

54. Lorenzi, R., Brickell, R., Katz, D. R., Kinnon, C. & Thrasher, A. J. Wiskott–Aldrich syndrome protein is necessary for efficient IgG-mediated phagocytosis. *Blood* **95**, 2943–2946 (2000).
55. Fadok, V. A. *et al.* A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature* **405**, 85–90 (2000).
56. Mitchell, J. E. *et al.* The presumptive phosphatidylserine receptor is dispensable for innate anti-inflammatory recognition and clearance of apoptotic cells. *J. Biol. Chem.* **281**, 5718–5725 (2006).
57. Harvey, K. & Tapon, N. The Salvador–Warts–Hippo pathway — an emerging tumour-suppressor network. *Nature Rev. Cancer* **7**, 182–191 (2007).
58. Saucedo, L. J. & Edgar, B. A. Filling out the Hippo pathway. *Nature Rev. Mol. Cell Biol.* **8**, 613–621 (2007).
59. Edgar, B. A. From cell structure to transcription: Hippo forges a new path. *Cell* **124**, 267–273 (2006).
60. Vita, M. & Henriksson, M. The Myc oncoprotein as a therapeutic target for human cancer. *Semin. Cancer Biol.* **16**, 318–330 (2006).
61. Dakubo, G. D., Jakupciak, J. P., Birch-Machin, M. A. & Parr, R. L. Clinical implications and utility of field cancerization. *Cancer Cell Int.* **7**, 2 (2007).
62. Hoglund, M. Bladder cancer, a two phased disease? *Semin. Cancer Biol.* **17**, 225–232 (2007).
63. Gurova, K. V. & Gudkov, A. V. Paradoxical role of apoptosis in tumor progression. *J. Cell Biochem.* **88**, 128–137 (2003).
64. Joensuu, H., Pylikäinen, L. & Toikkanen, S. Bcl-2 protein expression and long-term survival in breast cancer. *Am. J. Pathol.* **145**, 1191–1198 (1994).
65. Adams, J. M. & Cory, S. The Bcl-2 apoptotic switch in cancer development and therapy. *Oncogene* **26**, 1324–1337 (2007).
66. Muller, H. J. Artificial transmutation of the gene. *Science* **46**, 84–88 (1927).
67. Hartwell, L. H. & Kastan, M. B. Cell cycle control and cancer. *Science* **266**, 1821–1832 (1994).
68. Nurse, P. Genetic control of cell size at cell division in yeast. *Nature* **256**, 547–551 (1975).
69. Evans, T., Rosenthal, J., Youngblom, D., Distel, D. & Hunt, T. Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell* **33**, 389–396 (1983).
70. Ellis, H. M. & Horvitz, H. R. Genetic control of programmed cell death in the nematode *C. elegans*. *Cell* **44**, 817–829 (1986).
71. Land, H., Parada, L. F. & Weinberg, R. A. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. *Nature* **304**, 596–601 (1983).

Acknowledgements

To my father in law, Alfred Rhiner, who died from adrenal adenocarcinoma in 2007. We all miss you, Fred. I thank C. Rhiner, M. Serrano, J. M. López-Gay and I. Flores for critically reading the manuscript and for suggestions.

DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>
[BCL2](#) | [brk](#) | [BSK](#) | [CED-1](#) | [ced-3](#) | [chico](#) | [CSK](#) | [dlg1](#) | [dm](#) | [DPP](#) | [drpr](#) | [E2f](#) | [GBB](#) | [lgf](#) | [Orct2](#) | [PSR](#) | [Ras85D](#) | [Rbf](#) | [scrib](#) | [tkv](#) | [W](#) | [WASP](#)

FURTHER INFORMATION

Eduardo Moreno's homepage: <http://www.cnio.es/ing/grupos/plantillas/presentacion.asp?pag=124>

ALL LINKS ARE ACTIVE IN THE ONLINE PDF

Review we will focus on the extension of this approach that includes the integration of pet dogs with cancer into the development path of new cancer drugs and discuss studies that have used this integrated approach, primarily within the United States. The use of the dog as a model of cancer drug translation has not been exploited as widely in Europe as in the United States. There are many efforts underway in Europe and the rest of the world that suggest a strong interest in this field and we expect that future reviews will be able to directly reference these current efforts. Worldwide, important progress has and continues to be made in the study of cancer biology through the comparative evaluation of canine cancers (readers are referred to REFS 2, 15–25 for recent reviews and background information).

Cancers in the dog and human

Cancer has increased in the pet animal population in recent years, as have other age-related diseases, owing to increased life expectancy for pets resulting from advances in pet nutrition, vaccination for common infectious diseases, leash laws that reduce deaths caused by automobiles and overall advances in veterinary care. Pet owners are highly motivated to seek out new options for the management of cancer in their pets (FIG. 1). Their motivation is also projected as an interest in receiving care that is provided as part of clinical trials when conventionally available treatments do not meet their goals. Many features of cancer in pet dogs can uniquely contribute to our understanding of cancer pathogenesis, progression and therapy. Pet dogs are large and are relatively outbred compared with laboratory animals (BOX 1). In addition, the inclusion of dogs from different breeds in clinical trials provides a cross-sectional value that is often higher than that obtained in studies of inbred laboratory animals, by providing a background genetic diversity similar to that seen in human populations (on the basis of single nucleotide polymorphism frequency)²¹. Furthermore, the recent deciphering of the canine genome provides evidence of strong similarities with humans^{21,22,26}. For many gene families, most notably those associated with cancer, the similarities are significantly closer than the relationship between a mouse and human²⁷ (FIG. 2). Cancers developing in these animals are naturally occurring and develop in the context of an intact immune system where tumour, and host and tumour microenvironment are syngeneic. Tumour initiation and progression are influenced

SCIENCE AND SOCIETY

Translation of new cancer treatments from pet dogs to humans

Melissa Paoloni and Chand Khanna

Abstract | Naturally occurring cancers in pet dogs and humans share many features, including histological appearance, tumour genetics, molecular targets, biological behaviour and response to conventional therapies. Studying dogs with cancer is likely to provide a valuable perspective that is distinct from that generated by the study of human or rodent cancers alone. The value of this opportunity has been increasingly recognized in the field of cancer research for the identification of cancer-associated genes, the study of environmental risk factors, understanding tumour biology and progression, and, perhaps most importantly, the evaluation and development of novel cancer therapeutics.

Comparative oncology can be used to describe a discipline that integrates the study of naturally occurring cancers in animals into studies of human cancer biology and therapy¹. The term is most often used when referring to the study of cancers seen in companion (pet) animals. Cancers in companion species are well suited to uniquely inform investigations of cancer biology and drug development. Several companion species have successfully contributed to this effort, including cats, horses, ferrets and

other small animals^{2–8}. The long history of dogs in biomedical research, their strong anatomical and physiological similarities to humans, and the sheer number of pet dogs that are diagnosed and managed with cancer (estimated to be over 1 million per year in the United States) have focused early interest and efforts on the dog. Investigations of cancer in dogs are not novel; studies of dogs with cancer to answer questions about cancer therapy have been conducted and reported since the early 1960s^{9–14}. In this

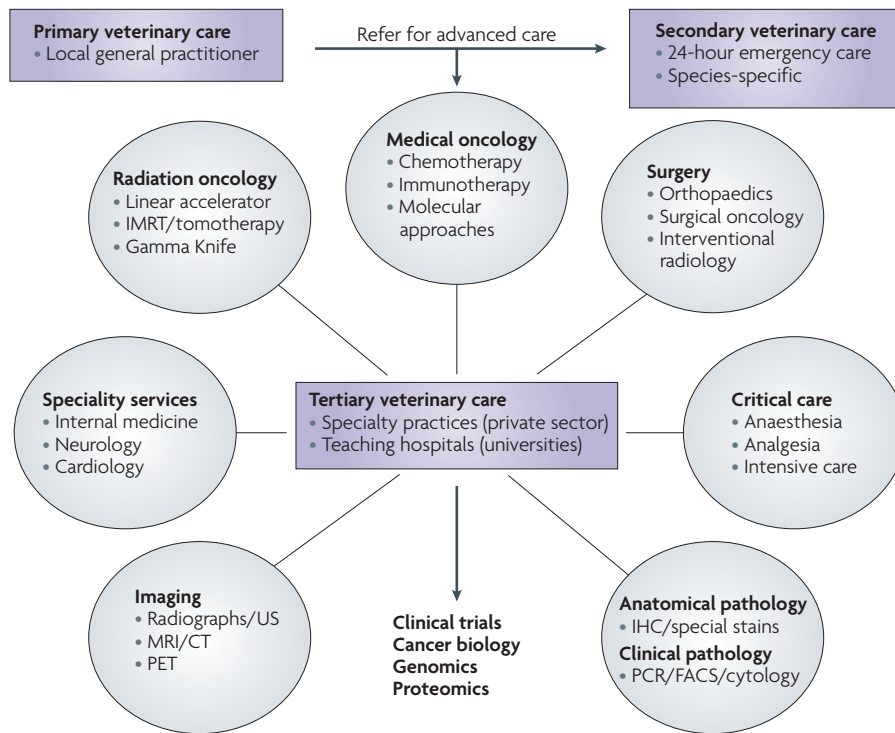


Figure 1 | Provision of veterinary care to animals with cancer. The advanced veterinary care for pet animals provides an established infrastructure for clinical trials in pet animals. Care provided to pet animals in much of the developed world includes primary care veterinary services, secondary care centres providing 24-hour care and tertiary centres that provide disease- or discipline-specific speciality care to animals. Tertiary veterinary care centres include veterinary teaching hospitals based within large universities and, increasingly, private referral veterinary hospitals that offer speciality care for pets. Care available in these centres includes critical care management, the use of sophisticated diagnostic capabilities and imaging modalities such as computerized tomography (CT), magnetic resonance imaging (MRI) and positron-emission tomography (PET), and advanced therapeutic options (nuclear medicine and radiation therapy). There are a variety of specialities in veterinary medicine, mirroring those traditionally known in human medicine. Although not an exhaustive list, these include internal medicine, surgery, critical care or emergency medicine, ophthalmology, dermatology, cardiology, neurology, radiology, pathology, medical oncology and radiation oncology. These specialities require advanced training after a veterinary degree is granted and commonly include standardized residency programmes (3–4 years), research experience and examination-based board certification. Training within these specialities are governed by specialist boards within each discipline (cancer-related specialities include the American College of Veterinary Internal Medicine, the European College of Veterinary Internal Medicine and the American College of Veterinary Radiology-Radiation Oncology (see Further information)). Information on certified specialities approved by the American Veterinary Medical Association and the European Board of Veterinary Specialization can be found at http://www.avma.org/education/abvs/specialty_orgs/all_orgs.asp and <http://www.ebvs.be>. The level of advanced care available through these speciality institutions provides the opportunity to conduct well-organized and advanced clinical trials in pet dogs that have many diseases, including cancer. FACS, fluorescent-activated cell sorting; IHC, immunohistochemistry; IMRT, intensity-modulated radiation therapy; US, ultrasound.

by similar factors in both human and canine cancers, including age, nutrition, sex, reproductive status and environmental exposures^{2,28–33}. The range of cancers seen in pet dogs is as diverse as the cancers seen in human patients. Some histologies of comparative interest include melanoma, non-Hodgkin lymphoma (NHL), leukaemia, osteosarcoma, soft tissue sarcomas, and prostate, mammary, lung, head and

neck, and bladder carcinomas^{1,15,34–41}, and the biological complexity of cancers in pet animals captures the essence of cancer in human patients. This is based in large part on the intratumoural (cell-to-cell) heterogeneity seen in these cancers. Natural consequences of this heterogeneity are the same deadly features of human cancers, including acquired resistance to therapy, recurrence and metastasis.

More importantly than histological similarities, to the extent that comparisons have been made, the genetic molecular alterations that drive cancers in dogs and humans are also highly analogous. Most tumour oncogenes and tumour suppressors that have been defined in human cancers have been shown to contribute to canine cancers². In some cases, specific genetic changes that drive the carcinogenic process in a given human cancer (that is, *p53* alterations in breast cancer, sarcomas and lymphoma) have been found within the cognate canine cancer^{8,42,43}. In other situations, similar if not identical cancer-causing gene mutations can also result in different cancers in humans and dogs. For example, similar mutations in *KIT*, a tyrosine kinase growth factor receptor, have been identified in both gastrointestinal stromal tumours (GIST) in humans and mast-cell cancers in dogs. The high prevalence of mast-cell tumours in dogs provides significant opportunities to study therapeutic strategies targeting KIT-driven cancers in dogs with mast-cell tumours, such as tyrosine kinase inhibition, which can be translated to human GIST and, more globally, to other cancers driven by mutated oncogenes^{44–48}.

Advances in technology and the public release of a high-quality sequence covering 99% of the canine genome (2.5 billion base pairs) have recently made it possible to apply in dogs many of the same high-throughput methodologies that are used to interrogate human cancer²¹. Breen *et al.* recently built a syntenic karyotype map between humans and canines that showed strong similarities between the cytogenetic aberrations associated with NHL in each species^{23,34}. On the basis of the high-throughput opportunities provided by the canine genome sequence, recent work has suggested significant similarities between genomic profiles in dog and human soft tissue sarcoma, glioma and others (M. Breen, personal communication). Using the commercial availability of a canine Affymetrix expression microarray, built on the same platform used for human and murine Affymetrix microarrays, informative comparisons between canine and human osteosarcoma have been possible. Preliminary results demonstrate a strong similarity in the global expression patterns of canine and human osteosarcoma (C.K., unpublished data). In fact, cluster analysis of orthologous gene signatures does not segregate human and canine tumours on the basis of species, suggesting that cancers from each species are not distinguished by these gene expression analyses. These genomic data

complement the known strong biological and clinical similarities between osteosarcoma in humans and dogs²⁴. Similar non-candidate comparisons between human and canine cancers are underway in lymphoma, bladder carcinoma and mammary carcinoma.

These biological similarities may predict the observed similarities in treatment responses between canine and human tumours². For example, the most active drugs used to treat canine NHL are those that comprise the CHOP-like (cyclophosphamide, doxorubicin, vincristine and prednisone) chemotherapy protocols commonly used in human NHL. Those agents demonstrated to be inactive in human NHL are similarly inactive in the management of canine NHL (that is, gemcitabine and cisplatin)^{1,2}. Similar parallels have been seen for investigative and targeted therapeutics.

Because there are no gold standards for the management of cancer in dogs, there is the added opportunity to evaluate novel therapeutics in less advanced, less heavily pretreated populations, compared with most human cancer patients participating in early-phase human trials. This allows the assessment of a new agent's activity when given alone or in combination with other therapies early in the development life of the drug. Furthermore, although the rates of cancer progression seen in pet dogs are more similar to humans than rodents, they are still relatively short. The relatively rapid cancer progression rates, compared with those in humans, provide the opportunity to answer questions about cancer progression (that is, time to metastasis or survival) in a much shorter period of time than trials conducted in human patients. For example, the disease-free intervals in recent studies of canine osteosarcoma and canine lymphoma were assessed in approximately 18 months, whereas similar endpoints have required more than 7 years in human osteosarcoma and lymphoma studies^{49,50–53}.

Cancer treatment in dogs

The cancer treatment options available for pet animals include surgery, radiation therapy, hyperthermia, photodynamic therapy, immunotherapy and chemotherapy². Techniques available for pathological evaluation of pet animal cancers include standard pathological and immunohistochemical descriptors, flow cytometry and other molecular diagnostics such as PCR and cytogenetics^{23,54–56}. In many ways, veterinary cancers are described in the same language as their human counterparts. Furthermore, the process of defining the extent of disease

Box 1 | Outbred and inbred

The germline genetic diversity (single nucleotide polymorphism frequency) of a population of dogs (from Chihuahua to Great Dane) with a given cancer is similar to the diversity seen in a well-mixed population of human patients with a given cancer²¹. This relatively outbred nature of dogs compared with most rodent models of cancer contributes to their relevance for studying new cancer treatments. Dogs of all breeds and mixed breeds develop cancer. However, there are some breeds that have a higher incidence of certain cancers. Over-represented breeds include the Boxer for mast-cell cancer and gliomas, Rottweilers and Greyhounds for osteosarcoma, Golden Retrievers for lymphoma and osteosarcoma, Scottish Terriers for transitional-cell carcinoma of the bladder, Flat-Coat Retrievers and Bernese Mountain Dogs for histiocytic sarcomas and Chow Chows for gastric carcinoma and melanoma^{2,22,106}. Interestingly, decreased overall cancer incidence has been reported in the Dachshund and Beagle. Among the genetic diversity of dogs are groups of highly related individuals that are part of the same breed or pedigree. Studying cancers within related dogs and dogs within a breed has been an effective strategy in the study of familial cancers and the identification of cancer-associated genes. German Shepherd dogs develop renal cystadenocarcinoma and nodular dermatofibrosis, a heritable cancer that is linked to the loss of tumour-suppressor gene function. Study of this disease in dogs recently led to the identification of a gene similar to the gene responsible for Birt–Hogg–Dubé syndrome in humans, which predisposes to kidney cancer, providing a proof of concept that similar cancer genes cause similar diseases in dogs and humans¹⁰⁷. Several groups are currently pursuing the identity of cancer-associated genes in dog breeds at increased risk for osteosarcoma, histiocytic sarcoma, bladder cancer, lymphoma and melanoma.

(stage) and the resultant consideration of local, regional and systemic therapies are similar to those involved in the management of human patients.

Surgical techniques and approaches are nearly identically applied to canine and human cancer patients. The state of the art in veterinary radiation therapy is highly sophisticated and includes the use of megavoltage radiation therapy delivered by linear accelerator, most of which are capable of electron and proton production. Additional technology including intensity-modulated radiation therapy and Gamma Knife radiation therapy are also available through an increasing number of veterinary radiation centres. Currently, stereotactic radiation therapy options are available at three veterinary radiation centres in the United States. Interestingly, the clinical use of intensity-modulated radiation therapy and tomotherapy technology was assessed in pet dogs with cancer in advance of its widespread use in human patients, underlining the opportunity to model medical device use within this species^{57–59}.

Many of the chemotherapy protocols used in veterinary medicine have been adopted from protocols used to treat human patients. Currently there are no agents approved for the treatment of cancer in dogs; as such, all conventional chemotherapeutics used to treat dogs are human drugs used off-label. However, it is expected that novel cytotoxic and non-cytotoxic cancer agents have and will be submitted for veterinary approval in Europe and the United States soon. The toxicity range of conventional

cytotoxic chemotherapy in dogs is similar if not identical to human patients; however, as the goal of maximizing quality of life is important for pet owners and veterinary oncologists, the dose intensity of chemotherapy given to dogs is generally lower than that delivered to human patients. For the most part, lowered dose intensity maintains a good quality of life during and following chemotherapy treatments. On average, 10% of dogs will experience adverse events from chemotherapy that require intervention by a veterinarian. Life-threatening adverse events, most commonly related to myelosuppression, are rare and seen in approximately 1% of veterinary patients^{2,60–63}. Not surprisingly, because of the reduced dose intensity used in veterinary chemotherapy protocols, cure rates are not as high as seen in human patients receiving similar treatment⁶⁴.

For canines, unlike in the human field, there are few established standards of care related to a given cancer. In the event that conventionally available options do not meet the goals of a pet owner, investigational studies including novel therapeutic options for cancer may be considered. The decision to pursue an investigational drug for the treatment of a pet dog is often influenced by the owner's expectations for outcome, the risks associated with the conventional treatment compared with the investigational treatment, and the degree of financial support afforded by the investigational trial. Equally important to many pet owners is a sense that they and their pet are contributing to the 'greater good' and future treatment of human and canine cancer patients.

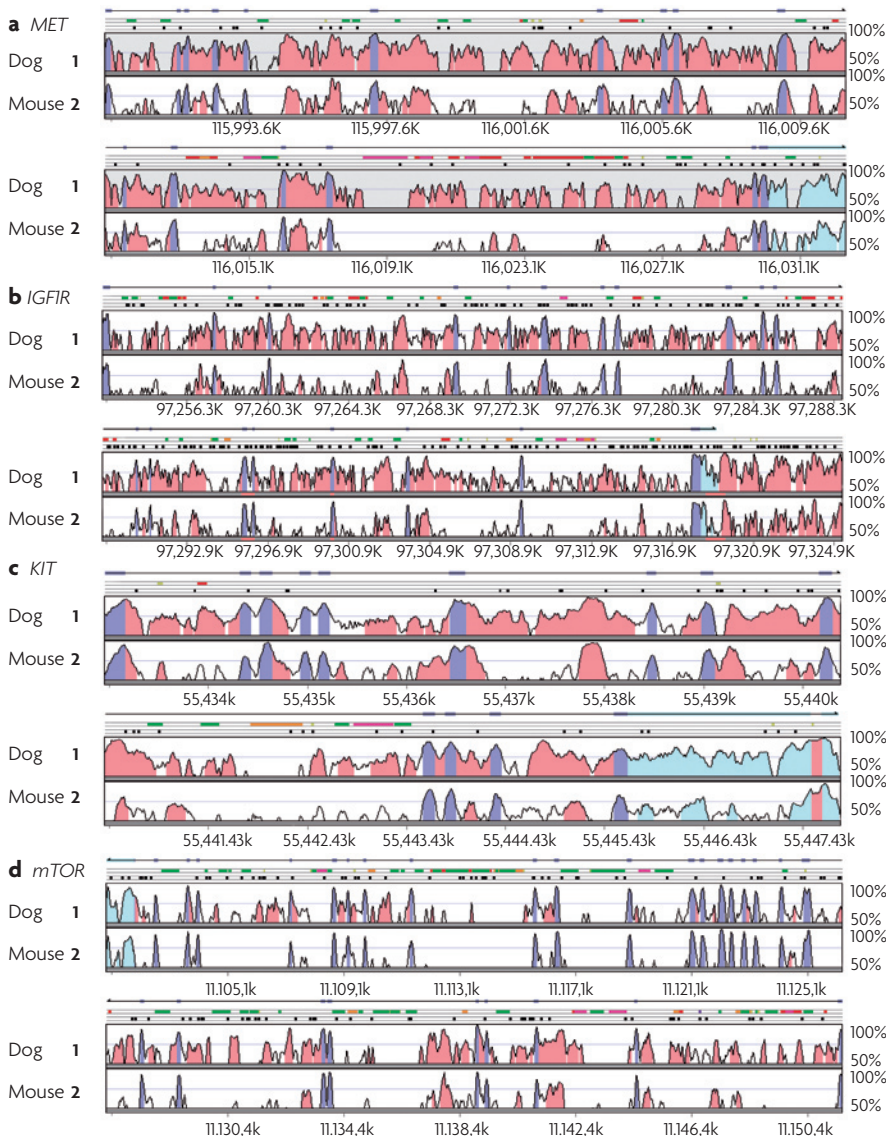


Figure 2 | Homology between dog, human and mouse for recognized cancer genes. Recent completion of the canine genome draft sequence has allowed demonstration of the strong similarities between canine and human cancer genes. VISTA graph displays (see VISTA in Further information) visually compare aligned canine and murine genes with their human orthologues. The y-axis of the graph represents the percent conservation of the canine or murine sequence against the human gene target. The y-axis ranges from 50% to 100% conservation with a threshold line drawn at 70%, which is a level denoting significant similarity. The colours of the peaks describe the function of the sequence, dark blue representing exons, pink non-coding regions and light blue untranslated regions. The plots compare the entire human sequence for the following cancer-associated genes with their respective canine and mouse orthologues. **a** | *MET*, an oncogene activated in canine and human sarcomas. **b** | Insulin-like growth factor 1 receptor (*IGF1R*), a receptor for IGF, which is an important growth factor in various tumours. **c** | *KIT*, a causative oncogene in human gastrointestinal stromal tumours (GISTs) and canine mast-cell tumours and GISTs. **d** | Mammalian target of rapamycin (*mTOR*, also known as *FRAP1*), an integral regulator of protein translation in various tumours and a therapeutic target of rapamycin. The graphs indicates that the dog nucleotide sequences are more highly conserved with human sequences than mouse sequences are for all four candidate genes. This is especially evident at the level of similarity within the non-coding regions.

Translation of new therapies from dogs
The development and delivery of new cancer drugs can be costly, inefficient and linear. Despite evidence of drug efficacy in mouse models of cancer, many new cancer drugs

fail in human cancers either because of unacceptable toxicity or a lack of efficacy^{65,66}. Mouse models of cancer have proved to be excellent tools for dissecting the biology and biochemistry of particular pathways involved

in cancer development and progression. However, they are limited in their representation of some of the features that define human cancer, including growth over long periods of time, genomic instability, and significant heterogeneity in both tumour cells and tumour microenvironment and stroma. As presented above, the pet dog with cancer lacks many of these shortcomings and might assist the transition between mouse models and human patients.

Informing preclinical drug development.
Over the past 30 years various clinical trials have been performed in dogs with cancer and have complemented the use of more traditional murine cancer models and human clinical trials (BOX 2) in the development of new drugs. Such studies have provided opportunities to answer questions that would have been difficult or impossible to answer in either mice or humans alone.

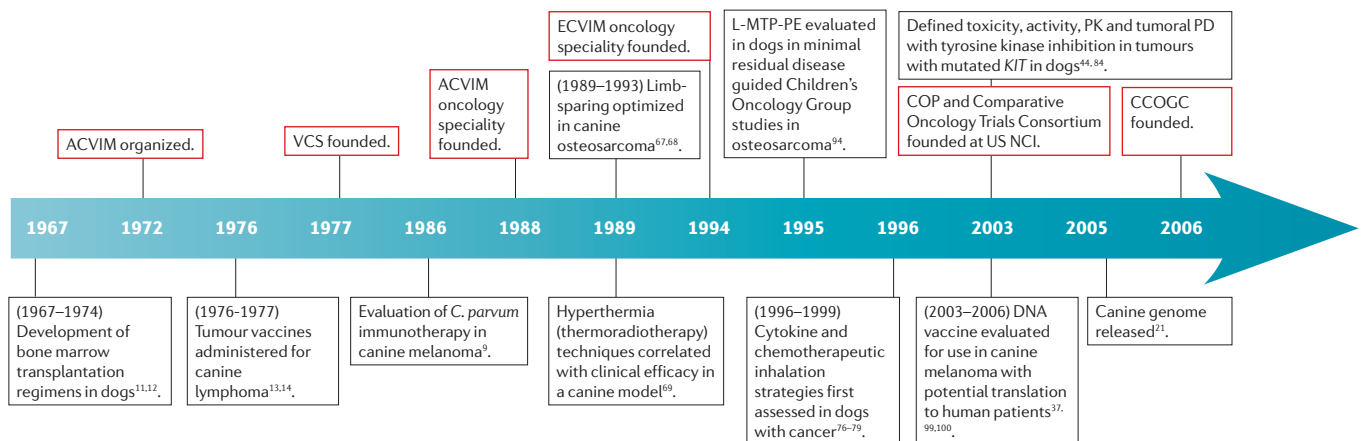
One example of such an effort includes the use of pet dogs with osteosarcoma in the assessment of pre-operative and operative techniques to optimize limb-sparing surgical procedures that are now used in the management of paediatric and adult patients^{2,67,68}. Similarities between humans and dogs with respect to their size, their osteosarcoma biology and the anatomy of the surgical site were essential in the development of these surgical interventions and could not have been easily recreated in other animal models.

Similar studies have relied on the size of dogs and their tumours to scale up therapeutic approaches (drugs and devices) to human application^{1,35,57,69–75}. The comparable respiratory anatomy and the relative size and distribution of primary lung cancer and cancers that metastasize to the lung allowed the assessment of novel anticancer inhalation therapies in dogs^{36,76–79}. Canine trials of inhaled cytokine immunotherapy preceded and anticipated the successful completion of early-phase trials of this novel treatment approach in human patients with pulmonary metastases^{36,77–79}. Similarly, inhalational cytotoxic chemotherapy trials in dogs informed phase I trials that followed in human patients^{76,80}. It is likely, without the strong evidence of anti-tumour activity and the opportunity to understand the biological mechanisms associated with this therapy in dogs, that these inhalation approaches would not have advanced into clinical development in humans as rapidly^{80–82}. The preclinical use of pet dogs in future inhalation studies is likely to answer questions that will allow optimal development of this therapeutic approach for humans.

Box 2 | Historical timeline for on the use of pet dogs in cancer research

Historically, the use of pet dogs as cancer models can be broken up into three phases. The first was the descriptive phase in which dogs were used as models for surgical or therapeutic intervention owing to their similarity to humans in size, anatomy and tumour histology. This included the development of bone-marrow transplant regimens and early evaluation of immunotherapies such as *Corynebacterium parvum* and cancer vaccines^{9,11,12}. Early efforts led to advances in limb-sparing techniques for children with osteosarcoma and assessment of the safety and efficacy of hyperthermia in heterogeneous tumours^{67–69,74}. Dogs were then used to define the activity of agents first in measurable disease and then in minimal residual disease. Examples of this work led to the development of cytokine and chemotherapeutic inhalation strategies for pulmonary metastatic disease and the evaluation of liposomal muramyl tripeptide phosphatidylethanolamine (L-MTP-PE) for adjunctive therapy in children with osteosarcoma^{76,78,79,94,108}. The present phase involves the use of dogs for biology-intensive studies often correlating pharmacokinetic (PK) and pharmacodynamic (PD) endpoints^{35,37,44,52,83,84,109}. A number of cooperative groups have provided an infrastructure for these studies. The Veterinary Cancer Society (VCS), founded in 1977, focuses on education and

sharing scientific knowledge within the veterinary oncology community (see Veterinary Cancer Society in Further information). VCS and the Veterinary Cooperative Oncology Group have encouraged largely retrospective multicentre collaborative studies. The Comparative Oncology Program (COP, see Further information) of the United States National Cancer Institute (US NCI), developed in 2003, represents an ambitious effort for using dogs as a comparative model. Through the NCI's COP, a multicentre collaborative network of 14 veterinary teaching hospitals (Comparative Oncology Trials Consortium) was established to conduct rigorously controlled and focused preclinical trials of new cancer drugs intended to inform the design of human studies. In 2006 the Comparative Oncology and Genomics Consortium (CCOGC), a not-for-profit entity, was established. The CCOGC includes a broad group of parties focused on the genetics and biology of naturally occurring cancers in dogs. The CCOGC has endeavoured to build the infrastructure necessary for studying and characterizing canine cancers using newly developed genomic and biological tools. A primary effort has been the development of a canine cancer biospecimen repository^{20,103,110}.



Black boxes indicate milestones in canine oncology; red boxes indicate the founding of societies and organizations. ACVIM, American College of Veterinary Internal Medicine; ECVIM, European College of Veterinary Internal Medicine.

Studying novel therapeutic approaches.

Biological similarities between pet animal cancers and human cancers have also provided significant rationale for the study of novel therapeutic approaches in dogs. One of the earliest uses of canine cancers as biological models of human cancer was the work of Storb and colleagues, who treated pet dogs with NHL using bone-marrow transplantation protocols^{10–12,73,74}. These studies were essential to the development of preparatory protocols used to limit graft-versus-host disease and maximize treatment outcome for human patients.

Recently, studies in pet dogs with cancer were undertaken to assist in the evaluation of anti-angiogenic peptide mimetics of thrombospondin 1 (TSP1, also known as *THBS1*) in parallel with human clinical trials^{52,83}. Initial studies in dogs demonstrated single-agent activity for anti-angiogenic TSP1 peptides against several types of

cancer.⁸³ Responding histologies in dogs provided supporting data for the selection of phase II human study populations to be included in the evaluation of these agents. The canine trials also provided guidance on the drug level and exposure durations (for example, in some cases over 90 days) that were required for the anti-tumour activity of this class of drug to be seen. The fact that responses were infrequent, often transient, and required long exposures predicted low single-agent response rates in conventional early human studies, and emphasized the importance of evaluating these agents in novel trial designs and in combination with other anticancer agents. Follow-up combination studies in dogs with lymphoma have now supported the potential for cooperative activity between cytotoxic chemotherapy and TSP1 anti-angiogenic therapy⁵².

The relationship between a specific cancer target, its modulation with a small-molecule

kinase inhibitor and clinical benefit was in fact first established in dogs with cancer. This clinical trial explored the safety and efficacy of a novel multi-targeted indolinone tyrosine kinase inhibitor⁸⁴. The orally bio-available compound, SU11654, exhibited potent inhibitory activity against receptor tyrosine kinases including vascular endothelial growth factor receptor (VEGFR, also known as *KDR*), platelet-derived growth factor receptor B (*PDGFRB*), *KIT* and *FLT3*, resulting in both direct anti-tumour and anti-angiogenic activity^{44,84}. Responding histologies in this study of 57 pet dogs included sarcomas, carcinomas, melanomas and mast-cell tumours⁸⁴. The highest response rate was observed in canine mast-cell tumours, which are often driven by *KIT* mutations. As discussed earlier, these mutations are remarkably similar to the *KIT* mutations found in GIST in human patients^{46,47,85–87}. Follow-up studies

in dogs correlated target inhibition with the mutational status of *KIT* and SU11654 plasma concentrations. As indicated earlier, this was the first target modulation study of its kind (human or dog) in which a direct association could be made between the blood level of a kinase inhibitor, actual inhibition of the specific target *in vivo* and an anti-tumour response⁴⁴. A compound related to SU11654, [sunitinib malate](#) (SU11248), which also targets VEGFR, PDGFRB, *KIT* and FLT3, was recently approved by the United States Food and Drug Administration (FDA) for the treatment of kidney cancer and GIST in humans⁸⁸.

These studies in dogs with mast-cell tumours have aided the translational development of this class of agents by defining toxicity, activity, and pharmacokinetic and tumour pharmacodynamic relationships that would be difficult to answer in other preclinical models or in human clinical trials alone^{88–93}. Furthermore, the spontaneous development of resistance to single-agent tyrosine kinase inhibitors has been observed in dogs with mast-cell tumours, and these dogs may now be included in studies designed to determine the optimal strategies to manage human cancers with similar acquired resistance^{88,93}. It is important to note that the value of the data collected in dogs using SU11654 was based on the drug–target interaction within a naturally occurring cancer rather than the regression of a specific cancer histology. Opportunities to use canine cancers as spontaneous molecular models, independent of histology, will continue to increase as the molecular profiles of cancers become better defined and the canine-based molecular toolkit expands (that is, through the efforts of the Comparative Oncology and Genomics Consortium and others).

One of the important advantages of dogs as models of human cancer is the ability to evaluate novel therapeutic agents in the setting of minimal residual disease. The kinetics of tumour progression in most murine models of cancer do not provide the opportunities to create meaningful periods of minimal residual disease and most early human clinical trials focus on patients with advanced disease that cannot be reduced or down-staged effectively by other therapies. For example, the opportunity to evaluate novel therapeutics for minimal residual disease has been important for the development of several immunotherapeutic options for cancer. MacEwen and colleagues demonstrated the activity of liposomal muramyl tripeptide phosphatidylethanolamine (L-MTP-PE) in dogs with osteosarcoma

following the resection of the primary tumour (that is, minimal residual disease)⁹⁴. These randomized, placebo-blinded studies of L-MTP-PE in dogs were part of the scientific rationale for the phase III evaluation of L-MTP-PE in osteosarcoma^{95–97}. Recent re-evaluation of the matured human data from these studies found remarkably similar results to these earlier canine studies, and these are now the basis for a request of FDA approval for L-MTP-PE (Junovan) by IDM Pharma for osteosarcoma in children (see IDM Pharma for Further information)^{95,96,98}.

The evaluation of a novel DNA-based vaccine strategy for melanoma is another important example of the evaluation of new therapeutics in the setting of minimal residual disease in dogs^{37,99,100}. The initial studies conducted to assist the development of the human vaccine also provided the safety data required for conditional approval of a veterinary biological by the United States Department of Agriculture for dogs with melanoma (final approval will require conclusive demonstration of efficacy). In June of 2007, the commercial launch of this DNA vaccine for dogs occurred (see Merial US for Further information). The opportunity for human-orientated research to also provide similar (but distinct) animal health products provides an additional incentive to conduct studies in dogs with cancer as a part of a two-species development plan.

Clinical trials in client-owned dogs are not constrained by traditional phase I, phase II and phase III trial designs. Novel agents can be offered to pet dogs as single agents before any conventional treatment has been provided or during the period of minimal residual disease^{35,94,101,102}. Moreover, such agents can readily be added to conventional treatment regimens such as chemotherapy and radiation therapy to determine optimal therapeutic combinations. These studies often take many years in the human arena and are hindered by the fact that the combination therapy of interest may be contrary to accepted treatment standards for humans.

Broadening the translational opportunity

Historically, translational studies that have included dogs with cancer have been observational in nature and have focused on treatment-induced regression of tumours (BOX 2). This focus on regression ignores the broader opportunity of studying the biology of cancer in large animals afflicted with cancer. The realization of this broadened opportunity is increasingly evident and is largely the result of the completion of the

canine genome sequence, a reduction in costs for reagent and assay development, and the needs of the pharmaceutical community for cancer models that can better inform drug development both before and after a new treatment has entered human clinical development. Collectively, the opportunity now exists to conduct detailed and biologically intensive studies in dogs that have cancer. Evidence of this includes the commercial availability of a canine oligonucleotide microarray (see Affymetrix for Further information), optimized conditions for proteomic studies, validated canine-specific antibodies and characterization of human antibodies that cross-react with canine epitopes. The flexibility in study designs and study-related interventions (for example, biopsies and imaging) in dog studies allow acceleration of the understanding of the biological principles that are required for successful drug development. An important attribute of cancer studies in dogs is the opportunity to gather serial biopsies from target and non-target lesions and repeated collection of body fluid (serum, whole blood and urine) from the same animal during exposure to an investigational agent. This serial sampling allows for the identification of tumoural and surrogate pharmacodynamic endpoints, or biomarkers that can be uniquely correlated to exposure, correlative endpoints (for example, imaging) and therapeutic response in ways that are often difficult or unacceptable in human trials (FIG. 3).

For example, Vail and colleagues have recently presented preliminary results of their evaluation of a novel tissue-specific prodrug cytotoxic agent in the treatment of dogs with lymphoma. The study design allowed collection of tumour biopsies before and after exposure to this novel therapy and included advanced positron-emission tomography and computerized tomography (PET–CT) imaging of responses. Results from this multi-layered study supported the safety and activity of this agent and validated the use of PET–CT as a predictor of future clinical response¹¹¹.

A similarly advanced study design was used in the evaluation of a novel vascular targeting therapy based on phage delivery of tumour necrosis factor- α (TNF α). The phage is delivered on the basis of the abundant expression of the integrins $\alpha_v\beta_5$ and $\alpha_v\beta_3$ on tumour cells and tumour vasculature using an Arg–Gly–Asp (RGD) localization motif on the phage (RGD–TNF α). Studies in mice validated the

selectivity of the phage and the expression of TNF α in tumours and tumour-associated vessels, and showed the sparing of normal organs. However, the preclinical development of this treatment approach required a large animal model in which relevant toxicity endpoints could be assessed and evidence of the localization of TNF α to tumour versus normal tissue could be confirmed. As non-tumour-bearing animals would not be expected to express high levels of $\alpha_v\beta_5$ and $\alpha_v\beta_3$ (owing to a lack of tumour and tumour-associated vessels), it would not be expected that TNF α would be delivered or expressed, so toxicity would probably be under-reported by these traditional models¹⁰³. Preliminary results of a dose-escalation and multiple-dose design of RGD-TNF α phage delivery to tumour-bearing dogs has established a robust safety profile for this agent in a relevant host. Tumour and normal tissue biopsies obtained before, during and after phage delivery have confirmed tumour-specific delivery of TNF α to tumour and sparing of normal tissue (S. Libutti, C.K. and colleagues, unpublished data). This study was conducted through the Comparative Oncology Trials Consortium of the Center for Cancer Research — Comparative Oncology Program (CCR-COP) and is expected to provide essential support for the Investigational New Drug (IND) application for this agent.

Perceived risks and concerns

In common with all novel perspectives or approaches, the concept of integrating studies that include pet dogs with cancer into the development of new cancer drugs is associated with some hesitation and the perception of risk. Understanding the source of such perceptions and defining the actual risks or weaknesses are an important part of successful integration. It is true that not all agents can or should be evaluated in tumour-bearing dogs and not all questions can be answered using this approach.

Study duration. Timelines for the completion of studies in pet dogs are longer than those in rodent models. The development of multicentre consortia focused on conducting cancer studies in pet dogs has mitigated part of this risk (see CCR-COP in Further information); furthermore, strategic inclusion of pet dog studies within a preclinical and clinical development path can prevent any delays in the conduct or completion of human clinical trials.

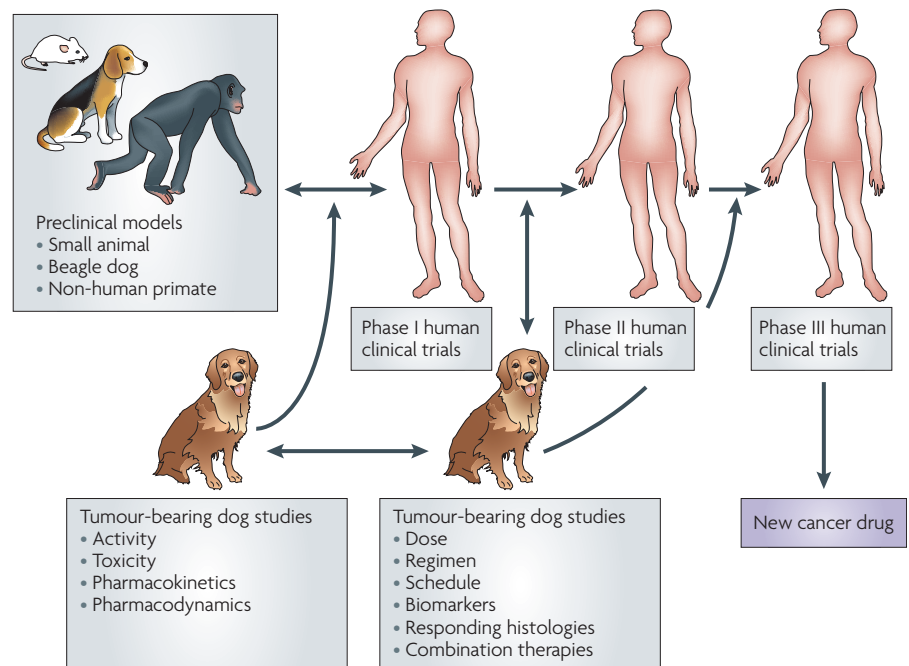


Figure 3 | Integrated approach. Current drug development efforts are largely uni-directional and non-integrated. New cancer drugs are evaluated in conventional preclinical models of efficacy and toxicity and then either succeed or fail in human clinical trials. An optimal drug development path would integrate both preclinical and clinical components of drug development so that questions that emerge in the human clinic could be answered in animals. Translational drug development studies in the pet dog with cancer are optimal for such an integrated approach, being an intermediary between conventional preclinical models (mouse, research-bred dog and non-human primate) and the human clinical trial. The opportunity for this approach is now feasible on the basis of the recent completion of the canine genome sequence, the availability of tools to study cancer biology and the biological consequences of therapy, and the urgent needs of the drug-development community for more effective cancer models. Through this integrated approach it is likely that important questions about a new drug candidate can be answered before it enters human studies, for example, toxicity, dose, regimen, pharmacokinetics, pharmacodynamics and activity. Perhaps equally important are the questions that emerge following the completion of an early-phase human study (often left unanswered) as the agent moves into later stages of development. It is likely that the totality of information generated through this comparative approach will contribute to the success and reduce late attrition of new cancer therapeutics. It is important to note that the inclusion of the dog within this integrated approach will not be reasonable, feasible or valuable for all drugs, drug targets or disease conditions.

Compliance and reporting of data. The reporting of data in a timely manner using good clinical practice guidelines is an important component of current and future trials that are intended to aid in the development of human drugs. An essential part of this is the reporting of adverse events and assigning the severity, duration and attribution (whether it is study- or drug-related) of these events. Using novel electronic reporting systems, data are available in real-time so as to encourage discussion between the study sponsor (that is, a pharmaceutical company) and study investigators at all phases of a dog trial. Such an electronic reporting system, with capture of data from remote sites, has been successfully used by the Comparative Oncology Trials Consortium in three clinical trials.

Compliance is also an important component of complete data reporting and study conduct. Using the United States Freedom of Information Act, review of the clinical trials that are directed at the registration of veterinary drugs reveals high study compliance (>90%). This study compliance rate includes adherence to all parts of a study protocol, including oral administration of drugs, completion of diet and intake reports, and consent to necropsy in the event of unexpected deaths (see CCR-COP in Further information).

Patient-to-patient variability. There is a concern that the ‘uncontrolled’ nature of pet dog studies may falsely associate a toxicity with a new cancer drug. It is true that studies in tumour-bearing dogs represent a

clinical population and cannot be compared to a population of purpose-bred research dogs that are receiving an investigational agent as part of a toxicology study.

Depending on the nature of the agent and the level of concern for toxicity, study entry requirements may be limited to pet dogs of a specific sex, size or age, and may limit all or some concurrent drugs and concurrent illnesses. By limiting the clinically relevant variables the study evaluation may appear more controlled, but as a result it will be less like the human populations that will eventually receive these agents. It is important to note that in over 50 cancer therapeutics (most often cytotoxic) used in clinical populations of pet animals no new toxicities have been identified beyond those identified in conventional research-bred dog studies (see CCR-COP in Further information). It is likely that the maximally tolerated dose of a given therapeutic will be higher in research-bred dogs than in pet dogs.

Regulatory oversight and reporting. The guidelines for regulatory oversight of non-clinical trials that include pet dogs with cancer and are intended to support the development of human drugs are not well defined. Meetings among interested parties including academic groups, pharmaceutical companies, governmental organizations and regulatory bodies are underway in both the United States and Europe to provide this needed clarity. In all cases the care of pet animals must be given great consideration and should include institutional Animal Care and Use Committee approval. For agents that are post-IND, it is reasonable that data from a tumour-bearing dog study would become part of a reporting package for the agent in question. Novel agents not yet submitted for IND status may be treated similarly to other preclinical studies in traditional model species. Specific guidance on the timing and nature of such reports is currently under discussion. It is clear that regulators understand and are excited by the potential value of additional non-clinical data informing the development of new cancer drugs; accordingly, it is reasonable that these data be reviewed on completion of a study or final resolution of specific findings that emerge from such a study.

Drug and budget requirements. The size of dogs requires a significantly greater drug supply than the traditional murine studies; however, manufacture does not require good manufacturing practice certification. For the most part, pet owners are not responsible for the cost of study drug or care provided

through participation in a clinical trial, as these costs are typically covered by the sponsoring company. Tumour-bearing dog studies are more expensive than mouse studies but they are within range of other large animal toxicity studies necessitated for IND application. The addition of serial biopsy of the tumour, imaging or other correlative endpoints incrementally adds to the study costs.

Cancer prevalence. Sarcomas and lymphoid neoplasms are more prevalent in dogs, whereas the most common cancers of humans, namely breast, prostate, gastrointestinal and lung carcinomas, are less common in dogs. However, the prevalence of cancers of the breast in dogs is higher in those that have not been spayed; this might be more similar to the human population. Clinical studies of these cancers that are rarer in dogs might need more time for completion or the addition of broader, potentially international clinical trial centres. In the future, it is likely that cancer therapeutics will not be defined by their activity within a cancer histology, but by a specific cancer biology or deregulation of a specific pathway or gene. As such, a focus on cancer histology might be antiquated.

Species concerns. Historically the dog has been and continues to be used as a model of toxicity assessment in most therapeutic areas and for many organ systems. The gastrointestinal sensitivity of the dog is recognized to be higher than human patients, particularly for orally administered drugs and for agents that stimulate all parts of the chemoreceptor trigger zone (an area of the brain involved in initiating vomiting)^{104,105}.

Future directions and conclusions

That dogs with cancer can effectively contribute to the development of new cancer drugs should be considered as a hypothesis. We believe that this hypothesis will be demonstrated to be true; however, caveats and nuances will be learned. With time, a clear understanding of the types of tumour targets, treatment agents, therapeutic approaches and disease entities that are most suitable to this approach will become clearer. Studies conducted within the field should continue to be focused on specific questions needed to advance the development path of new drugs. It is reasonable that in the future the field will view dogs and other animals with naturally occurring cancers as common, valued and necessary parts of the drug development process. The increasing availability of banked canine tumours

and associated 'omic' annotation for these cancers will allow for rapid identification of valid tumour targets in canine cancers. Additionally, we foresee the availability of large, highly trained, multi-disciplinary teams of investigators to design studies that are integrated within the development path of existing cancer drugs. Such studies will be conducted under clear regulatory guidance and results translated to studies in human patients. The outcome will be more optimal design of human clinical trials, early identification of liabilities that are related to a drug asset, reduced late attrition or failure of cancer drugs in human patients, and improved care of future human and canine cancer patients.

Melissa Paoloni and Chand Khanna are at the Comparative Oncology Program, Center for Cancer Research, National Cancer Institute, 37 Convent Drive, Room 2144, Bethesda, Maryland 20892, USA.

Correspondence to C.K. e-mail: khannac@mail.nih.gov

doi: 10.1038/nrc2273

Published online 18 January 2008

- Hansen, K. & Khanna, C. Spontaneous and genetically engineered animal models; use in preclinical cancer drug development. *Eur. J. Cancer* **40**, 858–880 (2004).
- Withrow, S. J. & Vail, D. M. *Withrow & MacEwen's Small Animal Clinical Oncology* 846 (Saunders Elsevier, St. Louis, 2007).
- Klein, W. R. *et al.* Equine sarcoïd: BCG immunotherapy compared to cryosurgery in a prospective randomised clinical trial. *Cancer Immunol. Immunother.* **21**, 133–140 (1986).
- Antinoff, N. & Hahn, K. Ferret oncology: diseases, diagnostics, and therapeutics. *Vet. Clin. North Am. Exot. Anim. Pract.* **7**, 579–625 (2004).
- Nasir, L. & Reid, S. W. Bovine papillomaviral gene expression in equine sarcoïd tumours. *Virus Res.* **61**, 171–175 (1999).
- Heinzerling, L. *et al.* Intratumoral injection of DNA encoding human interleukin 12 into patients with metastatic melanoma: clinical efficacy. *Hum. Gene Ther.* **16**, 35–48 (2005).
- Seltenhammer, M. H. *et al.* Comparative histopathology of grey-horse melanoma and human malignant melanoma. *Pigment Cell Res.* **17**, 674–681 (2004).
- Hershey, A. E., Dubielzig, R. R., Padilla, M. L. & Helfand, S. C. Aberrant p53 expression in feline vaccine-associated sarcomas and correlation with prognosis. *Vet. Pathol.* **42**, 805–811 (2005).
- MacEwen, E. G., Patnaik, A. K., Harvey, H. J., Hayes, A. A. & Matus, R. Canine oral melanoma: comparison of surgery versus surgery plus *Corynebacterium parvum*. *Cancer Invest.* **4**, 397–402 (1986).
- Weiden, P. L. *et al.* Treatment of canine malignancies by 1200 R total body irradiation and autologous marrow grafts. *Exp. Hematol.* **3**, 124–134 (1975).
- Tsoi, M. S., Weiden, P. L. & Storb, R. Lymphocyte reactivity to autochthonous tumor cells in dogs with spontaneous malignancies. *Cell Immunol.* **13**, 431–439 (1974).
- Storb, R., Epstein, R. B., Ragde, H., Bryant, J. & Thomas, E. D. Marrow engraftment by allogeneic leukocytes in lethally irradiated dogs. *Blood* **30**, 805–811 (1967).
- Crow, S. E. *et al.* Chemoimmunotherapy for canine lymphosarcoma. *Cancer* **40**, 2102–2108 (1977).
- Benjamini, E. *et al.* Tumor vaccines for immunotherapy of canine lymphosarcoma. *Ann. NY Acad. Sci.* **277**, 305–312 (1976).
- Porrello, A., Cardelli, P. & Spugnini, E. P. Oncology of companion animals as a model for humans. an overview of tumor histotypes. *J. Exp. Clin. Cancer Res.* **25**, 97–105 (2006).

16. Mueller, F., Fuchs, B. & Kaser-Hotz, B. Comparative biology of human and canine osteosarcoma. *Anticancer Res.* **27**, 155–164 (2007).
17. Waters, D. J. & Wildasin, K. Cancer clues from pet dogs. *Sci. Am.* **295**, 94–101 (2006).
18. Waters, D. J. High-grade prostatic intraepithelial neoplasia in dogs. *Eur. Urol.* **35**, 456–458 (1999).
19. Knapp, D. W. & Waters, D. J. Naturally occurring cancer in pet dogs: important models for developing improved cancer therapy for humans. *Mol. Med. Today* **3**, 8–11 (1997).
20. Khanna, C. *et al.* The dog as a cancer model. *Nature Biotechnol.* **24**, 1065–1066 (2006).
21. Lindblad-Toh, K. *et al.* Genome sequence, comparative analysis and haplotype structure of the domestic dog. *Nature* **438**, 803–819 (2005).
22. Ostrander, E. A., Giger, U. & Lindblad-Toh, K. *The Dog and its Genome 584* (Cold Spring Harbor Laboratory, New York, 2006).
23. Thomas, R. *et al.* Construction of a 2-Mb resolution BAC microarray for CGH analysis of canine tumors. *Genome Res.* **15**, 1831–1837 (2005).
24. Withrow, S. J., Powers, B. E., Straw, R. C. & Wilkins, R. M. Comparative aspects of osteosarcoma. Dog versus man. *Clin. Orthop. Relat. Res.* **270**, 159–168 (1991).
25. Vail, D. M. & MacEwen, E. G., Spontaneously occurring tumors of companion animals as models for human cancer. *Cancer Invest.* **18**, 781–792 (2000).
26. O'Brien, S. J. & Murphy, W. J. Genomics. A dog's breakfast? *Science* **301**, 1854–1855 (2003).
27. Hoffman, M. M. & Birney, E. Estimating the neutral rate of nucleotide substitution using introns. *Mol. Biol. Evol.* **24**, 522–531 (2007).
28. Patronek, G. J., Waters, D. J. & Glickman, L. T. Comparative longevity of pet dogs and humans: implications for gerontology research. *J. Gerontol. A Biol. Sci. Med. Sci.* **52**, B171–B178 (1997).
29. Bukowski, J. A., Wartenberg, D. & Goldschmidt, M. Environmental causes for sinonasal cancers in pet dogs, and their usefulness as sentinels of indoor cancer risk. *J. Toxicol. Environ. Health A* **54**, 579–591 (1998).
30. Hayes, H. M., Jr & Fraumeni, J. F., Jr. Epidemiological features of canine renal neoplasms. *Cancer Res.* **37**, 2553–2556 (1977).
31. Misdorp, W. & Hart, A. A. Canine mammary cancer. II. Therapy and causes of death. *J. Small Anim. Pract.* **20**, 395–404 (1979).
32. Mukaratirwa, S. Prognostic and predictive markers in canine tumours: rationale and relevance. A review. *Vet. Q.* **27**, 52–64 (2005).
33. Olson, P. N. Using the canine genome to cure cancer and other diseases. *Theriogenology* **68**, 378–381 (2007).
34. Thomas, R., Smith, K. C., Ostrander, E. A., 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).
35. Khanna, C. *et al.* A randomized controlled trial of octreotide pamoate long-acting release and carboplatin versus carboplatin alone in dogs with naturally occurring osteosarcoma: evaluation of insulin-like growth factor suppression and chemotherapy. *Clin. Cancer Res.* **8**, 2406–2412 (2002).
36. Khanna, C. & Vail, D. M. Targeting the lung: preclinical and comparative evaluation of anticancer aerosols in dogs with naturally occurring cancers. *Curr. Cancer Drug Targets* **3**, 265–273 (2003).
37. Bergman, P. J. *et al.* Long-term survival of dogs with advanced malignant melanoma after DNA vaccination with xenogeneic human tyrosinase: a phase I trial. *Clin. Cancer Res.* **9**, 1284–1290 (2003).
38. Mutsaers, A. J., Widmer, W. R. & Knapp, D. W. Canine transitional cell carcinoma. *J. Vet. Intern. Med.* **17**, 136–144 (2003).
39. Mohammed, S. I. *et al.* Effects of the cyclooxygenase inhibitor, piroxicam, on tumor response, apoptosis, and angiogenesis in a canine model of human invasive urinary bladder cancer. *Cancer Res.* **62**, 356–358 (2002).
40. Koenig, A., Bianco, S. R., Fosmire, S., Wojcieszyn, J. & Modiano, J. F. Expression and significance of p53, rb, p21/waf-1, p16/ink-4a, and PTEN tumor suppressors in canine melanoma. *Vet. Pathol.* **39**, 458–472 (2002).
41. Modiano, J. F., Ritt, M. G. & Wojcieszyn, J. The molecular basis of canine melanoma: pathogenesis and trends in diagnosis and therapy. *J. Vet. Intern. Med.* **13**, 163–174 (1999).
42. Setoguchi, A. *et al.* Aberrations of the p53 tumor suppressor gene in various tumors in dogs. *Am. J. Vet. Res.* **62**, 433–439 (2001).
43. Haga, S. *et al.* Overexpression of the p53 gene product in canine mammary tumors. *Oncol. Rep.* **8**, 1215–1219 (2001).
44. Pryer, N. K. *et al.* Proof of target for SU11654: inhibition of KIT phosphorylation in canine mast cell tumors. *Clin. Cancer Res.* **9**, 5729–5734 (2003).
45. Ozaki, K., Yamagami, T., Nomura, K. & Narama, I. Mast cell tumors of the gastrointestinal tract in 39 dogs. *Vet. Pathol.* **39**, 557–564 (2002).
46. London, C. A., Kisseberth, W. C., Galli, S. J., Geissler, E. N. & Helfand, S. C. Expression of stem cell factor receptor (c-kit) by the malignant mast cells from spontaneous canine mast cell tumours. *J. Comp. Pathol.* **115**, 399–414 (1996).
47. London, C. A. *et al.* Spontaneous canine mast cell tumors express tandem duplications in the proto-oncogene c-kit. *Exp. Hematol.* **27**, 689–697 (1999).
48. Kiupel, M., Webster, J. D., Kaneene, J. B., Miller, R. & Yuzbasiyan-Gurkan, V. The use of KIT and tryptase expression patterns as prognostic tools for canine cutaneous mast cell tumors. *Vet. Pathol.* **41**, 371–377 (2004).
49. Anderson, P. Liposomal muramyl tripeptide phosphatidyl ethanolamine: ifosfamide-containing chemotherapy in osteosarcoma. *Future Oncol.* **2**, 333–343 (2006).
50. Leahy, M. F., Seymour, J. F., Hicks, R. J. & Turner, J. H. Multicenter phase II clinical study of iodine-131-rituximab radioimmunotherapy in relapsed or refractory indolent non-Hodgkin's lymphoma. *J. Clin. Oncol.* **24**, 4418–4425 (2006).
51. Tomita, N. *et al.* Phase II study of CHOP-GR therapy for advanced-stage follicular lymphoma. *Leuk. Lymphoma* **47**, 1041–1047 (2006).
52. Rusk, A. *et al.* Cooperative activity of cytotoxic chemotherapy with antiangiogenic thrombospondin-1 peptides, ABT-526 in pet dogs with relapsed lymphoma. *Clin. Cancer Res.* **12**, 7456–7464 (2006).
53. MacDonald, V. S., Thamm, D. H., Kurzman, I. D., Turek, M. M. & Vail, D. M. Does L-asparaginase influence efficacy or toxicity when added to a standard CHOP protocol for dogs with lymphoma? *J. Vet. Intern. Med.* **19**, 732–736 (2005).
54. Avery, A. C. & Avery, P. R. Determining the significance of persistent lymphocytosis. *Vet. Clin. North Am. Small Anim. Pract.* **37**, 267–282 (2007).
55. Lana, S., Plaza, S., Hampe, K., Burnett, R. & Avery, A. C. Diagnosis of mediastinal masses in dogs by flow cytometry. *J. Vet. Intern. Med.* **20**, 1161–1165 (2006).
56. Lana, S. E., Jackson, T. L., Burnett, R. C., Morley, P. S. & Avery, A. C. Utility of polymerase chain reaction for analysis of antigen receptor rearrangement in staging and predicting prognosis in dogs with lymphoma. *J. Vet. Intern. Med.* **20**, 329–334 (2006).
57. Forrest, L. J. *et al.* The utility of megavoltage computed tomography images from a helical tomotherapy system for setup verification purposes. *Int. J. Radiat. Oncol. Biol. Phys.* **60**, 1639–1644 (2004).
58. Kippenes, H. *et al.* Spatial accuracy of fractionated IMRT delivery studies in canine paraspinal irradiation. *Vet. Radiol. Ultrasound.* **44**, 360–366 (2003).
59. Mackie, T. R. *et al.* Image guidance for precise conformal radiotherapy. *Int. J. Radiat. Oncol. Biol. Phys.* **56**, 89–105 (2003).
60. Rassnick, K. M. *et al.* Treatment of canine mast cell tumors with CCNU (lomustine). *J. Vet. Intern. Med.* **13**, 601–605 (1999).
61. Ogilvie, G. K. *et al.* Evaluation of single-agent chemotherapy for treatment of clinically evident osteosarcoma metastases in dogs: 45 cases (1987–1991). *J. Am. Vet. Med. Assoc.* **202**, 304–306 (1993).
62. Ogilvie, G. K. *et al.* Acute and short-term toxicoses associated with the administration of doxorubicin to dogs with malignant tumors. *J. Am. Vet. Med. Assoc.* **195**, 1584–1587 (1989).
63. Bergman, P. J. *et al.* Amputation and carboplatin for treatment of dogs with osteosarcoma: 48 cases (1991 to 1993). *J. Vet. Intern. Med.* **10**, 76–81 (1996).
64. Garrett, L. D., Thamm, D. H., Chun, R., Dudley, R. & Vail, D. M. Evaluation of a 6-month chemotherapy protocol with no maintenance therapy for dogs with lymphoma. *J. Vet. Intern. Med.* **16**, 704–709 (2002).
65. Kamb, A., Wee, S. & Lengauer, C. Why is cancer drug discovery so difficult? *Nature Rev. Drug Discov.* **6**, 115–120 (2007).
66. Kola, I. & Landis, J. Can the pharmaceutical industry reduce attrition rates? *Nature Rev. Drug Discov.* **3**, 711–715 (2004).
67. LaRue, S. M. *et al.* Limb-sparing treatment for osteosarcoma in dogs. *J. Am. Vet. Med. Assoc.* **195**, 1734–1744 (1989).
68. Withrow, S. J. *et al.* Intra-arterial cisplatin with or without radiation in limb-sparing for canine osteosarcoma. *Cancer* **71**, 2484–2490 (1993).
69. Dewhirst, M. W. Animal modeling and thermal dose. *Radiol. Clin. North Am.* **27**, 509–518 (1989).
70. Dow, S. W. *et al.* *In vivo* tumor transfection with superantigen plus cytokine genes induces tumor regression and prolongs survival in dogs with malignant melanoma. *J. Clin. Invest.* **101**, 2406–2414 (1998).
71. Dow, S. W. & Potter, T. A. Expression of bacterial superantigen genes in mice induces localized mononuclear cell inflammatory responses. *J. Clin. Invest.* **99**, 2616–2624 (1997).
72. Elmslie, R. E. & Dow, S. W. Genetic immunotherapy for cancer. *Semin. Vet. Med. Surg. (Small Anim.)* **12**, 193–205 (1997).
73. Ladiges, W. C. *et al.* Failure of anti-MHC antibodies to prevent GVHD in DLA mismatched unrelated canine marrow recipients. *Bone Marrow Transplant.* **5**, 43–46 (1990).
74. Ladiges, W. C., Storb, R. & Thomas, E. D. Canine models of bone marrow transplantation. *Lab. Anim. Sci.* **40**, 11–15 (1990).
75. Whelan, H. T. *et al.* The role of photodynamic therapy in posterior fossa brain tumors. A preclinical study in a canine glioma model. *J. Neurosurg.* **79**, 562–568 (1993).
76. Hershey, A. E. *et al.* Inhalation chemotherapy for macroscopic primary or metastatic lung tumors: proof of principle using dogs with spontaneously occurring tumors as a model. *Clin. Cancer Res.* **5**, 2653–2659 (1999).
77. Khanna, C. *et al.* Nebulized interleukin 2 liposomes: aerosol characteristics and biodistribution. *J. Pharm. Pharmacol.* **49**, 960–971 (1997).
78. Khanna, C. *et al.* Interleukin-2 liposome inhalation therapy is safe and effective for dogs with spontaneous pulmonary metastases. *Cancer* **79**, 1409–1421 (1997).
79. Khanna, C., Hasz, D. E., Klausner, J. S. & Anderson, P. M. Aerosol delivery of interleukin 2 liposomes is nontoxic and biologically effective: canine studies. *Clin. Cancer Res.* **2**, 721–734 (1996).
80. Otterson, G. A. *et al.* Phase I study of inhaled Doxorubicin for patients with metastatic tumors to the lungs. *Clin. Cancer Res.* **13**, 1246–1252 (2007).
81. Rao, R. D., Anderson, P. M., Arndt, C. A., Wettstein, P. J. & Markovic, S. N. Aerosolized granulocyte macrophage colony-stimulating factor (GM-CSF) therapy in metastatic cancer. *Am. J. Clin. Oncol.* **26**, 493–498 (2003).
82. Rao, R. D., Markovic, S. N. & Anderson, P. M. Aerosol therapy for malignancy involving the lungs. *Curr. Cancer Drug Targets.* **3**, 239–250 (2003).
83. Rusk, A. *et al.* Preclinical evaluation of antiangiogenic thrombospondin-1 peptide mimetics, ABT-526 and ABT-510, in companion dogs with naturally occurring cancers. *Clin. Cancer Res.* **12**, 7444–7455 (2006).
84. London, C. A. *et al.* Phase I dose-escalating study of SU11654, a small molecule receptor tyrosine kinase inhibitor, in dogs with spontaneous malignancies. *Clin. Cancer Res.* **9**, 2755–2768 (2003).
85. Zemke, D., Yamini, B. & Yuzbasiyan-Gurkan, V. Mutations in the juxtamembrane domain of c-KIT are associated with higher grade mast cell tumors in dogs. *Vet. Pathol.* **39**, 529–535 (2002).
86. Reguera, M. J., Rabanal, R. M., Puigdemont, A. & Ferrer, L. Canine mast cell tumors express stem cell factor receptor. *Am. J. Dermatopathol.* **22**, 49–54 (2000).
87. Anderson, P. M. *et al.* Aerosol granulocyte macrophage-colony stimulating factor: a low toxicity, lung-specific biological therapy in patients with lung metastases. *Clin. Cancer Res.* **5**, 2316–2323 (1999).
88. Norden-Zfoni, A. *et al.* Blood-based biomarkers of SU11248 activity and clinical outcome in patients with metastatic imatinib-resistant gastrointestinal stromal tumor. *Clin. Cancer Res.* **13**, 2643–2650 (2007).
89. Britten, C. D. *et al.* A phase I and pharmacokinetic study of sunitinib administered daily for 2 weeks, followed by a 1-week off period. *Cancer Chemother. Pharmacol.* **61**, 515–524 (2007).
90. Figlin, R. A. Newly approved therapies for RCC and their effect on the standard of care. *Clin. Adv. Hematol. Oncol.* **5**, 35–36, 66 (2007).

91. Hornick, J. L. & Fletcher, C. D. The role of KIT in the management of patients with gastrointestinal stromal tumors. *Hum. Pathol.* **38**, 679–687 (2007).
92. Rubin, B. P., Heinrich, M. C. & Corless, C. L. Gastrointestinal stromal tumour. *Lancet* **369**, 1731–1741 (2007).
93. von Mehren, M. Beyond imatinib: second generation c-KIT inhibitors for the management of gastrointestinal stromal tumors. *Clin. Colorectal Cancer* **6** (Suppl. 1), S30–S34 (2006).
94. Kurzman, I. D. *et al.* Adjuvant therapy for osteosarcoma in dogs: results of randomized clinical trials using combined liposome-encapsulated muramyl tripeptide and cisplatin. *Clin. Cancer Res.* **1**, 1595–1601 (1995).
95. Kleinerman, E. S., Gano, J. B., Johnston, D. A., Benjamin, R. S. & Jaffe, N. Efficacy of liposomal muramyl tripeptide (CGP 19835A) in the treatment of relapsed osteosarcoma. *Am. J. Clin. Oncol.* **18**, 93–99 (1995).
96. Kleinerman, E. S. Biologic therapy for osteosarcoma using liposome-encapsulated muramyl tripeptide. *Hematol. Oncol. Clin. North Am.* **9**, 927–938 (1995).
97. Meyers, P. A. *et al.* Osteosarcoma: a randomized, prospective trial of the addition of ifosfamide and/or muramyl tripeptide to cisplatin, doxorubicin, and high-dose methotrexate. *J. Clin. Oncol.* **23**, 2004–2011 (2005).
98. Nardin, A., Lefebvre, M. L., Labroquere, K., Faure, O. & Abastado, J. P. Liposomal muramyl tripeptide phosphatidylethanolamine: Targeting and activating macrophages for adjuvant treatment of osteosarcoma. *Curr. Cancer Drug Targets* **6**, 123–133 (2006).
99. Liao, J. C. *et al.* Vaccination with human tyrosinase DNA induces antibody responses in dogs with advanced melanoma. *Cancer Immun.* **6**, 8 (2006).
100. Bergman, P. J. *et al.* Development of a xenogeneic DNA vaccine program for canine malignant melanoma at the Animal Medical Center. *Vaccine* **24**, 4582–4585 (2006).
101. MacEwen, E. G., *et al.* Adjuvant therapy for melanoma in dogs: results of randomized clinical trials using surgery, liposome-encapsulated muramyl tripeptide, and granulocyte macrophage colony-stimulating factor. *Clin. Cancer Res.* **5**, 4249–4258 (1999).
102. Vail, D. M. *et al.* Liposome-encapsulated muramyl tripeptide phosphatidylethanolamine adjuvant immunotherapy for splenic hemangiosarcoma in the dog: a randomized multi-institutional clinical trial. *Clin. Cancer Res.* **1**, 1165–1170 (1995).
103. Mack, G. S. Clinical trials going to the dogs: canine program to study tumor treatment, biology. *J. Natl Cancer Inst.* **98**, 161–162 (2006).
104. Martirosov, K. S., Grigor'ev, G., Borovkov, M. V. & Zorin, V. V. [Experimental study of the role of blocking 5-HT₃-receptors of serotonin and D₂-receptors of dopamine in the mechanism of early radiation vomiting in dogs]. *Radiats Biol. Radioecol* **42**, 75–79 (2002) (in Russian).
105. Legeza, V. I., Shagoian, M. G., Kamynina, M. F., Markovskaia, I. V. & Martirosov, K. S. [Mechanism of the species characteristics of the sensitivity of monkeys and dogs to the emetic action of various pharmacological agents]. *Biull Eksp. Biol. Med.* **93**, 64–66 (1982) (in Russian).
106. Modiano, J. F. *et al.* Distinct B-cell and T-cell lymphoproliferative disease prevalence among dog breeds indicates heritable risk. *Cancer Res.* **65**, 5654–5661 (2005).
107. Lingaas, F. *et al.* A mutation in the canine BHD gene is associated with hereditary multifocal renal cystadenocarcinoma and nodular dermatofibrosis in the German Shepherd dog. *Hum. Mol. Genet.* **12**, 3043–3053 (2003).
108. MacEwen, E. G., *et al.* Current studies of liposome muramyl tripeptide (CGP 19835A lipid) therapy for metastasis in spontaneous tumors: a progress review. *J. Drug Target* **2**, 391–396 (1994).
109. Thamm, D. H. *et al.* Systemic administration of an attenuated, tumor-targeting *Salmonella typhimurium* to dogs with spontaneous neoplasia: phase I evaluation. *Clin. Cancer Res.* **11**, 4827–4834 (2005).
110. Mack, G. S. Cancer researchers usher in dog days of medicine. *Nature Med.* **11**, 1018 (2005).
111. Reiser, H. *et al.* GS-9219 - A novel acyclic nucleotide analog with potent anti-neoplastic activity in dogs with spontaneous non-Hodgkin's lymphoma. *Clin. Cancer Res.* (in the press).

Acknowledgements

The authors are grateful for the contributions of C. Mazcko to the COP and the preparation of this manuscript. We also acknowledge the commitment and dedication of the members of the COTC and CCOGC.

DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>
 FLT3 | FRAP1 | IGF1R | KDR | KIT | MET | p53 | PDGFRB | THBS1 | TNF

National Cancer Institute Drug Dictionary:

<http://www.cancer.gov/drugdictionary/sunitinib.malate>

FURTHER INFORMATION

Chand Khanna's homepage: <http://ccr.cancer.gov/staff/staff.asp?profileid=8295>

Affymetrix: <http://www.affymetrix.com>

American College of Veterinary Internal Medicine: <http://www.acvim.org/>

American College of Veterinary Radiology-Radiation Oncology:

http://www.acvr.org/members/radiation_oncology/

Center for Cancer Research — Comparative Oncology Program: <http://ccr.cancer.gov/resources/cop/>

Dog Genome Sequencing Project at the Broad Institute: <http://www.broad.mit.edu/mammals/dog/>

E. A. Ostrander at the National Human Genome Research Institute: <http://www.genome.gov/12513335>

European College of Veterinary Internal Medicine: <http://www.ecvim-ca.org>

IDM Pharma: <http://www.idm-biotech.com>

Merial US: <http://us.merial.com>

Veterinary Cancer Society:

<http://www.vetcancersociety.org/>

VISTA: <http://genome.lbl.gov/vista/index.shtml>

ALL LINKS ARE ACTIVE IN THE ONLINE PDF