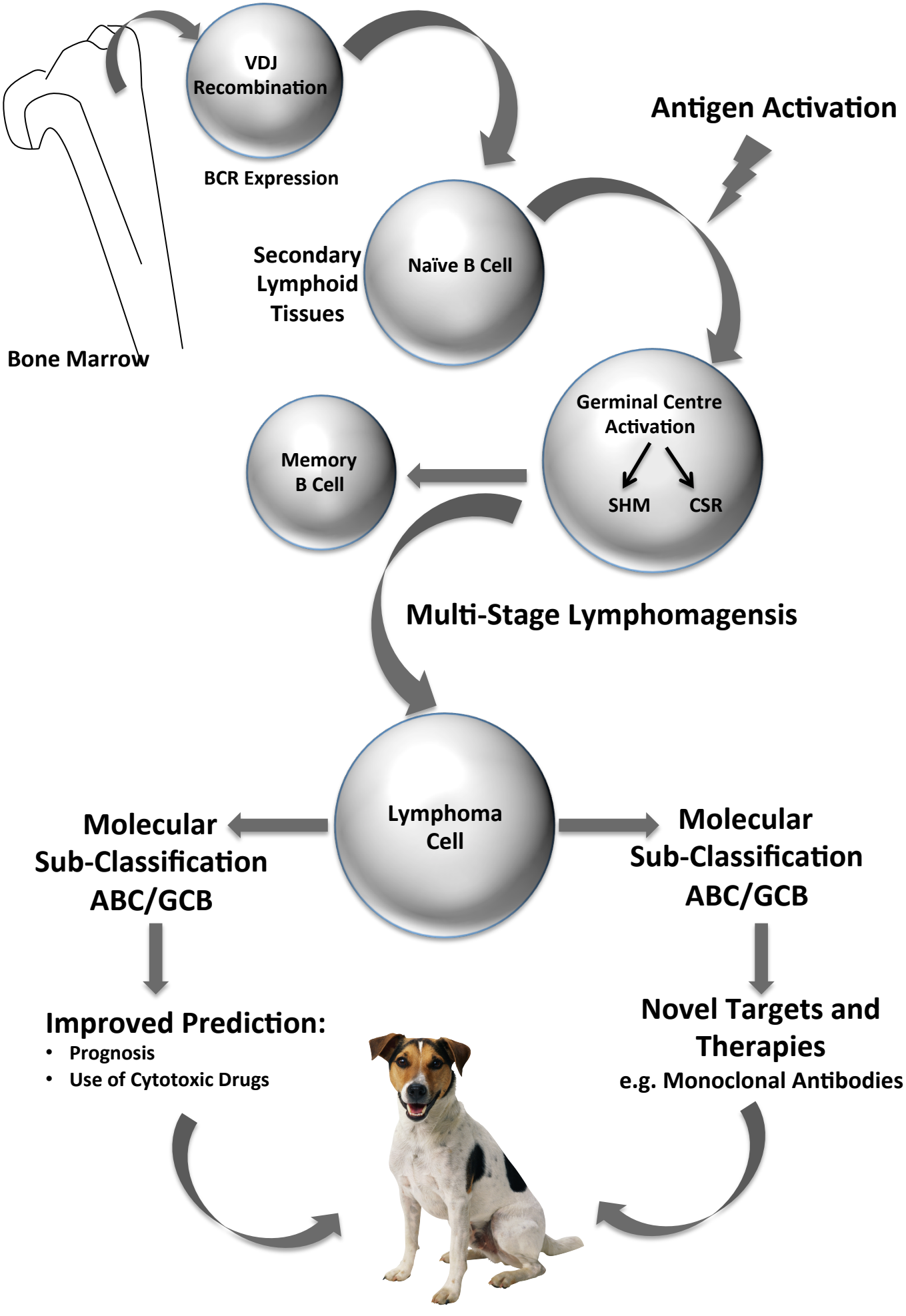
3. Rappaport H (1966): Altlas of Tumour Pathology. AFIP, section 3 fascicle 8, Washington, DC.
4. Lukes RJ, Collins RD (1974): Immunologic characterization of human malignant lymphomas. Cancer 34:1488-
5. Lennert K, Mohri N (1978): Malignant lymphomas other than Hodgkin's Disease: Histopathology and Diagnosis of Non-Hodgkin's lymphomas. Springer-Verlag, New York.
6. Valli VE, McSherry BJ, Dunham BM, Jakobs RM, Lumsden JH: Histology of lymphoid tumours in the dog, cat and cow. Vet Pathol 18:494-512.
7. Teske E, Wisman P, Moore PF, van Heerde P: Histological classification and immunophenotyping of canine non-Hodgkin lymphomas: unexpected high frequency of T cell lymphomas with B cell morphology
8. Harris N, Jaffe E, Stein H, Banks P, Chan Jm Cleary M, Delsol G, De Wolf-Peeters C, Falini B, Gatter K, Grogan T, Isaacson P, Knowles D, Mason D, Muller-Hermelink Hm Pileri S, Piris M, Ralfkiaer E Warnke R (1994): A Revised European-American classification of lymphoid neoplasms: a proposal for the international lymphoma study group. Blood 84: 1361-1392.
9. Harris N, Jaffe E, Diebold J, Flandrin Gm Muller-Hermelink H, Vardiman J, Lister T, Bloomfield C (1999): The World Health Organization classification system of neoplastic diseases of the haematopoietic and lymphoid tissues: report of the clinical advisory committee meeting – Airlie Housem Virginia. J Clin Oncol 17:3835-3849.

10. Vezzali E, Parodi AL Marcato PS, Bettini G (2010): Histopathologic classification of 171 cases of canine and feline non-Hodgkin lymphoma according to the WHO. Vet Comp Oncol; 8(1);38-49.
11. Valli VE, Jacobs RM, Norris A, Couto CG, Morrison WB, McCaw D, Cotter S Ogilvie G, Moore A (2000): The histologic classification of 602 cases of feline lymphoproliferative disease using the National Cancer Institute working formulation. J. Vet Diagn Invest 12(4), 295-306.
12. Valli VE, San Myint M, Barthel A, Bienzle D, Caswell J, Colbatzky F, Durham A, Ehrhart EJ, Johnson Y, Jones C, Kiupel M, Labelle P, Lester S, Miller M, Moore P, Moroff S, Roccabianca P, Ramos-Vara J, Ross A, Scase T, Tvedten H, Vernau W (2011: Classification of Canine Malignant Lymphomas According to the World Health Organization Criteria, Veterinary Pathology 48 (1), 198-211.
13. Madewell BR, Thesen GH (1987): Hematopoietic neoplasms, sarcomas and related conditions. In Theilen GH, Madewell BR, editors: Veterinary Cancer Medicine, ed2, Philadelphia, Lea and Febigor.
14. Rassnick KM, Moore AS, Collister KE, et al (2009): Efficacy of combination chemotherapy for treatment of gastrointestinal lymphoma in dogs. J Vet Intern Med 23:317-322.
15. Louwerens M, London CA Pedersen NC and Lyons LA (2005): Feline lymphoma in the post-feline leukaemia virus era. Journal of Veterinary Internal Medicine; 19; 329-335.
16. Ponce F, Magnol J, Ledieu D, Marchal T Turinelli V, Chalvet-Monfray K et al (2004): Prognostic significance of morphological subtypes in canine malignant lymphomas during chemotherapy. Veterinary Journal 167:158-166.
17. Valli VE, Vernau W, de Lorimier P, et al (2006): Canine indolent nodular lymphoma, Vet Pathol 43:241-256.

18. Hendrik Nogai, Bernd Dorken, and Georg Lenz (2011) Pathogenesis of Non-Hodgkin's Lymphoma. J Clin Oncol 29:1803-1811
19. Argyle D.J. and Blacking T.M. From Viruses to Cancer Stem Cells: Dissecting the Pathways to Malignancy. Vet J. 2008 Sep;177(3):311-23. Epub 2007 Nov 19
20. Pang LY, Argyle DJ. The evolving cancer stem cell paradigm: Implications in veterinary oncology. Vet J. 2015 Aug;205(2):154-60
21. Pang LY, Argyle DJ (2016). Veterinary oncology: Biology, big data and precision medicine. The Veterinary Journal Volume 213.
22. Garret LD, Thamm DH, Chun R, Dudley R and Vail DM (2002): Evaluation of a 6-month chemotherapy protocol with no maintenance therapy for dogs with lymphoma. Journal of Veterinary Internal Medicine; 16;704-709.
23. Mellanby RJ, Herrtage ME, Dobson JM (2003): Owner's assessments of their dog's quality of life during palliative chemotherapy for lymphoma.
24. Withrow SJ, Vail DM, Page RL (2013): In: Withrow& MacEwen's Small Animal Oncology. 5th ed. St. Louis, Elsevier.
25. Norton L, Simon R. (1986): The Norton-Simon hypothesis revisited. Cancer Treat Rep;70:163–9.
26. Rodrigues DS, de Arruda Mancera PF. (2013); Mathematical analysis and simulations involving chemotherapy and surgery on large human tumours under a suitable cell-kill functional response. Math Biosci Eng 10:221–34.
27. Gatenby RA (2009): A change of strategy in the war on cancer. Nature; 459;508-9.
28. Enriquez-Navas PM, Wojtkowiak JW, Gatenby RA (2015): Application of Evolutionary Principles to Cancer Therapy. American Association for Cancer Research; DOI: 10.1158/0008-5472.CAN-15-1337

29. Kam Y1, Das T, Tian H, Foroutan P, Ruiz E, Martinez G, Minton S, Gillies RJ, Gatenby RA. Sweat but no gain: inhibiting proliferation of multidrug resistant cancer cells with "ersatzdroges". Int J Cancer. 2015 Feb 15;136(4):E188-96. doi: 10.1002/ijc.29158. Epub 2014 Sep 2.
30. Hahn KA, Ogilvie G, Rusk T, Devauchelle P, Leblanc A, Legendre A et al (2008). Masitinib is safe and effective for the treatment of canine mast cell tumours. J Vet Intern Med; 22; 1301-1309.
31. Schwarz GK, LoRusso PM, Dickson MA, Randolph SS, Shaik MN Wilner KD et al (2011): Phase I study of PD 0332991, a cyclin-dependent kinase inhibitor, administered in 2-week cycles (Schedule 2/1). British Journal of Cancer; 104; 1862-1868.
32. Flaherty KT, LoRusso PM, Demichele A, Abramson VG, Courtney R, Randolph SS et al (2012). Phase I, dose-escalation trial of the oral cyclin-dependent kinase 4/6 inhibitor PD 0332991, administered using a 21-day schedule in patients with advanced cancer. Clinical Cancer Research; 18; 568-576.
33. Traulle C, Coiffier BB (2005): Evolving role of rituximab in the treatment of patients with non-Hodgkin's lymphoma. Future Oncol; 1; 297-306.
34. Jain S, Aresu L, Comazzi S, Shi J, Worrall E, Clayton J, Humphries W, Hemmington S, Davis P, Murray E, Limeneh AA, Ball K, Ruckova E, Muller P, Vojtesek B, Fahraeus R, Argyle D, Hupp TR. The Development of a Recombinant scFv Monoclonal Antibody Targeting Canine CD20 for Use in Comparative Medicine. PLoS One. 2016 Feb 19;11(2):e0148366. doi: 10.1371/journal.pone.0148366. eCollection 2016.
35. Jubala CM, Wojcieszyn JW, Valli VEO et al (2005): CD20 expression in normal canine B cells and in canine non-hodgkin lymphoma. 42;468-476.

36. Impellizeri JA, Howell K, McKeever KP et al (2006): The role of rituximab in the treatment of canine lymphoma: an ex vivo evaluation. Vet J; 171; 556-558.


VDJ Recombination
BCR Expression
Antigen Activation
Secondary Lymphoid Tissues
Naïve B Cell
Bone Marrow
Germinal Centre Activation
SHM
CSR
Memory B Cell
Multi-Stage Lymphomagensis
Lymphoma Cell
Molecular Sub-Classification ABC/GCB
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Improved Prediction:
• Prognosis
• Use of Cytotoxic Drugs
Novel Targets and Therapies
e.g. Monoclonal Antibodies