2011

Dog Models of Naturally Occurring Cancer

Donna O. McCarthy

Trends in Molecular Medicine, Vol. 17, No. 7 (July 2011): 380-388. DOI.

Dog Models of Naturally Occurring Cancer

Jennie L. Rowell
The Ohio State University College of Nursing, 1585 Neil Avenue, Columbus, OH
The Center for Human and Molecular Genetics at The Research Institute at Nationwide Children's Hospital
Donna O. McCarthy
The Ohio State University College of Nursing, 1585 Neil Avenue, Columbus, OH
Carlos E. Alvarez
The Center for Human and Molecular Genetics at The Research Institute at Nationwide Children's Hospital
Department of Pediatrics, The Ohio State University College of Medicine
Department of Veterinary Clinical Sciences, The Ohio State University College of Veterinary Medicine

Studies using dogs provide an ideal solution to the gap in animal models for natural disease and translational medicine. This is evidenced by approximately 400 inherited disorders being characterized in domesticated dogs, most of which are relevant to humans. There are several hundred isolated populations of dogs (breeds) and each has a vastly reduced genetic variation compared with humans; this simplifies disease mapping and pharmacogenomics. Dogs age five- to eight-fold faster than do

humans, share environments with their owners, are usually kept until old age and receive a high level of health care. Farseeing investigators recognized this potential and, over the past decade, have developed the necessary tools and infrastructure to utilize this powerful model of human disease, including the sequencing of the dog genome in 2005. Here, we review the nascent convergence of genetic and translational canine models of spontaneous disease, focusing on cancer.

The need for new models of complex disease

The greatest challenge facing clinical scientists is an incomplete understanding of the genetic basis for complex human diseases [1]. Despite numerous technological advances in genetics, progress has been slow. This is owed, in part, to intricate gene–gene interactions and poorly understood environmental effects [2]. The identification of these interactions and environmental influences is difficult to dissect in humans because of the high level of genetic heterogeneity [3]. Most genome-wide association studies (GWAS) have only identified a small fraction of the genetic bases of complex diseases [4]. Yet, disease heritability is crucial to understanding disease risk, the effects of environment and lifestyle on disease development and response to treatment.

Much of the research on human disease genetics relies on animal models. The most frequently used model, the mouse, has several advantages. Mice have short gestation times and are small, making their generation relatively rapid and inexpensive compared with other mammals. Moreover, technologies exist to manipulate the expression of genes in the entire organism or in selected cells or tissues [5]. However, mouse models of cancer have limitations. The most notable is that tumors arise spontaneously in humans, but must be induced in most mouse models. Whereas human disease is polygenic, genetic manipulations in mouse models often involve one or a few genes and/or environmental conditions that affect the expression of specific genes in an inbred mouse line with undetermined human relevance [3]. Mouse models of cancer in humans are thereby missing vast gene networks and interactions that are responsible for, or contribute to, disease in humans. Here, we discuss the advantages of tumor-bearing dogs as an alternative model for understanding the genetic bases of human disease [6], highlighting three cancer types as examples.

Advantages of dog models

Domesticated dogs (*Canis lupus familiaris*) are excellent models of human complex diseases for several reasons, including their easy accessibility and prominent status in diverse cultures. For instance, >73 million dogs live in ~40% of US households [7] and 54% of them are considered a 'family member' by their owners [8]. Over $40 billion is spent annually on dog health care [8], a level that is second only to humans in health care received [9]. That, combined with the shared environment of owners and dogs, can be exploited for epidemiological studies of diseases common to dogs and humans.

Next to humans, domesticated dogs have the most phenotypic diversity and known naturally occurring diseases of all land mammals [10]. For example, the average weights of Chihuahuas and English Mastiffs differ by 65-fold. Dogs share ~650 Mb of ancestral sequence in common with humans (which is absent in mice), and canine DNA and protein sequences are more similar to humans than are those of mice [11] (Figure 1a). The analysis of the 13 816 protein-coding genes with 1:1:1 orthology in humans, mice and dogs showed that the numbers of lineage-specific nonsynonymous substitutions (i.e. amino acid changing; K_A) are 0.017, 0.038 and 0.021, respectively [11]. Thus, many aspects of

human biology are presumably more relevant in dogs than they are in mice [12]. Approximately 400 inherited diseases similar to those of humans are characterized in dogs, including complex disorders such as cancer, heart disease and neurological disorders 13, 14. Indeed, more than 40 naturally occurring canine diseases have mutations in a homologous human gene associated with a similar disease [15]. Additionally, depending on breed size, dogs have a five- to eight-fold accelerated aging process compared with humans [http://www.avma.org/animal_health/care_older_pet_faq.asp]. Moreover, dogs are kept as companion animals well into their old age 16, 17. The most recently available data (2006) shows that ~45% of companion dogs were >6 years old [8], the human equivalent of ~60–95. Thus, dog models hold great promise for accelerating the understanding of genetic and environmental contributions to human disease, particularly those that are chronic or associated with aging.

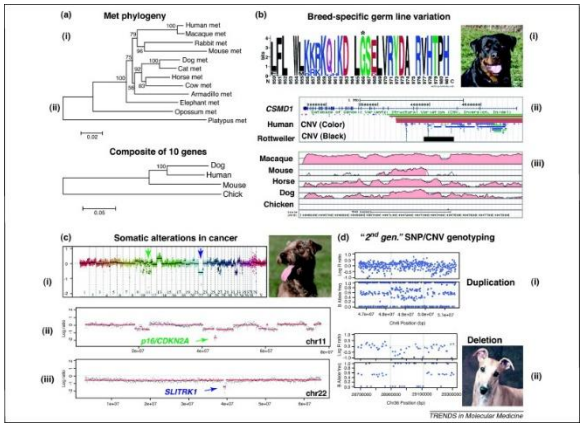

Figure 1. Dog cancer genetics. **(a)** Protein sequence conservation in dogs. **(i)** Phylogenetic tree of the mammalian c-Met receptor. The branching pattern corresponds well with the organismal relationships. For example, the Boreoeutheria clade comprises two sister taxa that include primates, rodents, rabbits and a taxa including carnivorans and most hoofed animals. Although mouse and human c-Met branch together, the branch length of mouse c-Met shows that the protein sequence is more divergent than that of human and dog (scale bar shows amino acid changes per site). **(ii)** Dog proteins are more similar to those of humans than are mouse proteins. Phylogenetic treeing analysis of a composite of 10 cancer proteins branches human and dog proteins apart from mouse with a bootstrap value of 100. The following proteins were included: MYC, ERBB2, KIT, ret proto-oncogene (RET), v-raf murine sarcoma viral oncogene homolog B1 (BRAF), PTEN, RB1, CDKN2A, breast cancer 1, early onset (BRCA1) and p53. [Neighbor-joining trees shown (500-replicate bootstrap values); maximum parsimony topology is the same.] **(b)** Examples of breed-specific germline variation with potential cancer relevance. **(i)** Common missense variant in Rottweiler c-Met receptor. WebLogo analysis shows a close-up of the consensus amino acid sequence of c-Met from 23 mammals. Letter height corresponds to the frequency of a given amino acid at each position, with the highest letters signifying complete conservation. A total of 70% of Rottweilers have a missense variant at Gly 966, which is located in the extracellular region and could thereby affect ligand binding or receptor signaling [70]. **(ii)** More than 60% of Rottweilers have a 273 kb copy number variant (CNV) in an intron of *CSMD1*, but it has not been observed in other breeds (UCSC Browser; human gene transcribed right to left) [71]. **(iii)** Close-up of one of several noncoding conserved elements within the *CSMD1* CNV (Vista Browser, conservation with human >60% shown by red coloring). The most conserved region within this area contains three candidate binding sites for the tumor suppressor transcription factor E2A (another conserved element contains p53-binding sites [71]). The conservation (which is absent in chicken) is reduced in the mouse in comparison to more distantly related mammals such as the horse and dog. **(c)** Somatic genome alterations in canine cancer. Kisseberth *et al*. isolated the OSW T-cell lymphoma cell line and identified several genomic alterations [72]. A single two-copy loss was found that affects the *CDKN2A* tumor suppressor gene. The subsequent analysis of OSW by high-resolution tiling oligonucleotide array CGH revealed many

additional alterations, including focal two-copy deletions affecting just a single gene [71]. (i) The whole genome display of the CGH analysis of OSW [71]. The midline shows a 1:1 DNA ratio to the reference genome of a Boxer. Deletion CNVs are segments below the midline and gains are above the midline (log 2 scale). 'Un' denotes unmapped contigs and is highly enriched for repetitive sequences; the Y chromosome is absent from the canFam2 genome assembly. **(ii, iii)** Close-up of the CGH analysis of chromosomes 11 and 22. Both chromosomes have two-copy microdeletions. One confirms the complete deletion of the tumor suppressor *p16/CDKN2A*. The other spans a single active gene *SLITRTK1*, which was previously implicated in malignant hematopoiesis [73]. This illustrates how dogs can be used as translational models of known human cancer genetics, as well as for the discovery of novel genes in the same genetic pathways. **(d)** Second generation genotyping technology allows the integration of SNP and CNV maps. CNVs from two Greyhounds are shown. This 170 k oligonucleotide array enables simultaneous SNP genotyping and DNA copy number determination (Illumina CanineHD). For each pair, the top window **(i)** shows DNA copy number as $\log_2 R$ ratios, with the midline generally corresponding to a copy number of two. The bottom windows **(ii)** show allele frequencies. A copy number gain is detected as an upward shift in the log*R* ratio and as a shift from B allele ratios of 1:1 (left and right segments) to 1:2 and 2:1 allele ratios (center segment). A copy number loss is detected as a downward shift in log*R* ratio and as a shift from allele ratios of 1:1 (left and right segments) to an allele ratio of 1:0 (or loss of heterozygosity; center).

The greatest advantage of dog models is the evolutionary history of canines, which has involved at least two severe population bottlenecks [14]. The first occurred when dogs were domesticated from wolves ~15 000–40 000 years ago [18]. The second was most pronounced ~200 years ago when most dog breeds were created by the selection of morphological and behavioral traits. Today, there are ~400 isolated populations or breeds. Breed creation has inadvertently selected many 'founder' mutations that are associated with specific traits and diseases; this translates into reduced disease and genetic heterogeneity, consistent with the fact that most breeds are predisposed to a distinct set of diseases. Because linkage disequilibrium is up to 100-fold greater in dogs than in humans, single breeds are powerful subjects for broad genetic mapping [14]. By contrast, related breeds that share a trait are powerful subjects for fine mapping. This advantage is illustrated by the recent analysis of polyneuropathy with juvenile onset in dogs, which is similar to human Charcot–Marie–Tooth (CMT) syndrome [19]. The comparison of seven affected and 17 related unaffected control Greyhounds identified a 19.5 Mb region that was homozygous in the affected dogs, and contained a 10 bp deletion in N-myc downstream regulated 1 (*NDRG1*), orthologous to a known human CMT gene. Pedigree information and the extended homozygosity suggest that the mutation arose in a popular sire in 1968. Now the disease can be eradicated from the breed through selective breeding, and the dog model can be used to better understand and treat human CMT [19]. Additionally, dogs might provide clues about the 'missing heritability' of human complex genetics. Recently, a group of 300 investigators performed a meta-analysis of GWAS (an approach using single nucleotide polymorphism (SNP) markers across the entire genomes of many people to find genetic variations associated with a particular disease) of 180 000 individuals characterized for height (known to be 80% heritable) [20]. They identified 180 loci that together explain 10% of height heritability. Similarly, Boyko *et al*. studied 57 quantitative morphological traits in 915 dogs that included samples from 80 breeds; traits included body size and external dimensions, and cranial/dental/long bone size and shape [21]. In contrast to human studies, they found that one to three quantitative trait loci explain the majority of phenotypic variation for most of the dog traits examined. The question now is whether canine complex diseases will turn out to have a similarly simplified genetic architecture.

Cancer development in dogs

Dogs are exceptional models of cancer because they naturally develop the same cancers as do humans [22]. Indeed, dog tumors are histologically similar to human tumors and respond similarly to conventional therapies [6]. Although disease course is reported to be more aggressive in dogs than in humans for some cancer types [6], it is not clear whether dog cancer is generally more aggressive than is human cancer. This issue is complicated because dog cancers are not treated as aggressively as human cancers and, therefore, they result in shorter survival times and faster evaluations of outcomes. Moreover, disease-bearing dogs tend to present for treatment at later stages than do humans. Regardless, the significantly shorter duration time of canine clinical trials is a major advantage [6] (Figure 2). The disease-free time interval in dogs treated for cancer is 18 months compared with the >7 years needed to assess treatment outcomes in humans [6]. Additionally, many histological types of cancer are associated with similar genetic alterations in humans and dogs. For instance, the statistical analysis of genomic alterations in human and dog colorectal tumors showed that samples were clustered according to stage, origin and instability status across species [23]. Strikingly, a cluster analysis of genome regions affected by DNA copy number alterations showed a branching together of human and dog tumors according to colorectal cancer subtypes (vs. species) [23]. This suggests that the same genetic pathways are affected in colorectal tumorigenesis in both species. By contrast, species-specific alterations tended to localize to evolutionarily unstable genome regions. These observations hint that the alterations common to both species are more likely to cause cancer than are those found in only one (i.e. the latter could be irrelevant species-specific mutation hotspots). In summary, dogs are useful in multiple approaches to cancer investigation [24]: breed-specific risk can be used to discover disease pathways; human cancer pathways can be tested for roles, and targeted for treatment, in canine disease; and canine somatic mutations and genome alterations can be used to narrow down human mutations (Figure 1b–d). Below we provide three examples of canine–human comparative oncology.

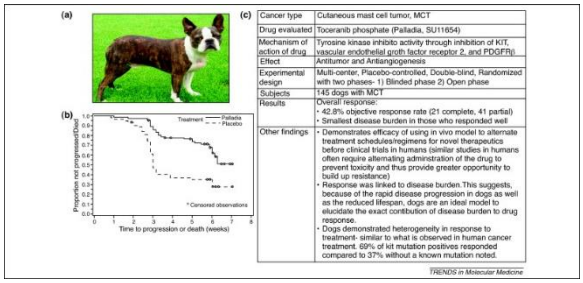

Figure 2. An example of the clinical relevance of dogs for cancer treatments. Canines are increasingly being used in clinical cancer drug trials to determine the efficacy of treatment given how closely many of the cancers they develop recapitulate the human cancer. **(a)** A picture of a Boston Terrier, a breed predisposed to the development of mast cell tumors. **(b)** London *et al*. conducted a clinical trial of an oral receptor tyrosine kinase inhibitor Palladia on dogs with recurrent mast cell tumors. Shown here is a Kaplan–Meier survival analysis demonstrating time-to-tumor progression in placebo-treated and Palladia-treated dogs with mast cell tumors [74]. **(c)** A breakdown of the clinical trial of Palladia, including the demonstrated advantages of dogs as models of pharmacologic cancer intervention. Reproduced, with permission, from [74].

Soft tissue sarcomas (STS)

STS comprise 1% of all newly diagnosed cancer types in humans [25] and represent a heterogeneous group of mesenchymal neoplasms that demonstrate a high degree of variation in clinical presentation and cellular morphology [26]. These genetically complex cancers include angiosarcomas (hemangiosarcomas in dogs), fibrosarcomas and histiocytomas. Recent advances in immunohistochemistry, cytogenetics and molecular genetic analysis have allowed a clinically relevant division of STS to improve diagnosis and treatment [27]. Based on clinical and biological variation among these neoplasms, STS can be broadly dichotomized into two groups. One is characterized by specific, balanced chromosomal translocations, whereas the other typically shows more extensive chromosomal rearrangements leading to recurrent, but nonspecific, chromosomal gains and losses [27]. Owing to their complex nature, the specific cells from which most of this group of cancers develop remain largely unknown. Although some strains of mice have developed spontaneous STS, rodent models generally require an induction of STS [28]. By contrast, dogs are an excellent model of STS because they have similar tumor genetic complexity to that of humans [29]. For instance, two poorly differentiated fibrosarcomas taken from Labrador Retrievers had large chromosomal rearrangements, amplifications and deletions similar to those observed in human fibrosarcomas [30]. Notably, these fibrosarcomas had a loss of heterozygosity affecting the cyclin-dependent kinase family 2A and 2B (*CDKN2A/CDKN2B*). Given that deletions of *CDKN2A* and *CDKN2B* have been reported in other cancer types, including STS in humans, this offers a novel target for discovering common pathways and genes affected in both dogs and humans that affects the development or progression of STS [29].

Another advantage of using canines for studying STS is breed predispositions to specific types of STS, including increased incidences in Flat-coated Retrievers and Rhodesian Ridgebacks [13]. For example, hemangiosarcomas are relatively common in dogs, accounting for ~5–7% of all observed tumors [31]. The dogs at greatest risk for hemangiosarcomas are Golden Retrievers (GRs), German Shepherds and Boxers [32]. One group recently compared gene expression profiles in hemangiosarcoma tumors from multiple dog breeds [22]. They found that the GR was unique in its overexpression of vascular endothelial growth factor 1 (*VEGF1*) compared with other breeds, whereas *VEGF2* was more highly expressed in the other breeds compared with the GR. When *VEGF2* expression was blocked in hemangiosarcoma-derived tumor cell lines, the rate of cell growth slowed – except in cell lines derived from GR tumors. This finding implies that the unique genetic background of the GR influenced the susceptibility of this breed to the development of hemangiosarcomas, suggesting that canine tumors can be used to understand how genetic background can influence the susceptibility of an individual to non-inherited cancers. Clinical trials involving tyrosine kinase inhibitor treatment of STS found that the most effective (e.g. Sorafenib) also targeted all VEGF isoforms [33]. Performing clinical trials on pedigree dogs, such as GRs, could provide novel information regarding genetic background effects on tumor progression. Thus, given the increased incidence of STS in dogs, the diversity of naturally occurring ‘complex’ and ‘simple’ sarcoma similarity in humans and dogs and the availability of different genetic backgrounds across breeds for clinical therapy testing, the canine model is more relevant than are other animal models for direct human STS applications.

Osteosarcoma (OSA)

In humans, the most commonly diagnosed primary malignant tumor of the bone is OSA. It is the third most frequent cause of cancer in adolescents and represents over 56% of all bone tumors. The prognosis for patients with metastatic OSA is poor, with only 20% surviving event-free for 5 years post-diagnosis [34] and >30% of patients failing to respond to chemotherapy [35]. Approximately 10 000 dogs are diagnosed with OSA yearly in the USA [36] compared with 2650 new cases of human primary bone cancer (including OSA, Ewing sarcoma, malignant fibrous histiocytoma and chondrosarcoma; http://www.cancer.gov/cancertopics/types/bone/). Because there is no consistent method for reporting cancer in dogs, we estimate OSA incidence is at least 13.9/100 000 8, 37 as opposed to the actual incidence of 1.02/100 000 in humans (across all ages) [38]. In both humans and dogs, OSA has a bimodal age distribution and the main cause of death is pulmonary metastasis. It accounts for 85% of malignancies originating in the bone [39] in large and giant dog breeds [40], which have an OSA risk 61 times higher than all breeds [32]. The canine disease is much more aggressive than the human disease, with surgical treatment alone producing a 5% survival rate [36]. The same treatments for OSA are used in both humans and dogs [41]. Dogs develop OSA at similar sites as do humans and both have similar histologies and responses to treatment 36, 42. Indeed, dogs have been a valuable model of OSA since they first participated in clinical trials pioneering limb salvage techniques that are now used in humans [43].

In addition to the similarity of tumor biological behavior of human and dog OSA, recent studies have identified parallel genetic features [44]. Both human and canine OSA have a 75% aneuploid DNA index, and both share similar genetic alterations [42]. Moreover, many candidate genes implicated in pediatric OSA have also been implicated in the canine disease: phosphatase and tensin homolog (*PTEN*), retinoblastoma 1 (*RB1*), ezrin (*EZR*), met proto-oncogene [hepatocyte growth factor (HGF) receptor; *MET*], v-erb-b2 erythroblastic leukemia viral oncogene homolog 2 (*ERBB2*) and tumor protein 53 (*TP53*) [45]. The commonly affected p53 tumor suppressor pathway has similar alterations in human and canine OSA [46]. Because human *TP53* is more similar to that of dogs than to that of mice [47], and because mutations occur naturally in dogs, the canine OSA model is presumably more relevant to humans. Additionally, recent work in dogs has focused on the differential OSA tumor expressions of genes associated with short- and long-term survival [48]. In experiments using cDNA microarrays, investigators found the deregulated expression of the following signaling pathways that were previously reported in human OSA: Wnt, chemokine/cytokine, apoptosis signaling, interleukin and Ras [48]. The coexpression of HGF and the proto-oncogenic receptor c-Met are implicated in growth, invasion and metastasis in human OSA. Although they are more frequently overexpressed in human OSA, another study found the coexpression of HGF and c-Met in all 59 OSA canine tumors studied, with the overexpression of both present in 24% of cases [49]. Other investigators have identified two genes, interleukin 8 (*IL8*) and solute carrier family member 3 (*SLC1A3*), that were uniformly expressed in all canine OSA tumors, but not in all human pediatric OSA tumors. However, pediatric patients who did overexpress *IL8* and *SLC1A3* had poorer outcomes then those who did not [50]. Yet another gene expression study of canine OSA tumors identified 10 significantly differentiated pathways between responders to treatment and nonresponders [51]. These pathways (including cAMP signaling, chemokines and adhesion and sonic hedgehog and parathyroid hormone signaling pathways in bone and cartilage development) are also disrupted in human cancers. These various findings suggest that

alterations in similar pathways occur in human and canine OSA, but that species-specific genetic changes might account for the overall disparity in incidence and aggressiveness. Related to that, Phillips *et al*. used a whole genome linkage approach to map OSA segregating in a four-generation pedigree of Scottish Deerhounds [52]. They found evidence of linkage (Zmax=5.766) consistent with a dominant OSA mutation in a 4.5 Mb region of chromosome 34q16.2–q17.1 (syntenic to human 3q26). Because OSA is relatively rare and most cases are sporadic in humans, inherited forms and different risks across dog breeds offer a great opportunity to identify pathogenetic pathways.

OSA tumors in dogs and humans also share DNA structural changes. Analyzing 38 OSA tumors from 29 Rottweilers and nine GRs, a recent study demonstrated that, as with its human counterpart, dog OSA has a tendency toward highly complex and chaotic karyotypes [53]. These comprise structural and numerical aberrations, including gene dosage imbalances of known oncogenes and tumor suppressors. The most frequently observed genome alteration was an amplification affecting both the *MYC* and *KIT* (*c-KIT*) oncogenes. This is consistent with observations of genome alterations in human OSA that are predictive of clinical outcome. Notably, *KIT* was recently proposed as a novel therapeutic target for pediatric OSA [54]. This supports not only the genetic relevance of the canine model, but also the clinical utility of including dogs in OSA clinical trials. Thus, the canine OSA model recapitulates the human cancer and, because OSA occurs 20 times more often in dogs than in humans [42], it provides an unparalleled opportunity for identifying key cellular pathways in this cancer [12].

Lymphomas

The group of cancers affecting the lymph tissue is collectively known as lymphomas. Lymphomas represent ~5% of all human cancers in the US and account yearly for treating totaling $4.6 billion (http://www.cancer.gov/aboutnci/servingpeople/snapshots/lymphoma.pdf). One specific class, namely non-Hodgkin's lymphoma (NHL), occurs in B- or T-cells, with >65 000 new cases reported in 2009 (for types of NHL, see http://www.cancer.gov/cancertopics/types/non-hodgkins-lymphoma). Notably, the incidence of NHL is increasing but the etiology remains obscure [55]. Thus, an alternative model of lymphoma is needed to elucidate the causes and identify clinically meaningful cancer biology. Dogs and humans have similar tumor biologies, tumor biological behaviors and genetic aberrations. The incidence of lymphoma in humans and dogs is similar [56]: 15.5–29.9 and 15–30 [57] per 100 000, respectively. The most common type of NHL is the same in both humans and dogs – diffuse large B-cell – and the same chemotherapy agents are used to treat it [55]. An additional advantage of the dog model is the increased prevalence of lymphoma within specific dog breeds. Lymphoma is the most common life-threatening cancer in all dogs, accounting for 24% of all canine cancers (http://www.akcchf.org/pdfs/2009FundingRequest.pdf). Approximately one in four Boxers and one in eight GRs develop lymphomas [32]. Additionally, there is a breed-specific distribution of B-cell and T-cell lymphomas [58] (Figure 3), whereas an excess incidence of T-cell lymphomas was noted in 10 breeds, the most striking occurring (in order of observed frequency) in Irish Wolfhounds, Siberian Huskies and Shih Tzus. By contrast, the breeds with an excessive occurrence of B-cell lymphomas were Cocker Spaniels and Basset Hounds. A second study conducted in Norway grouped all types of lymphomas and identified an excessive occurrence of lymphomas in specific breeds, lending credence to a breed-specific risk for lymphoma development [59]. They found the relative risk of lymphoma was highest in the Boxer and Flat-coated Retriever. More recently, a study examining records from the Veterinary Medical Database selected cases with an unspecified diagnosis of lymphoma type, giant follicular lymphoma and lymphosarcoma and used controls with any diagnosis other than lymphoma [60]. This study also identified a breed-specific risk for lymphoma with the highest breeds including Bullmastiff [odds ratio (OR) 4.83 vs. control], Boxer [OR 4.05 vs. control] and Bernese Mountain dog [OR 3.64 vs. control]. Notably, although the former and latter studies examined different subsets of lymphomas, they

included many of the same breeds and had similar findings. For instance, the Irish Wolfhound had the highest rate of T-cell lymphoma in the Modiano *et al*. study [58], and also had an OR of 3.23 for lymphoma compared with other dogs in the Villamil *et al*. study [60]. The underlying cytogenetic basis of lymphoma seems to be shared in humans and dogs. The examination of three canine hematological cancers, including Burkitt lymphoma and small lymphocytic lymphoma [61], showed that these canine cancers shared cytogenetic abnormalities with those characteristic of their human counterparts. This suggests that humans and dogs share common pathways or an ancestrally retained pathogenetic basis for lymphoma [61]. Consequently, by comparing the dog genome with the human genome, relevant genetic aberrations can be identified.

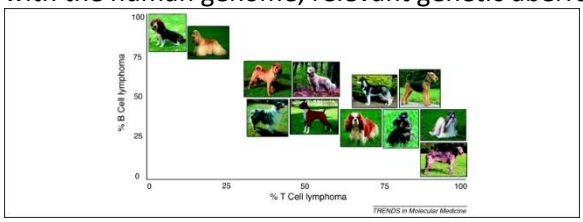

Figure 3. Prevalence of B- and T-cell lymphomas in dog breeds. A varying excess of T- and B-cell lymphomas, in a breed-specific manner, has been noted. Presented here is the observed percentage of T- vs. B-cell lymphomas by breed: Irish Wolfhounds (100:0 Siberian Huskies (88.9:11.1), Shih Tzus (81:19), Airedale Terriers (80:20), Cavalier King Charles Spaniels (80:20) and Yorkshire Terriers (80:20). By contrast, the breeds with an excessive occurrence of B-cell compared with T-cell lymphomas were Cocker Spaniels (93.2:6.8) and Basset Hounds (94.4:5.6) [58]. Photo sources follow: http://www.dublinirishfestival.org/animals/irishwolfhound.php; http://blogneffy.blogspot.com/2010/06/wanted-shih-tzu-breeders-in-davao-city.html; http://sentinelkennels.com/images/airedale.jpg; http://www.justdogbreeds.com/images/breeds/cavalier-king-charles-spaniel.jpg; http://www.petsflick.com/images/yorkshire-terrier.jpg; http://tidyyourdog.com/wp-content/uploads/2009/04/siberian-husky.jpg; http://www.petside.com/breeds/chinese-shar-pei.php; http://www.fordogtrainers.com/ProductImages/dog-breeds-muzzles/Australian-Shepherd-muzzle-Australian-Shepherd.jpg; http://www.breederretriever.com/photopost/pindex/516/; http://retrieverman.files.wordpress.com/2009/01/white-golden-retriever-wikipedia.jpg; http://www.dogtastic.org/dogtastic/images/BreedPics/cocker%20spaniel.jpg; http://a1.cdnsters.com/static/images/dogster/breeds/basset_hound.jpg.

Finally, the relevance of dogs as a lymphoma model is supported by use in clinical trials. Given that dogs develop spontaneous B-cell NHL and share many characteristics in common with human B-cell NHL [such as diagnostic criteria and response to a chemotherapy-based regimen that includes cyclophosphamide, doxorubicin, vincristine and prednisone (commonly referred to as CHOP chemotherapy)], dogs were recently enrolled in a clinical trial of a selective and irreversible Bruton tyrosine kinase (Btk) inhibitor PCI-32765, which blocks B-cell activation [62]. The activation of the B-cell antigen receptor signaling pathway contributes to the initiation and maintenance of B-cells [62]. This clinical trial research began when the same group described the synthesis of a series of Btk inhibitors that bind covalently to a cysteine residue, leading to the potent and irreversible inhibition of Btk enzymatic activity. In that study, after the additional analysis of this agent in both cell lines and mouse models, they initiated a canine clinical trial. Although the clinical trial is ongoing, eight dogs have been treated, with three demonstrating stable disease and three with partial responses including one dog with a 77% decrease in tumor size (this drug is now undergoing human clinical development in patients

with B-cell malignancies). Finally, a recent pilot study used antihuman leukocyte antigen (HLA)-DR monoclonal antibody (mAb) as a treatment for dogs with lymphomas [63]. Preliminary results have demonstrated that humanized IgG4 anti-HLA-DR, currently under evaluation preclinically for human trials, also bound malignant canine lymphocytes. These findings provide justification for using dogs with lymphomas in the safety and efficacy evaluations of therapy for both veterinary and human purposes [63] (Figure 4).

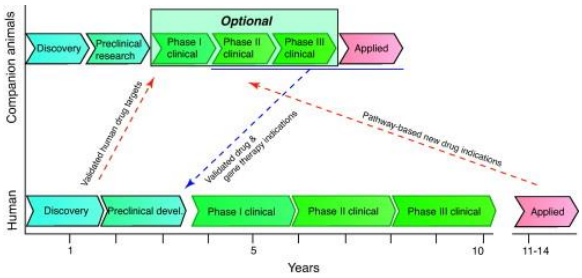

Figure 4. Translational potential of tumor-bearing dogs. At the bottom is the typical course of human drug R&D. There is no established paradigm for drug R&D in dogs and other companion animals [6]. Although our schematic mirrors the same process in pets, most drugs used on patient animals are taken from human drug development or are approved human drugs used off-label. Indeed, few regulations exist for phase I/II/III clinical trials before drugs are used in pets.

Potential utility of dogs in translational medicine

The naturally occurring relevance of the canine model to cancer in humans can be exploited to generate new treatments relatively quickly (Figure 4). Whereas there are strict FDA regulations concerning treatments to be used and commercialized, as well as for clinical trials in humans, there are fewer regulations for phase I/II/III clinical trials before drug use in pets [64] (http://prsinfo.clinicaltrials.gov). Rather, it is left to the discretion of the owner, who could approve the use of investigational therapeutics before conventional treatments. Several trends in drug development suggest the increased use of dogs as translational models. Two of these are the rising proportion of biological vs. chemical compounds and the growing focus on targeting genetic/biochemical pathways (or disease subtypes) vs. broad diseases or types of cancer. Here, we propose that dogs are ideal patients in which to develop novel therapeutics. Several facts indicate that using dogs in translational medicine can hugely accelerate drug development: reduced regulatory guidelines, vastly diminished and soon-to-be fully defined genetic variation within breeds (but similar levels of variation occur across all breeds as with humans), reduced disease heterogeneity (i.e. breed-specific risks of diseases are often associated with a single founder mutation) and accelerated aging/disease progression compared with humans. These genetic benefits translate into faster progress at every stage (e.g. identifying disease mutations in discovery, identifying biomarkers and endpoints in clinical trials and using pharmacogenetics from preclinical research to postapproval studies). Indeed, dogs have been instrumental in the rapid development of biological and biological-like therapeutics, including gene therapies (e.g. for specific inherited forms of muscular and retinal dystrophies [65]) and antisense morpholino oligonucleotides (e.g. to alter mRNA splicing and avert the nonsense-mediated decay of dystrophin [66]). However, we believe dog patients are greatly underutilized in the development of therapeutic interventions. Drug development is difficult and risky,

with the average drug costing approximately $800 million to develop. One of the most challenging go/no go decision points is determining whether a therapeutic agent is effective in humans. This is established by a small clinical study of select subjects that might respond to therapy. Dog breeds with known disease mutations are ideal lead-ins to such studies. Depending on the disease, such proof-of-concept studies could be robustly performed in even fewer than 10 subjects and at a pace proportional to the accelerated disease progression. Such studies would establish not only efficacy, pharmacokinetics/dynamics and toxicity, but also dosing, biomarkers/endpoints and adverse effects. This could dramatically reduce the failure rate of human proof-of-concept studies, and thereby save time and costs.

Concluding remarks

Dogs are uniquely suited as animal models for complex human diseases because of their phenotypic diversities and the similarity to human conditions of their naturally occurring diseases. The evolutionary histories of dogs, their positions as a family member in many households and the high levels of health care they receive offer tremendous opportunities. That, combined with recently developed genetic resources, makes dogs outstanding models for the study of known genetic pathways, discovery of genetic and environmental contributions to disease and translational studies in cancer risk, prevention and treatments 6, 14. The full utilization of canine models of cancer will require expertise in basic science, translation and direct clinical relevance. This will necessitate large collaborations across almost all aspects of veterinary and human medicine including molecular biology and genetics, epidemiology, pharmacology, bioinformatics, statistics and engineering. Developing these pipelines now will speed potential therapeutic outcomes. Although this review has focused on the relevance of the dog as a model for research in cancer genetics, biomedical research has long included canine models of numerous other diseases and their treatments [14]. For example, dogs are also increasingly used in behavioral research, including learning [67], social cognition [68] and the effects of diet and behavior enrichment on executive functioning [69]. The increased appreciation of the unique and comparative value of the dog as a model for diverse human diseases should accelerate research, leading to new treatments and improved health care for both humans and our best friends.

Acknowledgments

We thank C.A. London for critically reading the manuscript, and W.C. Kisseberth and C.G. Couto for providing their veterinary clinical expertise. CEA is supported by R210602710 from NIH/NIHGR and funding from The Research Institute at Nationwide Children's Hospital. JLR is supported by a fellowship award F31NR011559 from NIH/NINR.

References

1 E.S. Lander, N.J. Schork. **Genetic dissection of complex traits.** *Science*, 265 (1994), pp. 2037-2048

2 K. Strauch, *et al.* **How to model a complex trait. 1. General considerations and suggestions.** *Hum. Hered*., 55 (2003), pp. 202-210

3 E.K. Karlsson, K. Lindblad-Toh. **Leader of the pack: gene mapping in dogs and other model organisms.** *Nat. Rev. Genet*., 9 (2008), pp. 713-725

4 T.A. Manolio, *et al.* **Finding the missing heritability of complex diseases.** *Nature*, 461 (2009), pp. 747-753

5 Y. Gondo, *et al.* **Next-generation gene targeting in the mouse for functional genomics.** *BMB Rep*., 42 (2009), p. 315-323
6 M. Paoloni, C. Khanna. **Translation of new cancer treatments from pet dogs to humans.** *Nat. Rev. Cancer*, 8 (2008), pp. 147-156
7 APPA National Pet Owners Survey (2005). American Pet Products Manufacturers Association (APPMA).
8 US pet ownership & demographics sourcebook. (2007). American Veterinary Medical Association.
9 D.F. Patterson. **Companion animal medicine in the age of medical genetics.** *J. Vet. Intern. Med*., 14 (2000), pp. 1-9
10 M.P. Starkey, *et al.* **Dogs really are man's best friend – canine genomics has applications in veterinary and human medicine!** *Brief Funct. Genomic Proteomic*, 4 (2005), pp. 112-128
11 K. Lindblad-Toh, *et al.* **Genome sequence, comparative analysis and haplotype structure of the domestic dog.** *Nature*, 438 (2005), pp. 803-819
12 C. Khanna, *et al.* **The dog as a cancer model.** *Nat. Biotechnol*., 24 (2006), pp. 1065-1066
13 D.R. Sargan. **IDID: inherited diseases in dogs: web-based information for canine inherited disease genetics.** *Mamm. Genome*, 15 (2004), pp. 503-506
14 H.G. Parker, *et al.* **Man's best friend becomes biology's best in show: genome analyses in the domestic dog.** *Annu. Rev. Genet*., 44 (2010), pp. 309-336
15 E.A. Ostrander, *et al.* **Canine genetics comes of age.** *Trends Genet*., 16 (2000), pp. 117-124
16 B.J. Cummings, *et al.* **The canine as an animal model of human aging and dementia.** *Neurobiol. Aging*, 17 (1996), pp. 259-268
17 B.N. Bonnett, A. Egenvall. **Age patterns of disease and death in insured Swedish dogs, cats and horses.** *J. Comp. Pathol*., 142 (Suppl. 1) (2010), pp. S33-38
18 M. Germonpré, *et al.* **Fossil dogs and wolves from Palaeolithic sites in Belgium, the Ukraine and Russia: osteometry, ancient DNA and stable isotopes.** *J. Archaeological Sci*., 36 (2009), pp. 473-490
19 C. Drogemuller, *et al.* **A deletion in the N-myc downstream regulated gene 1 (NDRG1) gene in Greyhounds with polyneuropathy.** *PLoS ONE*, 5 (2010), p. e11258
20 H. Lango Allen, *et al.* **Hundreds of variants clustered in genomic loci and biological pathways affect human height.** *Nature*, 467 (2010), pp. 832-838
21 A.R. Boyko, *et al.* **A simple genetic architecture underlies morphological variation in dogs.** *PLoS Biol*., 8 (2010), p. e1000451
22 B.A. Tamburini, *et al.* **Gene expression profiles of sporadic canine hemangiosarcoma are uniquely associated with breed.** *PLoS ONE*, 4 (2009), p. e5549
23 J. Tang, *et al.* **Copy number abnormalities in sporadic canine colorectal cancers.** *Genome Res*., 20 (2010), pp. 341-350
24 M. Breen. **Update on genomics in veterinary oncology.** *Top Companion Anim. Med*., 24 (2009), pp. 113-121
25 D. Krikelis, I. Judson. **Role of chemotherapy in the management of soft tissue sarcomas.** *Expert Rev. Anticancer Ther*., 10 (2010), pp. 249-260
26 L. Guillou, A. Aurias. **Soft tissue sarcomas with complex genomic profiles.** *Virchows Arch*., 456 (2010), pp. 201-217
27 F. Mertens, *et al.* **Genomic characteristics of soft tissue sarcomas.** *Virchows Arch*., 456 (2010), pp. 129-139
28 S.M. Cohen, *et al.* **Hemangiosarcoma in rodents: mode-of-action evaluation and human relevance.** *Toxicol. Sci.,* 111 (2009), pp. 4-18
29 J. Aguirre-Hernandez, *et al.* **Disruption of chromosome 11 in canine fibrosarcomas highlights an unusual variability of CDKN2B in dogs.** *BMC Vet. Res*., 5 (2009), p. 27
30 D.R. Sargan, *et al.* **Chromosome rearrangements in canine fibrosarcomas.** *J. Hered*., 96 (2005), pp. 766-773
31 J. Modiano. ***Canine hemangiosarcoma – the road from despair to hope.*** National Canine Cancer Foundation (2008)

32 A.L. Shearin, E.A. Ostrander. **Leading the way: canine models of genomics and disease.** *Dis. Model Mech*., 3 (2010), pp. 27-34
33 J. Chao, W.A. Chow. **Novel targeted therapies in the treatment of soft-tissue sarcomas.** *Expert Rev. Anticancer Ther*., 10 (2010), pp. 1303-1311
34 V. Mialou, *et al.* **Metastatic osteosarcoma at diagnosis: prognostic factors and long-term outcome – the French pediatric experience.** *Cancer*, 104 (2005), pp. 1100-1109
35 H.J. Mankin, *et al.* **Survival data for 648 patients with osteosarcoma treated at one institution.** *Clin. Orthop. Relat. Res*. (2004), pp. 286-291
36 S.J. Withrow, D.M. Vail (Eds.), Withrow & MacEwen's *Small Animal Clinical Oncology* (4th edn), Saunders Elsevier (2007)
37 S.J. Withrow, G. MacEwan (Eds.), *Small Animal Clinical Oncology* (3rd edn), W.B. Saunders (2001)
38 L. Mirabello, *et al.* **Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program.** *Cancer*, 115 (2009), pp. 1531-1543
39 J. Harari. **The prevalence of and risk factors for canine appendicular osteosarcoma.(study recaps and comments).** *Vet. Med*., 103 (2008) 79(1)
40 G. Ru. **Host related risk factors for canine osteosarcoma.** *Vet. J*., 156 (1998), pp. 31-39
41 P.J. Messerschmitt, *et al.***Osteosarcoma.** *J Am. Acad. Orthop. Surg*., 17 (2009), pp. 515-527
42 S.J. Withrow, R.M. Wilkins. **Cross talk from pets to people: translational osteosarcoma treatments.** *ILAR J*., 51 (2010), pp. 208-213
43 S.M. LaRue, *et al.* **Limb-sparing treatment for osteosarcoma in dogs.** *J. Am. Vet. Med. Assoc*., 195 (1989), pp. 1734-1744
44 F. Mueller, *et al.* **Comparative biology of human and canine osteosarcoma.** *Anticancer Res*., 27 (2007), pp. 155-216
45 J. Kirpensteijn, *et al.* **TP53 gene mutations in canine osteosarcoma.** *Vet. Surg*., 37 (2008), pp. 454-460
46 R.A. Levine, M.A. Fleischli. **Inactivation of p53 and retinoblastoma family pathways in canine osteosarcoma cell lines.** *Vet. Pathol*., 37 (2000), pp. 54-61
47 L. Nasir, *et al.* **Nucleotide sequence of a highly conserved region of the canine p53 tumour suppressor gene.** *DNA Seq*., 8 (1997), pp. 83-86
48 G.T. Selvarajah, *et al.* **Gene expression profiling of canine osteosarcoma reveals genes associated with short and long survival times.** *Mol. Cancer*, 8 (2009), p. 72
49 H. Fieten, *et al.* **Expression of hepatocyte growth factor and the proto-oncogenic receptor c-Met in canine osteosarcoma.** *Vet. Pathol*., 46 (2009), pp. 869-877
50 M. Paoloni, *et al.* **Canine tumor cross-species genomics uncovers targets linked to osteosarcoma progression.** *BMC Genomics*, 10 (2009), p. 625
51 L.E. O'Donoghue, *et al.* **Expression profiling in canine osteosarcoma: identification of biomarkers and pathways associated with outcome.** *BMC Cancer*, 10 (2010), p. 506
52 J.C. Phillips, *et al.* **A novel locus for canine osteosarcoma (OSA1) maps to CFA34, the canine orthologue of human 3q26.** *Genomics*, 96 (2010), pp. 220-227
53 R. Thomas, *et al.* **Influence of genetic background on tumor karyotypes: evidence for breed-associated cytogenetic aberrations in canine appendicular osteosarcoma.** *Chromosome Res*., 17 (2009), pp. 365-377
54 N. Entz-Werle, *et al.* **KIT gene in pediatric osteosarcomas: could it be a new therapeutic target?** *Int. J. Cancer*, 120 (2007), pp. 2510-2516
55 Cancer Trends Progress Report – 2009/2010 Update, National Cancer Institute, NIH, DHHS, Bethesda, MD, April 2010.
56 K. Hansen, C. Khanna. **Spontaneous and genetically engineered animal models; use in preclinical cancer drug development.** *Eur. J. Cancer*, 40 (2004), pp. 858-880

57 K.A. Hahn, *et al.* **Naturally occurring tumors in dogs as comparative models for cancer therapy research.** *In Vivo*, 8 (1994), pp. 133-143

58 J.F. Modiano, *et al.* **Distinct B-cell and T-cell lymphoproliferative disease prevalence among dog breeds indicates heritable risk.** *Cancer Res*., 65 (2005), pp. 5654-5661

59 H. Gamlem, *et al.* **Canine neoplasia – introductory paper.** *APMIS Suppl*. (2008), pp. 5-18

60 J.A. Villamil, *et al.* **Hormonal and sex impact on the epidemiology of canine lymphoma.** *J. Cancer Epidemiol*. (2009), p. e591753

61 M. Breen, J.F. Modiano. **Evolutionarily conserved cytogenetic changes in hematological malignancies of dogs and humans – man and his best friend share more than companionship.** *Chromosome Res*., 16 (2008), pp. 145-154

62 L.A. Honigberg, *et al.* **The Bruton tyrosine kinase inhibitor PCI-32765 blocks B-cell activation and is efficacious in models of autoimmune disease and B-cell malignancy.** *Proc. Natl. Acad. Sci. U.S.A*., 107 (2010), pp. 13075-13080

63 R. Stein, *et al.* **Evaluation of anti-human leukocyte antigen-DR monoclonal antibody therapy in spontaneous canine lymphoma.** *Leuk. Lymphoma*, 52 (2011), pp. 273-284

64 C. Khanna, *et al.* **Guiding the optimal translation of new cancer treatments from canine to human cancer patients.** *Clin. Cancer Res*., 15 (2009), pp. 5671-5677

65 Z. Wang, *et al.* **Gene therapy in large animal models of muscular dystrophy.** *ILAR J.,* 50 (2009), pp. 187-198

66 T. Yokota, *et al.* **Efficacy of systemic morpholino exon-skipping in Duchenne dystrophy dogs.** *Ann. Neurol*., 65 (2009), pp. 667-676

67 A.M. Elgier, *et al.* **Communication between domestic dogs (*Canis familiaris*) and humans: dogs are good learners.** *Behav. Process*., 81 (2009), pp. 402-408

68 V. Morell. **Animal behavior. Going to the dogs.** *Science*, 325 (2009), pp. 1062-1065

69 C.W. Cotman, E. Head. **The canine (dog) model of human aging and disease: dietary, environmental and immunotherapy approaches.** *J. Alzheimers Dis*., 15 (2008), pp. 685-707

70 A.T. Liao, *et al.* **Identification of a novel germline MET mutation in dogs.** *Anim. Genet*., 37 (2006), pp. 248-252

71 W.K. Chen, *et al.* **Mapping DNA structural variation in dogs.** *Genome Res*., 19 (2009), pp. 500-509

72 W.C. Kisseberth, *et al.* **A novel canine lymphoma cell line: a translational and comparative model for lymphoma research.** *Leuk. Res*., 31 (2007), pp. 1709-1920

73 T. Milde, *et al.* **A novel family of slitrk genes is expressed on hematopoietic stem cells and leukemias.** *Leukemia*, 21 (2007), pp. 824-827

74 C.A. London, *et al.* **Multi-center, placebo-controlled, double-blind, randomized study of oral toceranib phosphate (SU11654), a receptor tyrosine kinase inhibitor, for the treatment of dogs with recurrent (either local or distant) mast cell tumor following surgical excision.** *Clin. Cancer Res*., 15 (2009), pp. 3856-3865